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Accumulation of Heavy Metals in Caspian Seals (*Phoca caspica*)

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Abstract. Concentrations of heavy metals (Fe, Mn, Zn, Cu, Pb, Ni, Cd, Co, and Hg) were determined in the muscle, liver, and kidney of 42 Caspian seals and fishes collected from the Caspian Sea in 1993. Higher Mn and lower Fe and Cu concentrations were found in the liver in comparison with other marine pinnipeds. Lower Cu concentrations in the liver appear to be a common feature in small seals belonging to subgenus *Pusa*, which include ringed, Baikal, and Caspian seals. However, low Fe and high Mn in livers were specific to Caspian seal. Concentrations of toxic metals such as Hg and Cd were relatively low. Pinniped species can be divided into two groups, based on accumulations of Cd or Hg in the liver. Interestingly, it was found that Cd-accumulating groups feed on invertebrates, whereas the preferred diet of Hg accumulators is fish. Caspian seals seemed to belong to the Hg-accumulating group. Cd and Hg concentrations in the liver and kidney of young animals increased with age. Mercury concentrations in adult animals increased with age continuously, whereas Cd concentrations in adult animals decreased. This trend might be due to preferential feeding habits and shift in ratio of Hg and Cd in the diet (*i.e.*, invertebrates to fish).

The Caspian Sea is the largest land-locked saltwater lake in the world. Among the various pollutants present in the Caspian Sea, Karpinsky (1992) reported elevated levels of heavy metals in Caspian Sea waters in which the concentrations of Cu (7.0 $\mu g\ L^{-1}$) increased by 11.5 times, Zn (22.5 $\mu g\ L^{-1}$) by 9.8 times, Pb (1.3 $\mu g\ L^{-1}$) by 5.6 times, and Cd (0.5 $\mu g\ L^{-1}$) by 4.9 times over the past 15 years. These heavy metals also accumulate in sediment and bottom-feeding organisms, showing that their concentrations have increased in the last two decades.

The Caspian seal (*Phoca caspica* Gmelin, 1788), which reaches a maximum length of 1.5 m, is one of the smallest seals and occupies the highest tropic level in the food web of the Caspian Sea ecosystem. Although contaminants such as organochlorines are known to be present in Caspian seals (Watanabe *et al.* 1999), studies of heavy metals had not been conducted prior to this work.

Caspian seals are a phocine relict inhabiting only the Caspian Sea and are isolated from other marine seals. This species is taxonomically closely related to ringed seals (*Phoca hispida*) and Baikal seals (*Phoca sibirica*). These three species compose the subgenus *Pusa* of the genus *Phoca*. Our previous studies have indicated specific accumulation of heavy metals in Baikal seals, a close relative to Caspian Seals (Watanabe *et al.* 1996; 1998). It is therefore expected that Caspian seals may have a unique accumulation of heavy metals. The objective of the present study was to elucidate accumulation of heavy metals in Caspian seals by comparing with those in Baikal seals from Lake Baikal and ringed seals from various seas and oceans.

Materials and Methods

Sixteen male and 30 female Caspian seals were caught in the northern part of Caspian Sea in November 1993 (Table 1). After biometric measurement, the specimens were immediately dissected. Samples were stored at −20°C until chemical analysis. Muscle, liver, and kidney were analyzed for all the animals collected. Age identification was carried out using dentinal and cemental growth layers in canine tooth following the method of Kasuya (1976). Fish (*Rutilus* sp.) were collected by net from the Caspian Sea and analyzed for heavy metal concentrations. The whole body of the fish was homogenized before the analysis.

About 1–10 g of soft tissue were digested into a transparent solution with a mixture of nitric, perchloric, and sulfuric acids. The resultant solutions were diluted to a known volume with deionized water and transferred to acid-washed sample tubes. The concentrations of Fe, Mn, and Zn were directly determined by flame atomic absorption spectrophotometry (F-AAS). Cu, Pb, Ni, Cd, and Co were measured by F-AAS after 4-methyl-2-pentanone and sodium *N*,*N*-diethyl-dithiocarbamate, trihydrate treatment (Honda *et al.* 1982). Mercury was determined by cold-vapor AAS (Akagi and Nishimura 1991) after digestion of sample (approx. 1 g fresh weight) with nitric, sulfuric, and perchloric acids in long-necked flasks. Accuracy of these analyses was examined by analyzing a standard reference material, NIES No. 1 (Okamoto *et al.* 1978). The standard deviation of triplicate analysis was less than 5% for each element. Concentrations are given on a wet-weight basis.

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Table 1. Biometric data of Caspian seals (mean ± SD and range)

	Age (years)	Body Length (cm)	Body Weight (kg)	Blubber Weight (kg)	Muscle Weight (kg)	Liver Weight (kg)	Kidney Weight (g)
All	11.5 ± 11.2 0.0–41.5 (40)	103 ± 17 36–126 (42)	35 ± 16 1.1–60 (42)	16 ± 9 0–34 (34)	7.4 ± 3.0 0.3–13.6 (29)	0.77 ± 0.33 0.02–1.83 (41)	113 ± 45 13–255 (42)
All except fetus	11.8 ± 11.2 0.5–41.5 (39)	105 ± 14 72–126 (41)	36 ± 16 10–60 (41)	17 ± 9 2–34 (37)	7.6 ± 2.7 1.6–13.6 (28)	0.78 ± 0.32 0.27–1.83 (40)	116 ± 42 62–255 (41)
Fetus	0.0 (1)	36 (1)	1.1 (1)	0.0 (1)	0.3 (1)	0.02 (1)	13 (1)
Immature (M+F)	3.1 ± 2.4 0.5–7.5 (21)	95 ± 13 72–126 (21)	25 ± 12 10–56 (21)	10 ± 5 2–22 (17)	5.8 ± 2.7 1.6–10.1 (12)	0.61 ± 0.22 0.27–1.13 (21)	90 ± 24 62–154 (21)
Adult male	18.0 ± 6.5 8.5–22.5 (4)	116 ± 5 111–122 (4)	51 ± 10 38–59 (4)	21 ± 9 9–28 (4)	10.7 ± 2.2 8.6–13.6 (4)	1.26 ± 0.47 0.75–1.83 (4)	173 ± 60 111–255 (4)
Adult female	23.1 ± 8.6 9.5–41.5 (14)	114 ± 5 105–123 (15)	46 ± 11 27–60 (15)	21 ± 8 6–34 (15)	8.3 ± 1.4 6.1–10.8 (11)	0.91 ± 0.21 0.58–1.20 (14)	138 ± 32 79–203 (15)
Pregnant	18.7 ± 8.2 9.5–28.5 (5)	115 ± 7 105–123 (6)	55 ± 5 45–60 (6)	29 ± 3 26–34 (6)	9.0 ± 1.2 7.5–10.8 (5)	0.93 ± 0.10 0.82–1.08 (5)	126 ± 16 103–140 (6)
Nonpregnant	25.6 ± 8.2 14.5–41.5 (9)	114 ± 4 107–120 (9)	40 ± 9 27–54 (9)	16 ± 6 6–26 (9)	7.8 ± 1.4 6.1–9.6 (6)	0.90 ± 0.25 0.58–1.20 (9)	146 ± 38 79–203 (9)

Number of samples is in parentheses. One male and one adult female were not identified for age.

Table 2. Heavy metal concentrations (mean ± SD and range $\mu g\ g^{-1}$ wet weight) in the muscle of Caspian seals

	Fe	Mn	Zn	Cu	Pb	Ni	Cd	Co	Hg
All 0–41.5years	200 ± 64 52–330 (42)	0.18 ± 0.06 0.08–0.37 (42)	30 ± 9 16–51 (42)	1.1 ± 0.2 0.7–1.7 (42)	0.027 ± 0.028 < 0.01–0.14 (22)	< 0.04 (0)	0.011 ± 0.017 < 0.001–0.099 (36)	0.010 ± 0.003 < 0.005–0.016 (10)	0.55 ± 0.30 0.19–1.8 (42)
All except fetus 0.5–41.5 years	200 ± 61 99–330 (41)	0.18 ± 0.06 0.08–0.37 (41)	30 ± 9 16–51 (41)	1.1 ± 0.2 0.7–1.6 (41)	0.027 ± 0.028 < 0.01–0.14 (22)	< 0.04 (0)	0.011 ± 0.017 < 0.001–0.099 (36)	0.010 ± 0.003 < 0.005–0.016 (10)	0.55 ± 0.31 0.19–1.8 (41)
Fetus	52 (1)	0.15 (1)	23 (1)	1.7 (1)	< 0.01 (0)	< 0.04 (0)	< 0.001 (0)	< 0.005 (0)	0.53 (1)
Immature (M+F) 0.5–7.5 years	200 ± 64 130–330 (21)	0.20 ± 0.06 0.08–0.37 (21)	28 ± 8 18–49 (21)	1.2 ± 0.2 0.8–1.6 (21)	0.024 ± 0.013 < 0.01–0.056 (15)	< 0.04 (0)	0.004 ± 0.003 < 0.001–0.011 (16)	0.010 ± 0.004 < 0.005–0.016 (5)	0.48 ± 0.14 0.27–0.71 (21)
Adult male 8.5–22.5 years	200 ± 39 160–250 (4)	0.19 ± 0.06 0.13–0.25 (4)	26 ± 6 20–33 (4)	1.1 ± 0.3 0.8–1.3 (4)	0.011 < 0.01–0.015 (2)	< 0.04 (0)	0.008 ± 0.005 0.003–0.015 (4)	0.012 < 0.005–0.016 (2)	0.65 ± 0.34 0.27–0.96 (4)
Adult female 9.5–41.5 years	210 ± 61 99–330 (15)	0.15 ± 0.05 0.09–0.25 (15)	33 ± 11 16–51 (15)	1.0 ± 0.1 0.7–1.2 (15)	0.042 ± 0.057 < 0.01–0.14 (5)	< 0.04 (0)	0.019 ± 0.024 0.005–0.099 (15)	0.008 ± 0.000 < 0.005–0.009 (3)	0.58 ± 0.44 0.19–1.8 (15)
Pregnant 9.5–28.5 years	200 ± 50 150–280 (6)	0.16 ± 0.07 0.09–0.25 (6)	36 ± 12 27–51 (6)	1.0 ± 0.1 0.9–1.2 (6)	0.020 ± 0.013 < 0.01–0.033 (3)	< 0.04 (0)	0.011 ± 0.005 0.005–0.019 (6)	0.009 < 0.005–0.009 (1)	0.47 ± 0.19 0.23–0.75 (6)
Nonpregnant 14.5–41.5 years	220 ± 70 99–330 (9)	0.14 ± 0.04 0.09–0.22 (9)	31 ± 10 16–45 (9)	1.0 ± 0.1 0.7–1.1 (9)	0.074 < 0.01–0.14 (2)	< 0.04 (0)	0.025 ± 0.030 0.005–0.099 (9)	0.008 < 0.005–0.008 (2)	0.66 ± 0.55 0.19–1.8 (9)

Number of samples with detectable concentration is in parentheses.

Results and Discussion

Concentrations in Muscle, Liver, and Kidney

Concentrations of heavy metal in muscle, liver, and kidney of Caspian seals are shown in Tables 2, 3, and 4, respectively. Fe, Mn, Zn, Cu, and Hg were detected in all the samples analyzed. Cd was detected in all the liver and kidney tissues. Pb and Co were not detected in some samples (< 0.02 and < 0.01 $\mu g\ g^{-1}$ wet weight, respectively). Ni was undetectable in all the muscle (<

Table 3. Heavy metal concentrations (mean ± SD and range $\mu g\ g^{-1}$ wet weight) in the liver of Caspian seals

	Fe	Mn	Zn	Cu	Pb	Ni	Cd	Co	Hg
All	470 ± 580	5.5 ± 1.3	49 ± 15	11 ± 5	0.068 ± 0.046	< 0.07	1.1 ± 1.7	0.042 ± 0.016	15 ± 26
0–41.5 years	77–2800	1.1–7.6	31–130	3–23	< 0.01–0.20	-	0.1–11	< 0.01–0.074	0.3–150
	(42)	(42)	(42)	(42)	(27)	(0)	(42)	(28)	(42)
All except fetus	480 ± 590	5.6 ± 1.1	47 ± 8	10 ± 5	0.067 ± 0.046	< 0.07	1.2 ± 1.7	0.042 ± 0.016	16 ± 27
0.5–41.5 years	77–2762	3.2–7.6	31–67	3–23	< 0.01–0.20		0.1–11	< 0.01–0.074	0.5–150
	(41)	(41)	(41)	(41)	(26)	(0)	(41)	(28)	(41)
Fetus	280	1.1	129	18	0.090	< 0.07	0.1	< 0.01	0.3
	(1)	(1)	(1)	(1)	(1)	(0)	(1)	(0)	(1)
Immature (M+F)	350 ± 440	5.9 ± 1.0	46 ± 7	10 ± 5	0.063 ± 0.041	< 0.07	0.7 ± 0.5	0.045 ± 0.017	6 ± 6
0.5–7.5 years	77–1900	4.0–7.6	31–57	3–23	< 0.01–0.16		0.2–2.0	< 0.01–0.073	0.5–22
	(21)	(21)	(21)	(21)	(14)	(0)	(21)	(13)	(21)
Male adult	290 ± 130	5.3 ± 1.4	51 ± 5	9 ± 5	0.089	< 0.07	1.0 ± 1.0	0.032 ± 0.005	9 ± 6
8.5–22.5 years	130–420	3.6–6.9	46–56	3–16	< 0.01–0.14		0.1–2.2	0.028–0.038	5–18
	(4)	(4)	(4)	(4)	(2)	(0)	(4)	(4)	(4)
Female adult	710 ± 780	5.5 ± 1.2	47 ± 9	11 ± 4	0.069 ± 0.052	< 0.07	1.9 ± 2.7	0.043 ± 0.017	30 ± 39
9.5–41.5 years	100–2800	3.2–7.6	33–67	5–17	< 0.01–0.20		0.2–11	< 0.01–0.074	3–150
	(15)	(15)	(15)	(15)	(10)	(0)	(15)	(11)	(15)
Pregnant	670 ± 810	5.1 ± 1.4	41 ± 6	10 ± 4	0.046 ± 0.024	< 0.07	1.1 ± 0.5	0.040 ± 0.013	16 ± 13
9.5–28.5 years	100–2,233	3.2–6.8	33–48	5–17	0.020–0.085		0.6–1.9	< 0.01–0.056	3–39
	(6)	(6)	(6)	(6)	(6)	(0)	(6)	(5)	(6)
Nonpregnant	730 ± 820	5.7 ± 1.1	51 ± 9	11 ± 4	0.10 ± 0.07	< 0.07	2.4 ± 3.4	0.045 ± 0.021	40 ± 48
14.5–41.5 years	120–2,800	4.5–7.6	40–67	6–17	< 0.01–0.20		0.2–11	< 0.01–0.074	7–150
	(9)	(9)	(9)	(9)	(4)	(0)	(9)	(6)	(9)

Number of samples with detectable concetration in parentheses.

Table 4. Heavy metal concentrations (mean ± SD and range on μg g-1, wet weight) in the kidney of Caspian seals

	Fe	Mn	Zn	Cu	Pb	Ni	Cd	Co	Hg
All	150 ± 42	1.0 ± 0.2	27 ± 7	3.3 ± 0.6	0.078 ± 0.097	0.070 ± 0.039	9.5 ± 11	0.038 ± 0.020	1.6 ± 1.3
0–41.5 years	90–250	0.4–1.4	19–48	2.4–5.1	< 0.02–0.58	< 0.06–0.16	0.01–55	< 0.01–0.097	0.3–8.4
	(42)	(42)	(42)	(42)	(31)	(10)	(42)	(36)	(42)
All except fetus	150 ± 42	1.0 ± 0.2	27 ± 6	3.3 ± 0.6	0.079 ± 0.099	0.070 ± 0.039	9.7 ± 11	0.038 ± 0.020	1.7 ± 1.3
0.5–41.5 years	90–250	0.7–1.4	19–48	2.4–5.1	< 0.02–0.58	< 0.06–0.16	0.7–55	< 0.01–0.097	0.5–8.4
	(41)	(41)	(41)	(41)	(30)	(10)	(41)	(36)	(41)
Fetus	96	0.4	37	2.8	0.040	< 0.06	0.01	< 0.01	0.3
	(1)	(1)	(1)	(1)	(1)	(0)	(1)	(0)	(1)
Immature (M+F)	140 ± 38	1.1 ± 0.2	24 ± 3	3.4 ± 0.7	0.056 ± 0.023	0.068 ± 0.024	4.0 ± 3.1	0.027 ± 0.013	1.3 ± 0.3
0.5–7.5 years	90–220	0.7–1.4	19–30	2.6–5.1	< 0.02–0.092	< 0.06–0.10	0.7–11	< 0.01–0.061	0.5–1.9
	(21)	(21)	(21)	(21)	(16)	(5)	(21)	(17)	(21)
Adult male	150 ± 46	1.1 ± 0.2	34 ± 6	3.0 ± 0.4	0.12	< 0.06	12 ± 7	0.058 ± 0.033	1.8 ± 0.8
8.5–22.5 years	110–220	0.9–1.2	30–43	2.6–3.4	< 0.02–0.13	(0)	6–22	0.017–0.097	0.9–2.7
	(4)	(4)	(4)	(4)	(2)		(4)	(4)	(4)
Adult female	170 ± 43	1.0 ± 0.1	29 ± 7	3.1 ± 0.6	0.11 ± 0.16	0.072 ± 0.053	16 ± 14	0.046 ± 0.016	2.1 ± 2.0
9.5–41.5 years	100–250	0.7–1.1	21–48	2.4–4.2	< 0.02–0.58	< 0.06–0.16	3–55	< 0.01–0.071	0.5–8.4
	(15)	(15)	(15)	(15)	(11)	(5)	(15)	(14)	(15)
Pregnant	190 ± 47	1.0 ± 0.2	27 ± 3	2.9 ± 0.3	0.050 ± 0.018	0.047 ± 0.019	14 ± 9	0.053 ± 0.012	1.4 ± 0.6
9.5–28.5 years	130–250	0.7–1.1	23–32	2.4–3.3	< 0.02–0.081	< 0.06–0.069	6–30	0.037–0.071	0.7–2.3
	(6)	(6)	(6)	(6)	(5)	(3)	(6)	(6)	(6)
Nonpregnant	150 ± 36	1.0 ± 0.1	31 ± 9	3.2 ± 0.7	0.15 ± 0.21	0.11	18 ± 18	0.041 ± 0.018	2.5 ± 2.4
14.5–41.5 years	100–210	0.9–1.1	21–48	2.4–4.2	< 0.02–0.58	< 0.06–0.16	3–55	< 0.01–0.061	0.5–8.4
	(9)	(9)	(9)	(9)	(6)	(2)	(9)	(8)	(9)

Number of samples with detectable concentration in parentheses.

0.04 $\mu g\ g^{-1}$ wet weight) and liver (< 0.07 $\mu g\ g^{-1}$ wet weight) samples but was detected in a few kidney samples. Frequency of detectable concentrations of Pb, Ni, and Co in females was higher than those in males.

The highest concentrations of Fe, Mn, Zn, Cu, and Hg were found in the liver, and that of Cd was in the kidney. Higher detection rates of Pb, Ni, and Co were found in the kidney (Table 4). Baikal seal, taxonomically a close relative of Cas-

Table 5. Correlation between heavy metal concentrations in the muscle, liver, and kidney in Caspian seals

Muscle	Mn-Zn (–0.37)*	Mn-Hg (–0.32)*	Zn-Hg (0.46)*				
Liver	Zn-Cu (0.45)*	Fe-Hg (0.47)*					
Kidney	Fe-Cu (–0.52)**	Mn-Cu (0.32)*	Zn-Cd (0.52)**	Fe-Cd (0.36)*	Zn-Pb (0.37)*	Cd-Co (0.38)*	Pb-Hg (0.40)*

* $p < 0.05$.
** $p < 0.001$.
r^2 Value by Spearman's rank correlation test is in parentheses.

pian seal, exhibited unique accumulation of Cu where the concentration was higher in the kidney than in other tissues except hair (Watanabe *et al.* 1996). However, this tendency was not observed in Caspian seal.

A significant gender difference in metal concentrations was observed for hepatic Cu ($p < 0.01$), Co ($p < 0.05$), and Hg ($p < 0.05$) using Mann-Whitney U test. For all these metals, concentrations in females were higher than those in males (Table 3).

Significant positive correlations were found for Cd ($p < 0.001$) and Hg ($p < 0.001$) concentrations between liver and muscle; Fe ($p < 0.05$), Mn ($p < 0.01$), Cu ($p < 0.01$), Cd ($p < 0.001$), and Hg ($p < 0.001$) concentrations between kidney and muscle; and Fe ($p < 0.01$), Mn ($p < 0.001$), Zn ($p < 0.01$), Cd ($p < 0.001$), and Hg ($p < 0.001$) concentrations between liver and kidney. A significant positive correlation among liver, kidney, and muscle was found for Cd and Hg. This suggests that these metals have accumulated concurrently in the organs and tissues of Caspian seals.

Intermetal correlations in the muscle, liver, and kidney of Caspian seals are shown in Table 5. In Baikal seals, relation among Fe, Mn, and Cu concentrations were significant. However, these tendencies were not observed in Caspian seals. This suggests a difference in the accumulation of essential metals between Caspian seal and Baikal seal, although both species have a similar taxonomical niche. Positive correlation between Cd and Zn concentrations in the kidney has been well documented in marine mammals (Honda and Tatsukawa 1983; Honda *et al.* 1987b; Noda *et al.* 1995). This tendency is due to the presence of the Cd-binding protein metallothionein. In this study, a significant positive correlation was also found in the kidney of Caspian seals, which was not observed in Baikal seals (Watanabe *et al.* 1996). This difference might be due to low exposure to Cd in ecosystems of the Lake Baikal and suggests that Cd levels in the Caspian Sea are higher than those in Lake Baikal.

Comparison with Marine Pinnipeds

Essential Metals: Relatively low Fe and high Mn concentrations were found in the liver of Caspian seals as compared with marine pinnipeds (Figure 1). In Baikal seals, on the contrary high Fe and low Mn accumulation was noted (Watanabe *et al.* 1996). Concentrations of Fe were negatively correlated with those of Mn in various tissues of birds (Honda *et al.* 1990), cetaceans (Honda *et al.* 1983), and pinnipeds (Watanabe *et al.* 1996). Interestingly, negative correlation was found not only for the same species but also for some phocine species. In addition, almost all Caspian seals exhibiting a wide range of Fe concentrations in the liver were adult females. This implies that adult females need more hepatic Fe than others and thus showed unique pattern of Fe accumulation in the liver. Our previous study (Watanabe *et al.* 1996) suggested that Fe levels in seal body varied according to the diving ability. Baikal seals (inhabiting deep lake) acquired high diving ability and thus contained high Fe concentration, and Fe concentrations in Caspian seal (inhabiting shallower water) were lower. This tendency implies that the diving ability of Caspian seals is less, and the habitat and niches arising from evolution of animals might affect the accumulation of essential metals in their bodies.

Zn concentrations in the muscle, liver, and kidney of Caspian seals were comparable to those in other species of marine mammals (Figure 2), and similar levels of Zn were observed among all pinniped species. On the other hand, relatively low concentrations of Cu were found in the liver of Caspian seals when compared with other pinnipeds (Figure 3). In general, hepatic Cu concentrations in pinniped are higher than those in cetaceans (Watanabe and Tanabe 2000). Observed levels in Caspian seal were comparable to those in cetacean species. Low levels of hepatic Cu were also reported in Baikal seals (Watanabe *et al.* 1996) and ringed seals (Harms *et al.* 1978; Perttila 1986; Frank *et al.* 1992; Zeisler *et al.* 1993) belonging to the same subgenus, *Pusa*. An interesting relation was found between the body size and hepatic Cu concentrations in pinnipeds (Figure 3). The subgenus *Pusa*, including Caspian, Baikal, and ringed seals, which have shorter body lengths, showed lower Cu concentrations in comparison with other seals with greater body lengths. This difference found in Cu levels between subgenus *Pusa* and other pinnipeds was significant ($p < 0.05$ by Mann-Whitney U test). It is well known that body size of animals relates to various physiological factors, *e.g.*, energy metabolism, metabolic rates, and oxygen consumption (Schmidt-Nielsen 1984). Although no plausible explanation is available, Cu levels in pinnipeds appear to be affected by certain factors related to body size.

Toxic Metals: Relatively low concentrations of toxic metals such as Hg and Cd were found in Caspian seals. The highest concentrations of both metals were found in the liver (150 $\mu g\ g^{-1}$ on wet-weight basis for Hg) and kidney (55 $\mu g\ g^{-1}$ on wet-weight basis for Cd). It is generally known that Hg and Cd levels in marine mammals are affected by the diet (Sergeant and Armstrong 1973; Honda *et al.* 1987a, 1987b; Bowles 1994; Watanabe *et al.* 1996). Mercury and Cd concentrations in fish (*Rutilus* sp.) collected from Caspian Sea were low (Table 6). Thus, low Hg and Cd concentrations in Caspian seals can be explained by low levels in diet fish.

Among pinnipeds, animal can be classified into two groups on the basis of Hg and Cd concentrations (Figure 4). One group

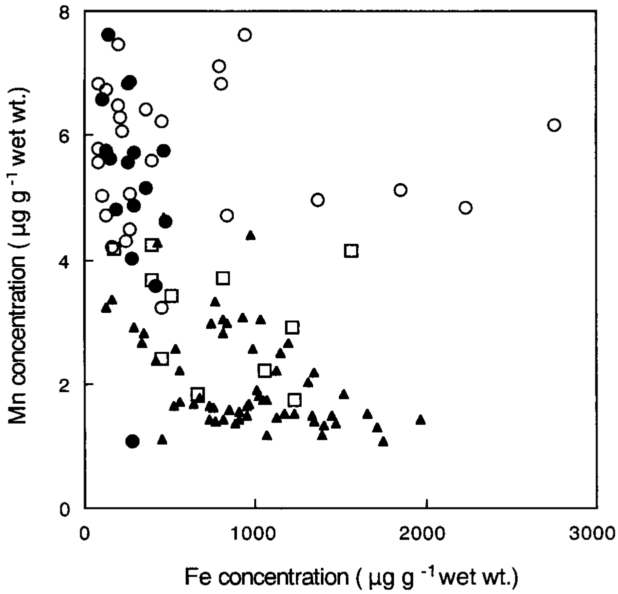

Fig. 1. Relationship between mean concentrations of Fe and Mn in the liver of various pinnipeds. Closed circle: male Caspian seal; open circle: female Caspian seal; closed triangle: Baikal seal; open square: mean value of other pinnipeds. Cited from Denton *et al.* 1980; Yamamoto *et al.* 1987; Frank *et al.* 1992; Noda *et al.* 1993, 1995; Zeisler *et al.* 1993; Watanabe *et al.* 1996

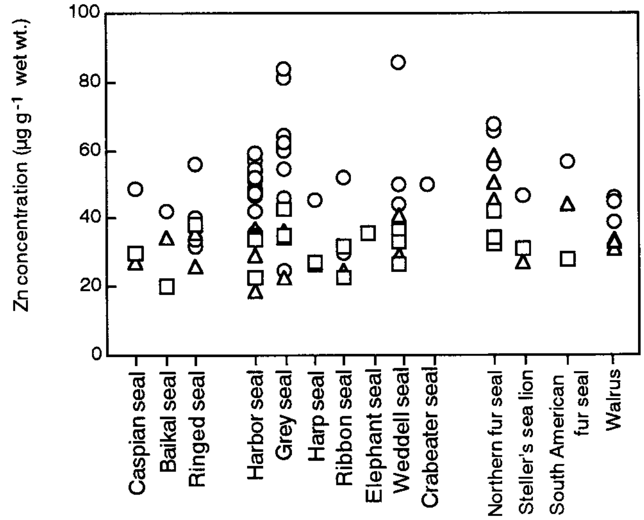

Fig. 2. Mean Zn concentrations in the muscle, liver, and kidney of various pinnipeds. Squares, circles, and triangles indicate muscle, liver, and kidney, respectively. Data were cited from Holden 1975; Drescher *et al.* 1977; Harms *et al.* 1978; Hamanaka 1983; Goldblatt and Anthony 1983; Wagemann and Muir 1984; Perttila 1986; Tohyama *et al.* 1986; Wagemann *et al.* 1988; Yamamoto *et al.* 1987; Wagemann 1989; Morris *et al.* 1989; Taylor *et al.* 1989; Skaare *et al.* 1990; Law *et al.* 1991, 1992; Frank *et al.* 1992; Zeisler *et al.* 1993; Noda *et al.* 1993, 1995; Marcovecchio *et al.* 1994

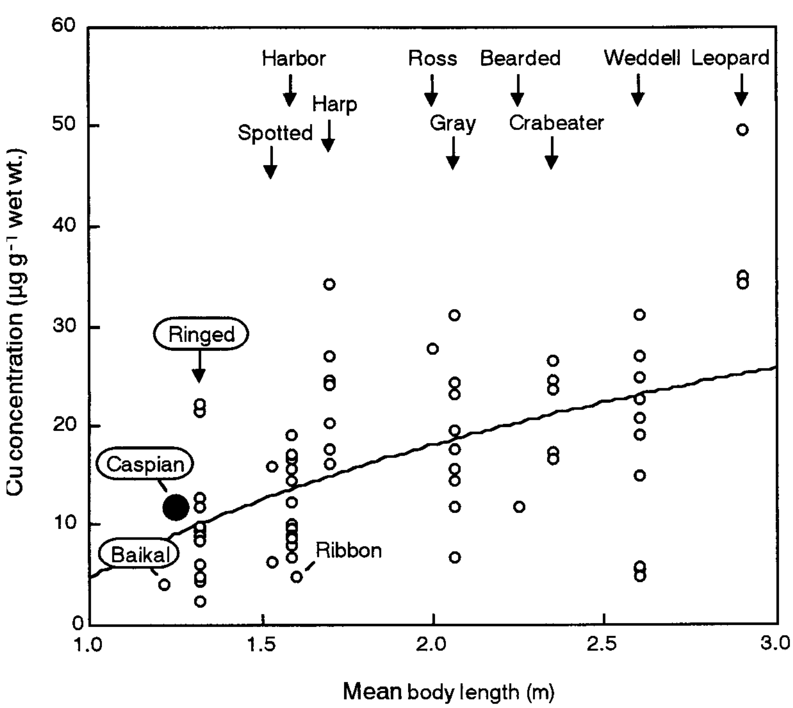

Fig. 3. Relationship between mean body weights and mean hepatic Cu concentrations of phocids. Data were cited from Holden 1975; Drescher *et al.* 1977; Harms *et al.* 1978; Burrell 1981; McClurg 1984; Wagemann and Muir 1984; Ronald *et al.* 1984; Mochizuki *et al.* 1985; Schneider *et al.* 1985; Perttila 1986; Steinhagen-Schneider 1986; Tohyama *et al.* 1986; Wagemann *et al.* 1988; Yamamoto *et al.* 1987; Wagemann 1989; Morris *et al.* 1989; Skaare *et al.* 1990; Law *et al.* 1991, 1992; Frank *et al.* 1992; Zeisler *et al.* 1993; Szefer *et al.* 1994; Noda *et al.* 1993, 1995; Marcovecchio *et al.* 1994

that accumulated higher concentrations of Hg than Cd, which includes gray seals, harbor seals, Caspian seals, and sea lions (California sea lion and south American sea lion). In contrast, another group accumulated higher concentrations of Cd than Hg; this group includes Antarctic seals (Wedded and leopard seal), harp seals, ringed seals, fur seals (northern fur seal and South American fur seal), and walrus. It was noted earlier that marine mammals feeding on invertebrates such as crustaceans, krill, clam, bivalves, and squids accumulated high Cd levels. For example, in the Antarctic ecosystem, which has simple

Table 6. Comparison of heavy metal concentrations (mean ± SD and range μg g^{-1} wet weight) in the whole body of fish and invertebrates from various waters

Species	n	Fe	Mn	Zn	Cu	Pb	Ni	Cd	Co	Hg	References
Caspian Sea											
Rutilus rutilus	4	21 ± 7	2.7 ± 0.4	30 ± 4	0.92 ± 0.06	0.03 ± 0.02	0.051 ± 0.014	0.016 ± 0.006	< 0.012	0.043 ± 0.041	Present study
		14–31	2.2–3.0	25–35	0.84–0.98	0.01–0.05	0.031–0.063	0.007–0.021		0.021–0.10	
Lake Baikal											
Comephorus baicalensis	3	14 ± 4	0.8 ± 0.1	12 ± 5	0.36 ± 0.07	0.56 ± 0.45	0.024 ± 0.008	0.012 ± 0.002	0.010 ± 0.003	0.034	Watanabe *et al.* 1996
		12–19	0.7–0.9	8–18	0.32–0.43	0.20–1.1	0.015–0.029	0.011–0.015	< 0.012–0.013		
C. dybowskii	3	55 ± 70	1.3 ± 0.4	9 ± 1	0.50 ± 0.13	3.3 ± 4.6	0.093 ± 0.064	0.033 ± 0.003	0.047	0.010	Watanabe *et al.* 1996
		11–140	1.1–1.8	8–10	0.38–0.65	0.4–8.5	0.042–0.16	0.031–0.037	< 0.012–0.047		
Cottocomephorus grewinki	3	22 ± 6	2.5 ± 1.1	25 ± 7	0.67 ± 0.10	0.39 ± 0.19	0.046 ± 0.014	0.020 ± 0.004	0.033 ± 0.002	0.008	Watanabe *et al.* 1996
		18–29	1.6–3.8	19–33	0.60–0.78	0.20–0.59	0.036–0.062	0.015–0.023	0.031–0.035		
C. inermis	2	22	2.0	14	0.55	0.10	0.018	0.011	0.026	0.018	Watanabe *et al.* 1996
		15–30	1.6–2.5	14–14	0.51–0.59	0.08–0.11	0.008–0.028	0.007–0.015	0.024–0.028		
Arctic water											
Arctic cod	50							0.40 ± 0.19			Muir *et al.* 1992
Boreogadus saida	20					0.62 ± 0.22					Muir *et al.* 1992
NW Pacific											
Myctophid, large	4			9				0.02			Honda and Tatsukawa 1983
Myctophid, small	10			13				0.03			Honda and Tatsukawa 1983
Antarctic water											
T. bernacchii	30	15	1.2	15	0.43	0.06	0.11	0.15		0.11	Honda *et al.* 1987
		7–47	0.7–1.9	11–21	0.27–0.70	0.04–0.19	0.03–0.26	0.06–0.34		0.04–0.31	
P. borchgrevinki	22	7	0.6	11	0.65	0.15	0.08	0.06		0.03	Honda *et al.* 1987
		4–9	0.3–1.0	9–14	0.38–1.1	0.02–0.31	0.05–0.12	0.01–0.12		0.01–0.05	
Notothenioidei	7	6	0.4	10	1.1	0.03	0.10	0.12		0.17	Honda *et al.* 1987
		4–7	0.3–0.5	9–11	0.4–1.9	0.01–0.07	0.04–0.17	0.05–0.23		0.12–0.26	
Myctophid	2	12	0.7	10	2.3	0.09	0.12	0.23		0.45	Honda *et al.* 1987
		11–14	0.7–0.7	9–10	1.9–2.7	0.05–0.13	0.11–0.12	0.22–0.23		0.44–0.45	

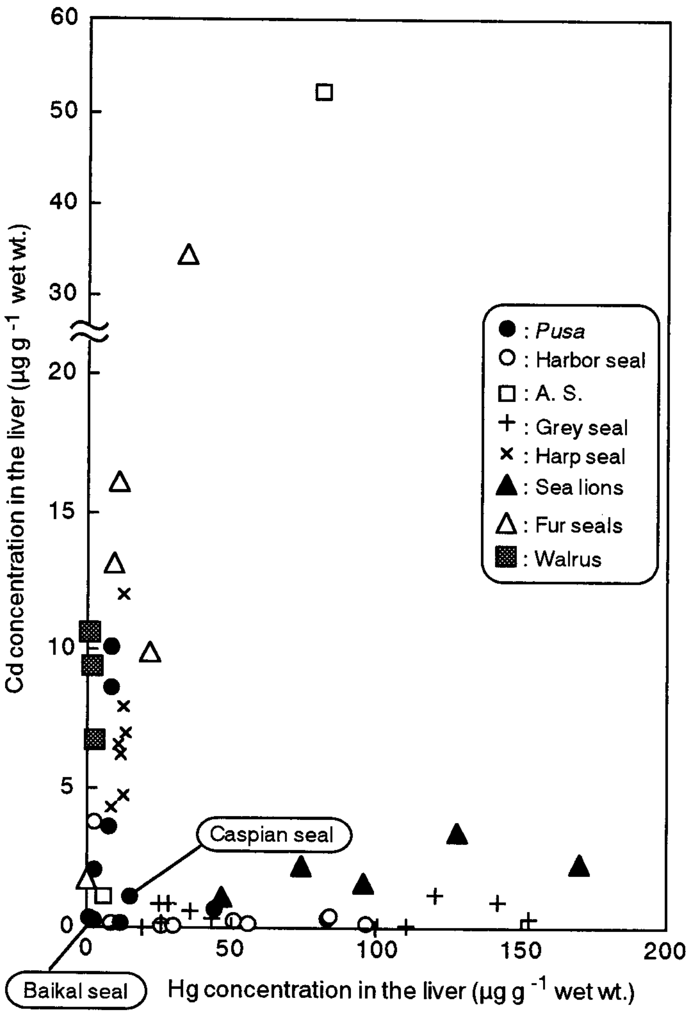

Fig. 4. Relationship between mean Hg and Cd concentrations in the liver of various pinnipeds. Data were cited from Anas 1974; Buhler *et al.* 1975; Holden 1975; Smith and Armstrong 1975, 1978; Drescher *et al.* 1977; Caines 1978; Mekie *et al.* 1980; Goldblatt and Anthony 1983; Ronald *et al.* 1984; Perttila 1986; Tohyama *et al.* 1986; Harms *et al.* 1987; Yamamoto *et al.* 1987; Wagemann *et al.* 1988; Taylor *et al.* 1989; Wagemann 1989; Dietz *et al.* 1990; Skaare *et al.* 1990; Law *et al.* 1991, 1992; Frank *et al.* 1992; Muir *et al.* 1992; Zeisler *et al.* 1993; Kemper *et al.* 1994; Marcovecchio *et al.* 1994; Noda *et al.* 1995. A. S. Antarctic seals (Wedded seal and leopard seal).

ecological food webs in comparison with other ecosystems, krill is a staple species and accumulates high Cd and low Hg concentrations. Therefore, top predators in the Antarctic ecosystem—such as whales, seals, and birds—accumulate apparently higher levels of Cd than Hg (Honda *et al.* 1987a; Bowles 1994). Noda *et al.* (1995) explained high levels of Cd in northern fur seals by the presence of high Cd levels in its diet, which is known to specifically accumulate Cd (Hamanaka *et al.* 1982). In addition, walrus accumulating higher concentrations of Cd than Hg feeds on mollusks, such as bivalves and gastropods. It implies that species that accumulate more Hg than Cd feed preferentially on fish rather than invertebrates. This supports the theory that Caspian seals feed primarily on fish (Popov 1982).

Age-Related Accumulation

The age of sexual maturity for Caspian seals is estimated to be 4–5 years for female and 6–7 years for males. However, most females have their first pup after 7 years (Popov 1982). As two of the females aged 7.5 years were not pregnant in the present samples, we assumed that the maturity was after 8 years for both sexes.

Positive correlations with age were found for muscular Fe ($p < 0.01$) and Cd ($p < 0.001$), hepatic Fe ($p < 0.05$) and Hg ($p < 0.001$), and renal Fe ($p < 0.01$), Zn ($p < 0.01$), Cd ($p < 0.001$), Co ($p < 0.01$), and Hg ($p < 0.05$) in Caspian seals. On the contrary, muscular and renal Cu concentrations decreased with age. Elevated concentrations of Fe were found in some marine mammals such as dugongs (Denton *et al.* 1980), striped dolphins (Honda *et al.* 1983), minke whales (Honda *et al.* 1987b), and Baikal seals (Watanabe *et al.* 1998). It is well known that the diving ability of marine mammals develops with growth, and muscular Fe of marine pinnipeds is related to the diving ability (Blessing 1972; Watanabe *et al.* 1998). Hence, an age-dependent increase in Fe concentrations in Caspian seals might be attributable to its diving ability.

Hg and Cd concentrations in body tissues, especially the accumulative tissues (such as liver and kidney), increased with age in various animals (Honda 1985; Eisler 1984; Bowles 1994). In Caspian seals, Hg concentrations in the liver, kidney and muscle increased with age (Figure 5). Similarly, Cd concentrations in younger animals increased with age until about 15 years, even after excluding the highest concentration ob-

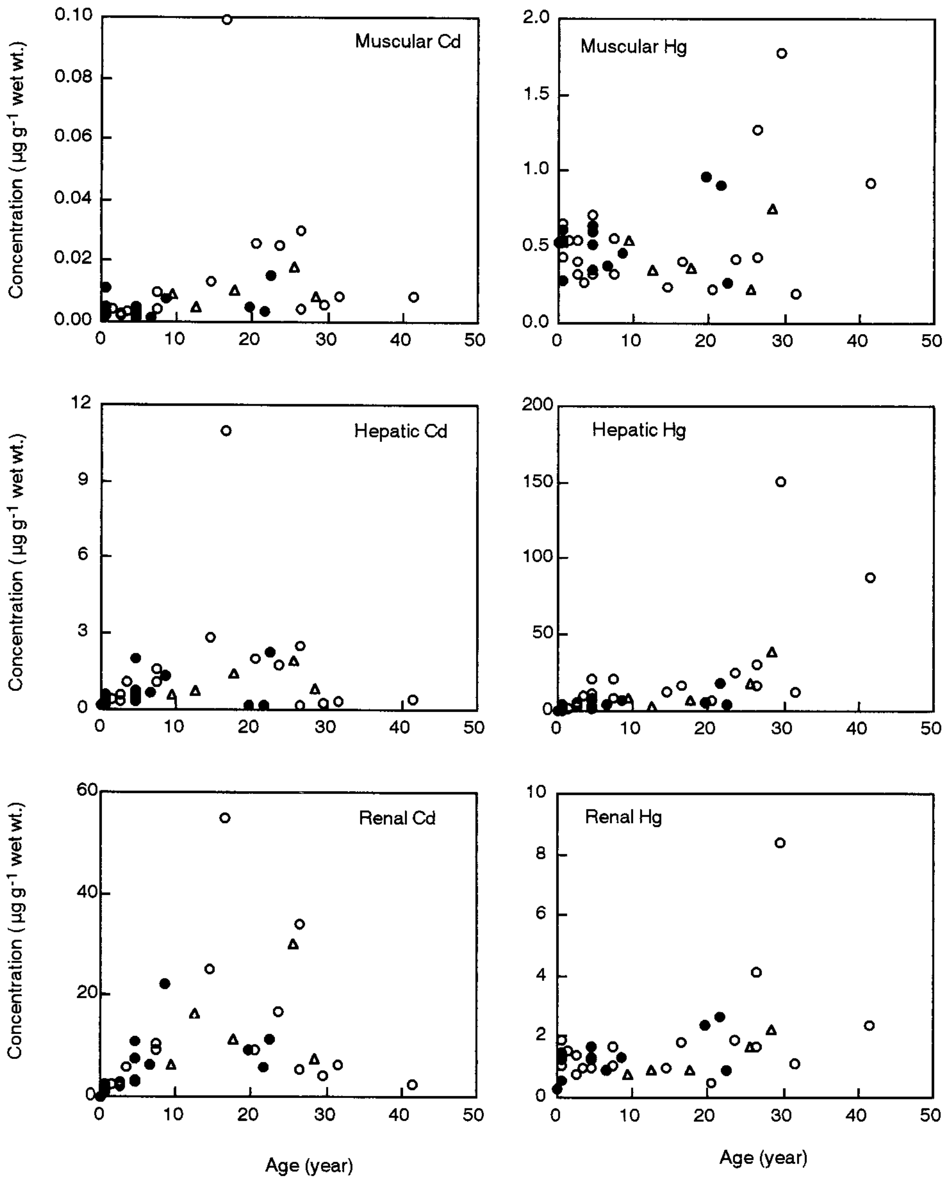

Fig. 5. Age-related variations of Cd and Hg concentration in the tissues of Caspian seals. Closed circle: male; open circle: nonpregnant female; open triangle: pregnant female

served in a female aged 16.5 years. However, Cd concentrations in older animals were lower. Such a decreasing pattern of Cd concentrations in older animals was also reported in minke whales (Honda *et al.* 1987b) and Baikal seals (Watanabe *et al.* 1998). Interestingly, in these animals, Hg concentrations concurrently decreased with age. Honda *et al.* (1987b) and our previous study (Watanabe *et al.* 1998) explained these decreased concentrations at older age by changes in the amount of diet intake. However, these explanations may not apply to the results of Caspian seals, because only Cd concentrations decreased in older animals (Figure 5). Although a possible reason for this is still unclear, one causative factor is exposure rate of Hg and Cd via diet intake, *i.e.*, prey of Caspian seal contains relatively higher levels of Hg than Cd. Earlier, we assumed that Caspian seal belongs to Hg accumulating group of pinnipeds, which is supported by their feeding preferences. In addition, the preferential diet of Caspian seals may change with growth, *e.g.*, invertebrates to fish, and thus the exposure rate to Hg and Cd may vary with age. From the discussion, Hg and Cd accumulation rate might be a useful indicator to estimate the feeding habit of marine mammals.

Specific Accumulation in Reproductive Processes

Six pregnant female seals and a fetus were analyzed to elucidate specific accumulation of heavy metals during reproductive processes. Concentrations of muscular Mn and Zn and renal Fe and Co were higher in pregnant females than those in nonpregnant females, whereas those of other metals in the muscle, liver, and kidney of pregnant females were lower than those in nonpregnant females (Table 2, 3, and 4). In a discussion of mineral transfer from mother to fetus, metal burden should be considered. In this study, heavy metal burden was calculated by tissue weight and concentrations. Interestingly, pregnant fe-

Table 7. Heavy metal burdens (calculated by concentrations in various tissues and their weight mesured) in the liver, kidney, and muscle of adult female Caspian seals

	Fe (g)	Mn (mg)	Zn (g)	Cu (mg)	Pb (μg)	Ni (μg)	Cd (mg)	Co (μg)	Hg (mg)
Liver									
Nonpregnant female	0.80 ± 1.0	5.1 ± 2.3	0.045 ± 0.015	11 ± 5	31 ± 39	NC	1.6 ± 1.8	45 ± 27	35 ± 46
	0.07–3.3	2.8–9.2	0.030–0.080	4–20	NC-120		0.2–6.4	NC-89	5–150
	(11)	(11)	(11)	(11)	(9)	(0)	(11)	(7)	(11)
Pregnant female	0.75 ± 0.9	4.6 ± 1.7	0.037 ± 0.009	10 ± 4	19 ± 16	NC	0.9 ± 0.2	35 ± 13	15 ± 16
	0.09–2.2	2.8–7.3	0.028–0.052	5–15	NC-40		0.6–1.2	NC-49	2–42
	(5)	(5)	(5)	(5)	(4)	(0)	(5)	(4)	(5)
Kidney									
Nonpregnant female	0.022 ± 0.008	0.13 ± 0.04	0.0040 ± 0.0008	0.43 ± 0.11	14 ± 14	13	1.9 ± 1.5	5.5 ± 2.3	0.34 ± 0.38
	0.009–0.036	0.07–0.19	0.0029–0.0055	0.31–0.66	NC-46		0.5–4.6	NC-8.8	0.08–1.3
	(11)	(11)	(11)	(11)	(8)	NC-17 (2)	(11)	(10)	(11)
Pregnant female	0.024 ± 0.008	0.12 ± 0.03	0.0034 ± 0.0005	0.37 ± 0.07	6.2 ± 2.1	5.8 ± 2.1	1.7 ± 1.0	6.5 ± 1.1	0.18 ± 0.10
	0.015–0.035	0.08–0.16	0.0027–0.0041	0.25–0.47	NC-10		0.9–3.5	5.2–8.3	0.09–0.31
	(6)	(6)	(6)	(6)	(5)	NC-8.1 (3)	(6)	(6)	(6)
Muscle									
Nonpregnant female	1.7 ± 0.5	1.3 ± 0.3	0.23 ± 0.08	7.4 ± 1.5	480 ± 590	NC	0.10 ± 0.06	55 ± 5	5.0 ± 5.0
	1.0–2.3	0.8–1.7	0.15–0.40	5.9–10	NC-1,400		0.03–0.18	NC-59	1.4–17
	(8)	(8)	(8)	(8)	(4)	(0)	(8)	(3)	(8)
Pregnant female	1.9 ± 0.4	1.6 ± 0.7	0.33 ± 0.09	9.4 ± 1.6	180 ± 100	NC	0.09 ± 0.03	67	4.0 ± 1.8
	1.6–2.5	0.8–2.7	0.24–0.47	7.1–11	NC-250		0.05–0.14	NC-67	1.7–6.5
	(5)	(5)	(5)	(5)	(3)	(0)	(5)	(1)	(5)

Number of samples in parentheses. NC: not calculated.

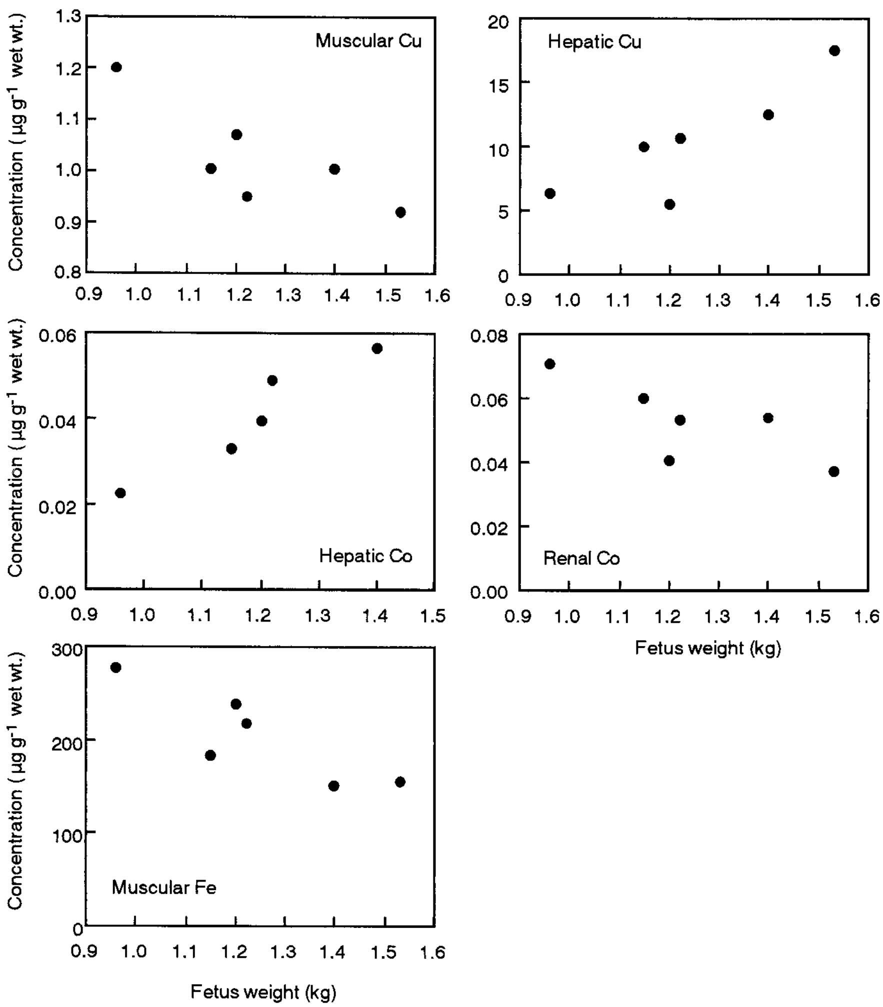

Fig. 6. Variations of essential element concentrations in the tissues of mother Caspian seals with increase of fetus body weight

males had comparatively smaller weight of muscle, liver, and kidney than nonpregnant females (Table 1). However, larger burdens of muscular Fe, Mn, Zn, and Cu and renal Fe and Co were observed in the body of pregnant females compared with nonpregnant females (Table 7). Moreover, muscular Fe and Cu and renal Co concentrations in mother decreased, but hepatic Cu and Co concentrations in mother significantly increased with growth of fetus (Figure 6). These trends suggested that essential metals, such as Fe, Zn, Cu, and Co, are needed for the growth of fetus, and hepatic Fe and Zn and renal Zn in mother would be transferred to fetus. It is well known that Hg can be transferred from mother to fetus (Honda 1985; Wagemann *et al.* 1988). In case of Caspian seals, Hg concentrations (Table 2, 3, 4, and Figure 5) and burdens (Table 7) in pregnant females were lower than those in nonpregnant females ($p < 0.05$: all differences were tested by Mann-Whitney U test). This clearly indicates that significant burden of Hg is transferred from mother to fetus.

Conclusions

Heavy metal concentrations were determined in muscle, liver, and kidney of Caspian seals to elucidate the status of toxic metal accumulations and to understand possible pollution by heavy metals in the Caspian Sea. Relatively low concentrations of toxic metals (such as Hg and Cd) were found. However these levels were higher than those of seals in Lake Baikal, which is also a land-locked water. Based on Hg and Cd concentrations in the liver, pinniped species were separated into two groups. One group accumulated higher levels of Hg than Cd, and another accumulated higher Cd levels than Hg. Caspian seals were likely to belong to high-Hg-accumulating group. This might be attributable to their dietary habit of feeding on fish in the Caspian Sea. Hg and Cd concentration ratio is suggested as a useful indicator to understand the feeding habit of marine mammals.

Age-related accumulation of toxic metals was found in the liver, kidney, and muscle tissues of Caspian seals. Cd concentrations increased up to 16.5 years and then decreased with age. Hg concentrations increased continuously. This trend might be due to the different rate of Hg and Cd uptake from diet arising from the shift in prey items with age.

In the case of essential metals, higher Mn and lower Fe concentrations were found in the liver of Caspian seals as compared to marine pinnipeds. This feature was different from that of Baikal seals, which is a taxonomically close relative with high diving ability. This specific pattern of Fe accumulation is likely to be explained by the habitat of Caspian seals, which evolved in shallower waters in the Caspian Sea. Lower Cu concentrations found in the liver of Caspian seals when compared with other marine pinnipeds was similar to that in Baikal and ringed seals. The low accumulation of Cu might be a characteristic of subgenus *Pusa*.

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