Heavy Metal Concentrations in Muscle, Liver and Kidney Tissue of Striped Dolphin, *Stenella coeruleoalba*, and Their Variations with Body Length, Weight, Age and Sex

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The concentrations of Fe, Mn, Zn, Cu, Pb, Ni, Cd and Hg in the muscle, liver and kidney of fifty nine striped dolphins captured during 1977~1980 were determined by atomic absorption spectrophotometry. The levels of most the metals examined were higher in the liver than the other tissues, except for the highest renal Cd. The frequency distribution of the metal concentrations was mostly log-normal. For all of the organs, correlation of the metal concentrations to the body length, weight and age was positive for Fe, Pb, Ni, Cd and Hg, and negative for Mn, Zn and Cu. A higher correlation of the concentrations of Fe, Mn, Zn and Cu to the body length and weight than to the age indicates that the metabolic turnover is more important than age or exposure time in determining the levels of those metals. Whilst the concentration of Pb, Ni, Cd and Hg correlated closely with the age of dolphins, and this suggests that the age or exposure time is a dominant factor for accumulation of these metals.

The concentrations of Pb, Ni, Cd and Hg in the muscle, liver and kidney increased with age until 1 year, leveled off through 1 to 18 years by physical dilution with increased body weight, and thereafter steadily increased year by year, but in case of Hg such physical dilution effects were not significant. Relatively wide variation of the metal concentrations in the pup and 8~25 years females was probably due to the effects of suckling, and of parturition and lactation, respectively.

The accumulation of heavy metals in marine organisms has increasingly drawn attention because of heavy metal pollution in the marine environment. In particular, mercury which caused "Minamata disease" is known to be highly concentrated in top predators in the marine food chain such as seal, dolphin, tuna, swordfish and shark. It is one of the main concerns among environmental scientists to elucidate the origin and mechanism of mercury accumulation in these species. The mercury and the other heavy metal concentrations in marine mammals have been extensively

studied by many workers for various species in different localities.^{1~17)} The nature of tissue or organs, size and age of animals, and season or locality for sample collection are very critical for the investigation of large animals such as marine mammals. However, a detailed analysis of tissue and organ distribution of metals and their variation with age, sex, reproductive activities and season largely remained to be studied.

Among marine mammals, the striped dolphin, *Stenella coeruleoalba*, which predominantly feed on squid, myctophid fishes and

shrimp, is an abundant and important component of the upper part of the pelagic food web surrounding Japan.¹⁸⁾ Since it is a long-living marine mammal, *i.e.* more than 40 years, the animal is useful as an "indicator species" to learn complex long-term accumulation characteristics of heavy metals in the marine environment.

We have already reported the distribution characteristics of heavy metals in bone, blubber and skin of the striped dolphin, and the nature of the organ(s) of dolphins to be selected for ecological and hygienic comparison was discussed.²³⁾

The main objectives of this paper, therefore, are as follows: (1) the measurement of metal levels in muscle, liver and kidney, (2) the variation and frequency distribution of these metal concentrations and (3) the relationship between metal concentration, body length, weight, age and sex. The heavy metals studied were Fe, Mn, Zn, Cu, Pb, Ni, Cd and Hg.

MATERIALS AND METHODS

Fifty nine striped dolphins were captured alive at Kawana on the east coast of the Izu Peninsula in December 1977, and at Taiji on the southern tip of the Kii Peninsula during the winter open seasons in December 1978~1980. Since the animals are seasonal migrators, the open season in these areas is between October and January.¹⁹⁾ All specimens appeared to be in a good healthy condition, with no macroscopic pathological symptoms. The animals were frozen at -20°C as soon as field conditions permitted (1~8 hr).

Body length, weight, sex and basic morphometric data were recorded for all specimens. The mandibular teeth were taken from each animal and were aged from the dentinal growth layers following the methods of Kasuya *et al.*²⁰⁾ After detailed anatomy, the weight of various organs and tissues was measured and recorded, and these data with the relevant biometrics have already been reported by Miyazaki *et al.*²²⁾ Representative muscle samples for metal determination were taken from a site about 10 cm in width under the dorsal fin, as described by Honda *et al.*²³⁾ Liver samples were excised from the medio-lateral lobe; samples of kidney were from medio-lateral region of the left kidney. These samples were packed in polyethylene bags and kept frozen at -20°C until analysis.

One to ten grams of tissue from each sample were digested with a concentrated sulfuric, nitric and perchloric acid mixture. Measurements of the heavy metals (Fe, Mn,

Zn, Cu, Pb, Ni and Cd) were performed by atomic absorption spectrophotometry, and the concentration of Hg was determined by flameless atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Metal levels in muscle, liver and kidney

Table I shows the mean concentration of heavy metals ($\mu\text{g/wet g}$) in muscle, liver and kidney of the striped dolphin. The level of the metals examined was generally higher in the liver than the other tissues, and markedly so for Hg. The highest level of Cd, however, was in the kidney where the mean concentration ($24.8 \mu\text{g/wet g}$) was approximately four times higher than that in the liver ($6.26 \mu\text{g/wet g}$). The characteristics accumulation of Hg in liver and Cd in kidney has been reported by many workers for other species of marine mammals from different localities.^{1~6),9~17)} Correlation coefficients among the metals in all the three tissues were calculated to see if there was any relationship between the metals, and the results are shown in Table II. The concentrations of Fe, Pb, Ni, Cd and Hg gave a positive correlation and these concentrations gave a negative one to the concentration of Mn, Zn and Cu except for the positive correlation between Cd and Zn in the kidney.

The literature concerning heavy metals, particularly Hg, in seals is extensive,^{1~6,15~17,26,37)} but information on the levels in small toothed cetacean is very sparse. The levels of Fe and Hg in striped dolphin from Kawana, Japan,³⁷⁾ West Indies,³⁴⁾ and Western Mediterranean³³⁾ are in a similar range to ours. Most of the metal levels in striped dolphin also agree with those of harbour porpoises, a beluga whale and a bottlenose whale.^{6,26)} However, Cd levels in the muscle, liver and kidney of striped dolphins were one or two orders higher than those of harbour porpoises and a beluga whale, and also significantly higher compared with those ($<0.2 \mu\text{g/wet g}$) in seals such as harbour seal,⁴⁾ common seal²⁵⁾ and ringed seal.⁷⁾ Such a high Cd level in the striped dolphin is explained by the fact that they feed

TABLE I. HEAVY METALS ($\mu\text{g}/\text{wet g}$) IN MUSCLE, LIVER AND KIDNEY OF STRIPED DOLPHIN, *S. coeruleoalba*

	(N) ^a	Muscle		(N) ^a	Liver		(N) ^a	Kidney	
		Range Mean	CV%		Range Mean	CV%		Range Mean	CV%
Fe	(59)	47~222 159	20.8	(57)	55.8~95.5 215	44.4	(30)	39.6~267 143	30.3
Mn	(59)	0.15~0.46 0.27	23.0	(57)	1.30~6.71 3.18	31.9	(30)	0.45~1.32 0.82	26.7
Zn	(59)	6.86~20.4 11.4	21.5	(57)	26.5~109 44.5	29.7	(30)	22.8~41.2 30.1	15.5
Cu	(59)	1.28~3.37 2.04	17.5	(57)	3.57~15.2 8.09	23.1	(30)	1.46~6.05 3.13	40.0
Pb	(59)	0.04~0.26 0.18	40.1	(57)	0.03~0.64 0.22	65.9	(30)	0.01~0.71 0.17	99.4
Ni	(59)	0.04~0.25 0.14	36.3	(57)	0.05~0.49 0.22	47.4	(30)	0.07~0.63 0.22	76.3
Cd	(59)	0.01~0.25 0.10	64.2	(57)	0.04~11.1 6.26	37.0	(30)	0.06~69.6 24.8	65.2
Hg	(51)	0.46~15.7 7.02	57.9	(45)	1.70~485 205	67.5	(20)	0.91~17.6 8.71	69.7

^a Number of samples analyzed.

on squid in which the Cd concentration is extraordinarily high.^{32,35)}

Moreover, Pb ($>2 \mu\text{g}/\text{wet g}$) in the liver of California sea lion,³⁾ elephant seal⁷⁾ and common seal,²⁴⁾ and Fe ($200 \sim 5000 \mu\text{g}/\text{wet g}$) in the liver of weddell seal, leopard seal, crabeater seal and dugong exceeded significantly the concentrations in our striped dolphins. Hepatic Fe in marine mammals is stored as hemosiderin which is considered to accumulate as a result of excessive Fe intake.^{7,38)} Seals live inshore where occasional exposure to high Pb pollution will accumulate a high level of Pb, and conversely, striped dolphins live offshore in a low level of Pb.

Frequency distribution of metal concentrations in muscle, liver and kidney

A wide variation (CV%) in all metal concentrations of striped dolphins was observed in the liver and kidney, and Pb, Ni, Cd and Hg concentrations in the muscle also varied widely (Table I). The frequency distribution of the metal concentrations in the muscle, liver and kidney are shown in Fig. 1. Here the data are combined for both males and females, the

skewness of concentration distribution being measured by Fisher's measure (g).

The pattern of frequency distribution differs slightly from an ordinary bell-shaped normal distribution, has a tailing to the right for most of the metals, and to the left for both the muscular Fe and hepatic Cd. To examine the frequency distribution pattern, cumulative frequency was plotted on probability paper (Fig. 2). A straight line was obtained on logarithmic probability paper and this indicates that the concentrations of most the metals examined obey a log-normal distribution. Plots of Cd concentrations in the muscle are shown in Fig. 2, as an example.

The frequency distribution of heavy metal concentrations in marine mammals has not been previously reported. Ting *et al.*²⁹⁾ have reported that the frequency distribution of trace elements in two species of marine fish and one marine algae was long-normal. Giesey *et al.*³⁰⁾ have examined the frequency distribution of concentrations of Cd, Cr, Cu, Fe and Zn in the whole bodies of five fresh water species of fish taken from an in-poundment in South Carolina, USA, and demonstrated that the

TABLE II. CORRELATION MATRIX OF: AGE, BODY LENGTH AND WEIGHT vs.
METAL CONCENTRATION; METAL vs. METAL IN THE MUSCLE, LIVER
AND KIDNEY OF STRIPED DOLPHIN, *S. coeruleoalba*

		Fe	Mn	Zn	Cu	Pb	Ni	Cd	Hg
Age	M	0.534 ^b	0.097	-0.375 ^a	0.057	0.630 ^b	0.480 ^b	0.780 ^b	0.817 ^b
	L	0.277	-0.406 ^b	-0.582 ^b	-0.432 ^b	0.492 ^b	0.485 ^b	0.398 ^b	0.823 ^b
	K	0.299	-0.516 ^b	-0.097	-0.446 ^b	0.117	0.107	0.245	0.687 ^b
Body length	M	0.775 ^b	0.150	-0.464 ^b	0.033	0.386 ^a	0.453 ^b	0.548 ^b	0.736 ^b
	L	0.375 ^a	-0.530 ^b	-0.721 ^b	-0.144	0.386 ^a	0.328 ^a	0.369 ^a	0.722 ^b
	K	0.542 ^b	-0.591 ^b	-0.129	-0.569 ^b	0.104	0.157	0.300	0.610 ^b
Body weight	M	0.717 ^b	0.134	-0.408 ^b	-0.029	0.388 ^a	0.466 ^b	0.579 ^b	0.793 ^b
	L	0.285	-0.503 ^b	-0.706 ^b	-0.118	0.397 ^a	0.397 ^a	0.380 ^a	0.732 ^b
	K	0.493 ^b	-0.548 ^b	-0.202	-0.565 ^b	0.068	0.115	0.294	0.641 ^b
Fe	M		0.233	-0.292	0.176	0.159	0.289	0.412 ^a	0.666 ^b
	L		-0.400 ^b	-0.305	-0.055	0.204	0.009	0.139	0.170
	K		-0.359 ^a	-0.078	-0.424 ^b	0.115	0.322 ^a	0.148	0.363 ^a
Mn	M			0.212	0.430 ^b	-0.049	-0.110	-0.139	-0.129
	L			0.611 ^b	0.353 ^a	-0.229	-0.031	-0.104	-0.295
	K			0.325 ^a	0.605 ^b	-0.119	-0.129	-0.142	-0.656 ^b
Zn	M				0.137	-0.155	-0.020	-0.217	-0.230
	L				0.473 ^a	-0.390 ^a	-0.291	-0.343 ^a	-0.459 ^b
	K				0.203	0.243	-0.007	0.528 ^b	-0.049
Cu	M					-0.082	-0.060	-0.226	-0.054
	L					-0.180	-0.062	-0.182	-0.025
	K					0.292	-0.501 ^b	-0.042	-0.625 ^b
Pb	M						0.265	0.492 ^b	0.432 ^b
	L						0.510	0.242	0.581 ^b
	K						0.357 ^a	0.154	0.051
Ni	M							0.294	0.444 ^b
	L							0.262	0.469 ^b
	K							0.129	0.017
Hg	M								0.686 ^b
	L								0.258 ^a
	K								0.391 ^a

^a Significant level, $p < 0.05$.

^b Significant level, $p < 0.001$.

M, muscle; L, liver; K, kidney.

log-normal, normal, Weibull and exponential distributions had a best fit to 57, 17, 26 and 0% of the species-element groupings respectively. Taguchi *et al.*²⁸⁾ have also reported that the frequency distribution of concentrations of Fe, Zn, Cd and Cu in the muscle of dogfish was log-normal.

Our results, examined for eight metals in the muscle, liver and kidney of striped dolphins also showed a log-normal distribution similar to those of the other marine organisms.

Therefore, positive or negative skewness causes some discrepancy in the confidence limits for the mean. To avoid a large error in confidence limits for populations in which the primary deviation from normality is a strongly positive or negative skew, Cochran³⁶⁾ has suggested that the sample size (n) must be large enough so that $n > 25g^2$, where g is Fisher's measure of skewness. In accordance with this rule, the number of our samples analysed was large enough for most of the metals, but Pb

FIG. 1. Frequency Distribution of Fe, Mn, Zn, Cu, Pb, Ni, Cd and Hg Concentration in the Muscle, Liver and Kidney of Striped Dolphin.

The data for males and females are combined.

FIG. 2. Cumulative Frequency: Cd Concentration ($\mu\text{g/wet g}$) in the Muscle, Liver and Kidney of Striped Dolphin.

●—●, normal scale; ○—○, logarithmic scale.

and Ni in the kidney showed high skewness ($g^2=4.12$ for Pb and 4.80 for Ni). When the distribution of heavy metal concentrations in biology is non-normal and highly skewed, the

sample size should be sufficiently large.

Changes of metal concentrations with age and sex

Significant positive correlation of the metal concentrations with age for striped dolphin was found in the muscular Fe, Pb, Ni, Cd and Hg, in the hepatic Pb, Ni, Cd and Hg, and in the renal Hg, whilst the hepatic Mn, Zn and Cu and the renal Mn and Cu were negatively correlated with age ($p<0.001$, Table II). The concentrations of metals in the muscle, liver and kidney are plotted against age (Figs. 3(a) and 3(b)), with the data for males and females being separately plotted.

Remarkable differences in most of the metal concentrations in the muscle, liver and kidney were observed between the ages of 0~1 year (pup) and the older specimens (immature and mature). The concentrations of Fe, Pb, Ni, Cd and Hg were higher in the latter than the former, and those of Mn, Zn and Cu, except for Mn and Cu in the muscle and Zn in the

FIG. 3(a). Relationships between the Concentration of Fe, Mn, Zn and Cu in Muscle, Liver and Kidney, and the Age for Striped Dolphin.

●, male; ○, female.

kidney, were higher in the pup. After the rapid change of metal concentrations in pups, the values of Mn, Zn and Cu in immature and mature dolphins remain relatively unchanged, but those of Fe, Pb, Ni, Cd and Hg characteristically change with age.

The concentration of Fe in the muscle in-

creased with age until 8 years and thereafter remained constant. A similar tendency also was observed with Fe in the liver and kidney. The concentrations of Pb, Ni and Cd in the muscle, liver and kidney increased with age until 1 year, and then remained constant through 1 to 18 years. After about 18 years,

FIG. 3(b). Relationships between the Concentration of Pb, Ni, Cd and Hg in Muscle, Liver and Kidney, and the Age for Striped Dolphin.

●, male; ○, female.

the concentration of Pb, Ni and Cd further increased with age. A steady increase of Hg concentration in the liver against age during the whole life span was observed, whereas Hg in the muscle and kidney reached a plateau after about 18 years.

The increase of body length and weight of

the striped dolphin ceases at about 8 and 18 years respectively (Fig. 4, discussed in detail later), the weaning and maturity ages being 0~1 and 8 years respectively.²¹⁾

A fast change of metal concentration from birth up to 1 year indicates that particular metals such as Pb, Ni and Cd are transferred *via*

FIG. 4. Relationships between the Body Length and Weight, and the Age for Striped Dolphin.

●, male; ○, female.

milk from the mother to her pup. The plateau of the concentrations of Pb, Ni and Cd during 1~18 years is explained by physical dilution from the increase in body weight. Hg did not show such similar dilution effects as Pb, Ni and Cd and this implies a higher absorption rate of Hg in the digestive tract of striped dolphin and a larger persistence of Hg than those of other metals. Moreover, a plateau in the concentration of Hg in the muscle and kidney comes earlier than that in the liver. This indicates a longer biological half-life of Hg in the liver.

Sexual differences in the metal concentration of striped dolphins are not particularly great (Figs. 3(a) and 3(b)). The concentrations of muscular Pb and Ni and hepatic Fe, Zn, Cu, Pb and Ni were higher in mature male than in mature female, although the hepatic Mn concentration in female was higher than that in male. Furthermore, the concentration of metals in 8~25 year females varied more widely than that in males and this probably reflects such reproductive activities as parturition and lactation.

Relationships between body length, weight and metal concentration

There were also some significant positive or negative correlations for metal levels against body length and weight. The concentrations of Fe, Mn, Zn and Cu in the muscle, liver and kidney showed a high correlation with body length, high with body weight and slight with age. In the case of Pb, Ni, Cd and Hg, however, the correlation with age was always stronger than those with body length and weight (Table II).

The concentration of Fe in the muscle was proportional to the body length, roughly to the cube root of the body weight, and slightly higher in female than male (Fig. 5). Similar results were obtained on the concentrations of Fe, Mn and Zn in the liver and kidney. This is understandable by the regression equation between the body length and weight which shows $BW = 2.19 \times 10^{-5} BL^{2.88}$ ($r^2 = 0.968$).³⁸⁾ The concentration of Hg in the muscle, liver and kidney also correlated with the body length and weight, higher in female than in male, although it showed strong positive skewness with larger size. Similar results (not shown) were found for Pb, Ni and Cd in the muscle and liver. Plots of Hg concentration in the liver to the body length and weight are shown in Fig. 6, as an example.

Although Hg concentration in marine mammals as well as many fish is known to increase with age and size, clear tendencies for other metals have not been established. The correlation of Fe, Mn, Cu and Cd concentrations to the age and size of harbour seal⁴⁾ and dugong⁷⁾ agree well with our results for striped dolphin.

However, no significant correlation of Pb and Ni concentrations with age and size was observed for harbour seal⁴⁾ and dugong.⁷⁾ These papers also showed that the concentration of Zn in the liver and kidney of dugong positively correlated with the age, while a specific increase of Zn concentration in harbour seal was not found. In our striped dolphins, Pb and Ni correlated with age and Zn showed a negative correlation against age.

FIG. 5. Relationships between the Concentration of Fe in Muscle and the Body Length and Weight for Striped Dolphin.

●, male; ○, female.

FIG. 6. Relationships between the Concentration of Hg in Liver and the Body Length and Weight for Striped Dolphin.

●, male; ○, female.

This difference may be due to the smaller sample sizes of young harbour seals and dugongs compared with ours, because the pup of striped dolphin contained relatively high Zn in the liver and low Pb and Ni in the muscle,

liver and kidney.

Studies by Olsson³⁷⁾ on northern pike, *Esox lucius* L., are of interest since he found that fish of the same size but of different ages (as a measure of exposure time) had similar Hg levels. He found also that males had significantly greater Hg levels than females. Gaskin *et al.*⁶⁾ have found clear correlations of Hg concentration with age in the muscle, kidney, cerebrum and liver of harbour porpoises. They also found that Hg levels, except in the liver, were less well-correlated with weight and body length than with age, and not significantly correlated with weight and length within the same age classes. Our results here agree well with the results on harbour porpoises⁶⁾ but not with those on the northern pike.³⁷⁾ Such correlation of metal concentration to body length, weight and age indicates that the metabolic turnover (correlated with size) is more important than age or exposure time in determining the levels of Fe, Mn, Zn, and Cu, and that the age or exposure time is a dominant factor for accumulation of Pb, Ni, Cd and Hg in marine mammals.

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