Changes of Hepatic Mercury Accumulations of Southern Minke Whales in Past Fifteen Years

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ABSTRACT

Liver (n=534) and muscle (n=353) samples are used to examine the accumulation level of mercury (Hg) in the southern minke whale *Balaenoptera acutorostrata*. For the analysis samples were grouped by sex, geographical position, periods within a austral summer season and years. Hepatic Hg concentrations are correlated positively to those in muscle. Hg concentration in liver was taken as an indicator of the concentration of Hg in the minke whale. No significant differences in the hepatic mercury levels were observed between male and female, nor between geographical position in the Antarctic feeding ground, nor between periods within a summer season. Marked inter-annual changes of age-related Hg concentration were found. In the 1980/81-1981/82 austral summer seasons, no correlation was observed between hepatic Hg concentration and age. However, in recent years (1995/96), the Hg concentrations increased with age. This confirm the previous view of an increasing trend of Hg intake in the minke whale, after the first half of the 1980's, as a result of a greater availability of food.

INTRODUCTION

Marine mammals have been used frequently as indicator species to determine the degree of pollution of chemicals such as heavy metals and organochlorine compounds. This is because these animals are located in the highest position of their food chain and therefore highly accumulated for these contaminants (Holden, 1978; Wagemann & Muir, 1984; Honda, 1985; Tanabe *et al.*, 1994). It is known that minke whale accumulated heavy metals, especially for Cd and Hg (Honda *et al.*, 1986; Yamamoto, 1988). Hg is accumulated in cetaceans and pinnipeds with their age (Koeman *et al.*, 1972; Anas, 1974; Smith and Armstrong, 1975; Drescher *et al.*, 1977; Holden, 1978; Harms *et al.*, 1978; Arima and Nagakura, 1979; Gaskin *et al.*, 1979; Itano *et al.*, 1980). However, in the case of southern minke whales there are no evidences that Hg increased with their age, in spite of Hg was accumulated in minke whales through the Antarctic food chain, and it suggested the increase of Hg intake, perhaps due to the increase of food intake (Honda *et al.*, 1987b) . In this paper, we examined the inter-annual changes of Hg accumulations in southern minke whales during past 15 years, using commercial and recent JARPA samples.

MATERIALS AND METHODS

Data and samples used

The number of samples used in this study are shown in Table 1. Data on Hg in muscle and liver of minke whales for the 1980/81, 1981/82, 1984/85 and 1985/86 seasons were those reported by Honda *et al.* (1986, 1987b), Yamamoto (1988) and Watanabe *et al.* (in prep.). These samples were collected during Japanese commercial whaling operations. Recent samples (1988/89, 1989/90 and 1994/95 seasons) were collected in the Japanese Whale Research Program Under Special Permit (JARPA) and analyzed for Hg accumulation. A total of 534 liver and 353 muscle samples were used in this study. Sampling locations of these whale samples are shown in Fig. 1, by season.

Method of Hg analysis

Muscle and liver samples were homogenized, and 5 to 10g of samples were mineralized with a mixture of nitric and sulfuric acid mixture in a flask equipped with a Liebig condenser and followed by potassium permanganate ($KMnO_4$) digestion. The excess of $KMnO_4$ was reduced with a 20% hydroxylamine hydrochloride solution and the mercury was reduced to Hg^0 with tin(II) chloride. The presence of Hg was determined by cold vapor atomic absorption spectrophotometry (Honda *et al.*, 1982; 1985; 1986; 1987b). Recoveries ranged 90.0 to 99.9%. The precision of this analysis method was examined by analysis of Hg in dolphin muscle, the coefficients of variation (CV%) were below 7.0%.

RESULTS

Relationship of Hg concentrations between liver and muscle samples

It was known that tissue distributions of Hg in the whale body. Levels of Hg was high in liver and kidney but most of Hg content in the body were allocated in muscle and liver tissues in cetaceans (Honda *et al.*, 1982, 1985; Fujise, 1987; Fujise *et al.*, 1988). In the case of southern minke whale, 80.6% of Hg burden was located in these two tissues (Yamamoto, 1988). We compared Hg concentrations in these tissues. Fig. 2 shows the relationship of Hg concentrations in liver and muscle of minke whales in three sampling seasons (84/85-85/86, 89/90 and 94/95 seasons). The results of regression analysis are shown below;

84/85-85/86 seasons	$y=2.5239x+9.7478$	$r=0.6275$	$n=139$
89/90 season	$y=2.4654x+9.3126$	$r=0.6433$	$n=78$
94/95 season	$y=2.7761x+0.7815$	$r=0.7923$	$n=49$

These results suggest that the Hg level in liver was correlated highly to that in muscle. The hepatic Hg levels could be an indicator of the total body burden of Hg and their changes.

Sexual differences of Hg accumulation

Hepatic Hg concentrations in male and female minke whales were compared. For this comparison, data are grouped to four age-class groups (i.e. 1-5, 6-15, 16-25, and 26+), taking into consideration the possibility of age-related accumulation of Hg, as reported on other cetaceans (Koeman *et al.*, 1972; Anas, 1974; Smith and Armstrong, 1975; Drescher *et al.*, 1977; Holden, 1978; Harms *et al.*, 1978; Arima and Nagakura, 1979; Gaskin *et al.*, 1979; Itano *et al.*, 1980).

Table 2 shows the mean, standard deviation and ranges of hepatic Hg concentrations by sex, age-class and season. Table 2 also shows the results of Wilcoxon Mann-Whitney U test to Hg concentration in males and females. Although all age-classes could not tested for all survey year, no significant differences were observed in Hg concentrations between male and females in most of age class and survey season.

Geographical differences of Hg accumulation

Hg concentrations of minke whales are compared between IWC management Areas. Fig. 3 shows age trends of Hg levels in minke whales in Areas III to VI in 1980/81-1981/82 seasons. Figs. 4 to 6 are for 1984/85-1985/86, 1988/89-1989/90, and 1994/95 seasons, respectively. No geographical difference was observed for all seasons by taking into account of their age-related changes of the metal, although it was observed large variation of the age-trend.

Seasonal changes of Hg accumulations

It is known that the Hg accumulation levels of cetaceans reflect the long-term intake of Hg though their food (Honda, 1985; Honda *et al.*, 1987; Fujise, 1987; Yamamoto, 1988). In order to examine the changes of hepatic Hg accumulations within a short-term period, we compared the hepatic Hg concentrations and their sampling date (Fig. 7). In this comparison, we used minke whales aged 10 years old or more. Although it is observed large variation of Hg levels, no trend of Hg concentration was observed with their sampling date.

Inter-annual changes of Hg accumulation

Watanabe *et al.* (in prep.) reported that the intake of Hg of southern minke whales increased at least during first 4 years of 1980's. In order to examine this phenomena, age trend of hepatic Hg concentrations was compared for four survey season classes (1980/81-81/82, 1984/85-85/86, 1988/89-1989/90 and 1994/95 seasons). In this comparison, all data was used because the sexual, geographical and seasonal changes of hepatic Hg concentrations were not observed for southern minke whales.

Age-related accumulation of hepatic Hg concentrations in four yearly groups is shown in Fig. 8. Remarkable inter-annual changes of Hg levels were observed. In 1980/81-1981/82 seasons, it seems that Hg concentrations are not increased significantly on the age-group of 10 years old or more. A different trend is observed for the 1988/89-1989/90 season and 1994/95 season, in which Hg concentrations increased for whale aged 10 years or more. It seems that Hg accumulated with year in all age classes as reported on other cetaceans. This maybe reflect that the increasing of Hg intake of minke whales (Watanabe *et al.*, in prep.) is continued after first half of 1980's.

DISCUSSIONS

Although significant sexual, geographical and seasonal changes of hepatic Hg concentrations were not observed for southern minke whales, it was observed a marked inter-annual changes of hepatic Hg during past 15 years. At this time there are two possible explanations for these changes. One is that these changes reflected a change of Hg in their food (i.e.. krill). Few data are available on the Hg concentration of Antarctic krill (*Euphausia superba*). Honda *et al.* (1987a) and Yamamoto *et al.* (1987) reported the Hg levels of krill collected from the stomach of the same whale samples in 1984/85-1985/86 seasons, and the levels were 3.6 ppb (range: 1.9-9.1, n=76) and 8.0 ppb (range: 4.0-23.0, n=53), respectively. The Hg levels of the krill (n=38) from stomach of minke whales collected in 1988/89 season is 7.9 ppb with a range of

4.5-13.6 (Y. Fujise, unpublished data). Thus it is hard to conclude that the change of Hg is caused by the Hg levels of the food. Other possibility is the changes of feeding circumstances for southern minke whale. It is well known that populations of larger baleen whale species such as blue, fin and sei whales were depleted by the past commercial whaling. It also known that growth rate of minke whales has increased and the age at sexual maturity decreased (Kato, 1985). It has been suggested that these changes of growth for minke whales have been caused by a decrease of stock of large baleen whales such as blue whale (Kato, 1985). Honda *et al.* (1987b) reported no significant linear correlation between hepatic Hg and Cd in southern minke whale and age, and they expected that this maybe reflected a greater availability of food which caused by a structural disturbance in the Antarctic marine ecosystem due to the commercial whaling. If it is true, the present results may show the increased Hg (food) intake may continued after 1980's. This is most reasonable to explain these changes of age trend of Hg accumulation in liver of minke whales in the Antarctic.

However, it should be noted that the lower level of hepatic Hg concentration seems to be constant among all seasons (ca. 30 ppb). This suggest that whales have a constant food (Hg) intake throughout the seasons. Because no information are available, further examination are required.

On the other hand, if we look at a part of younger age class in Fig. 9, level of Hg in liver were lower in the 1994/95 than those in 1980's. This could suggest the possibility that the increased Hg intake has begun to decrease in the recent year. From another kind of information (Kato and Fujise, unpublished data), it tend to decrease yearly for thickness of blubber even if it was used the whales which their age are 20 years old or more (Fig. 10). This is most remarkable for females. Furthermore, similar decreasing pattern for fattyness index of minke whales was reported by Ohsumi *et al.* (1997). Further examination is required to examine this phenomena.

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Table 1. List of samples used in this study

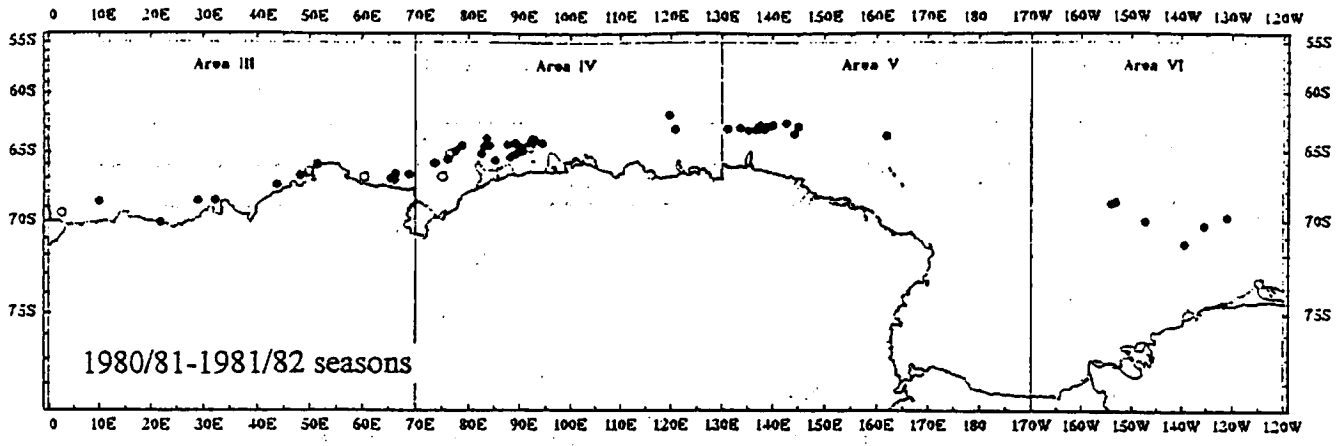
Season	Operation*	Area	Period	Male		Female	
				Muscle	Liver	Muscle	Liver
80/81	CC	III-V	80/11/18-81/2/28	0	75	0	22
81/82	CC	IV,VI	81/11/19-82/3/25	49	20	0	0
84/85	CC	IV-VI	84/11/28-85/3/12	20	20	17	17
85/86	CC	IV-VI	85/12/3-86/3/20	74	49	66	59
88/89	RT	V	89/1/12-89/3/27	0	68	0	76
89/90	RT	IV	89/12/10-90/2/14	78	78	0	0
94/95	RT	V	94/12/15-95/3/11	48	49	1	1

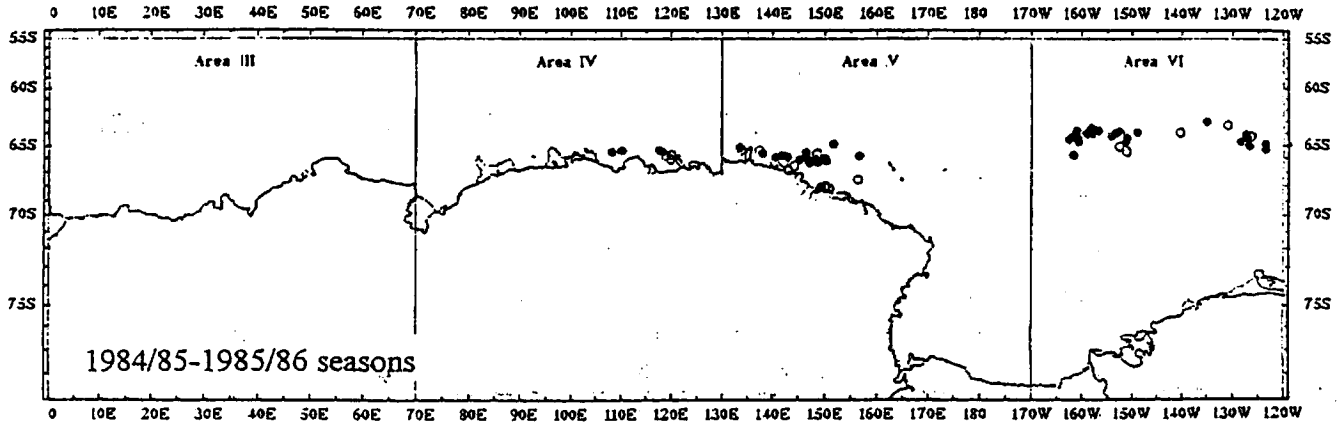
*: CC: Comercial catch, RT: Research take

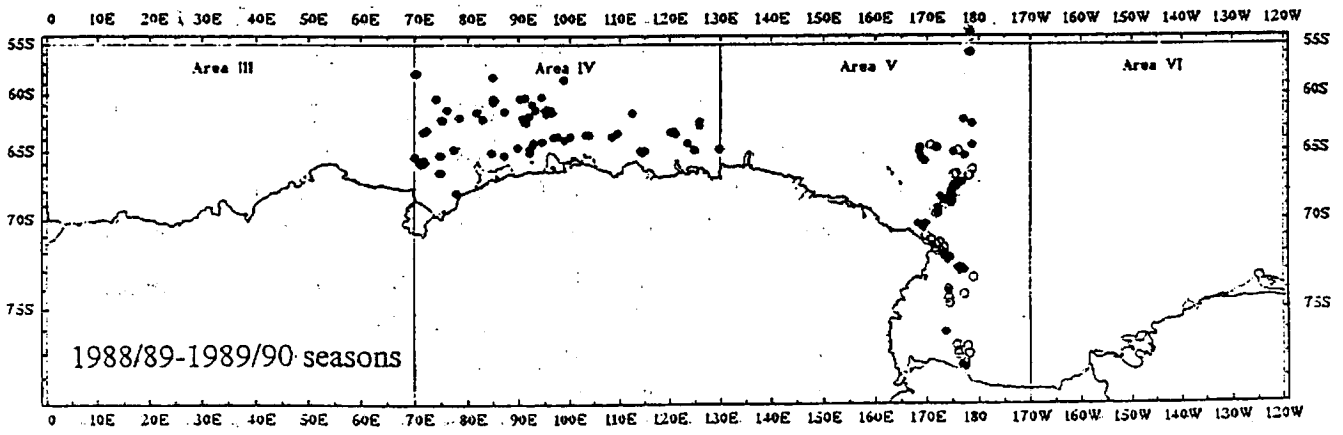
Table 2. Comparison of hepatic mercury concentrations between male and female minke whales in each season and age class.

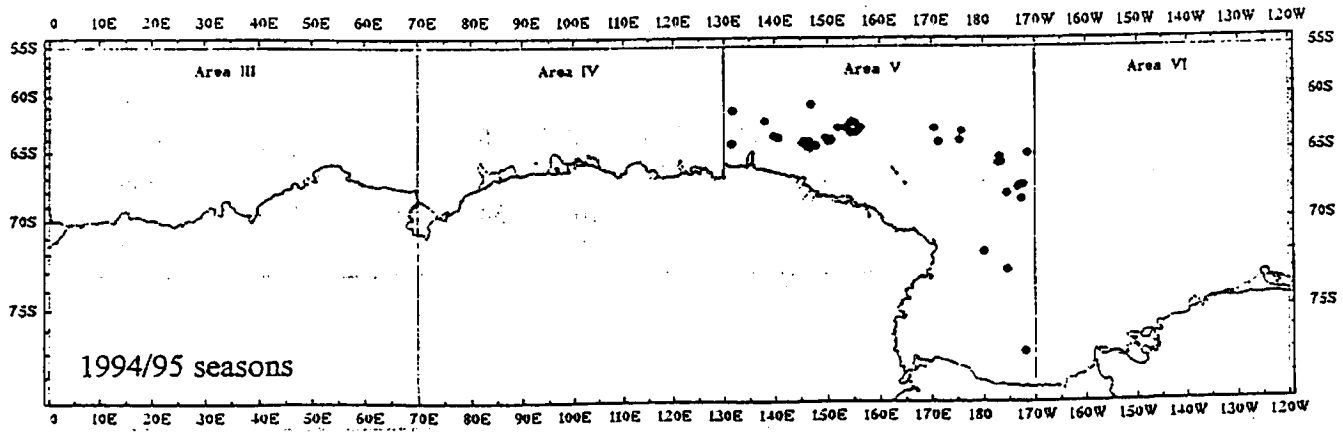
Season	Area	Age class	Male					Female					WMW-test*
			Mean	SD	Min	Max	n	Mean	SD	Min	Max	n	p
80/81-81/82	III	1-5	40.5	3.6	37.0	45.6	6					0	
		6-15	58.8	19.9	47.1	88.5	4	53.5	20.9	27.0	78.3	9	>0.01
		16-25	51.6	7.6	46.2	57.0	2					0	
		26-	63.4	16.7	42.6	77.5	4					0	
	IV	1-5	48.5	17.6	26.7	70.4	7					0	
		6-15	70.0	22.6	40.7	115.6	15	58.4	16.9	35.9	87.7	8	>0.01
		16-25	63.5	29.5	32.9	121.0	7					0	
		26-	56.0	9.2	45.0	67.9	6					0	
	V	1-5	44.5	11.5	20.6	57.0	8					0	
		6-15	54.2	15.6	36.9	92.6	11	60.8	17.8	44.1	86.6	5	>0.01
		16-25	49.5				1					0	
		26-	62.0	13.1	45.8	75.6	4					0	
	VI	1-5					0					0	
		6-15	44.4	13.7	24.2	57.8	5					0	
		16-25	41.3	10.6	23.2	61.7	10					0	
		26-	62.6	26.0	31.8	93.7	5					0	
84/85-85/86	VI	1-5					0					0	
		6-15					0					0	
		16-25	77.5	16.2	66.0	88.9	2	56.7	31.7	34.3	79.1	2	
		26-	47.8	6.8	40.1	52.8	3	53.0	5.2	49.3	56.6	2	>0.01
	V	1-5	38.1	9.7	29.9	49.7	4	64.3	18.3	42.3	92.4	7	>0.01
		6-15	79.6	31.4	36.1	113.0	5	61.1	31.6	24.0	112.0	8	>0.01
		16-25	60.6	40.3	29.6	163.0	9	79.6	32.1	33.3	160.0	16	>0.01
		26-	74.2	32.6	41.6	130.9	6	68.3	39.0	24.5	169.0	14	>0.01
	VI	1-5	49.8	19.8	21.4	72.1	6	39.4	20.0	14.7	76.6	11	>0.01
		6-15	69.3	18.8	40.3	94.7	10	53.6	33.6	22.6	129.0	11	>0.01
		16-25	89.9	30.5	45.3	136.2	11	57.3	13.4	39.9	68.9	4	>0.01
		26-	90.8	49.5	20.7	176.2	8	65.3				1	
88/89-89/90	IV	1-5					0					0	
		6-15	89.4	34.8	27.4	165.5	29					0	
		16-25	77.6	34.0	33.0	143.6	32					0	
		26-	87.9	39.9	38.5	148.9	17					0	
	V	1-5	64.2	63.8	6.0	346.0	26	57.3	38.7	8.0	158.0	26	>0.01
		6-15	70.7	29.8	23.0	136.0	23	81.4	39.1	31.0	198.0	28	>0.01
		16-25	79.0	32.9	30.0	127.0	12	101.8	54.8	44.0	273.0	19	>0.01
		26-	95.4	72.4	23.0	227.0	7	135.0	65.1	83.0	208.0	3	>0.01
94/95	V	1-5	32.8	20.4	15.2	56.3	5					0	
		6-15	79.6	32.8	41.9	146.8	13					0	
		16-25	85.8	40.9	45.9	182.5	16					0	
		26-	116.6	48.8	51.8	241.2	15	83.8	-	-	-	1	

* Wilcoxon Mann-Whitney test









Fig, 1. Locations of minke whales which were used in this study.

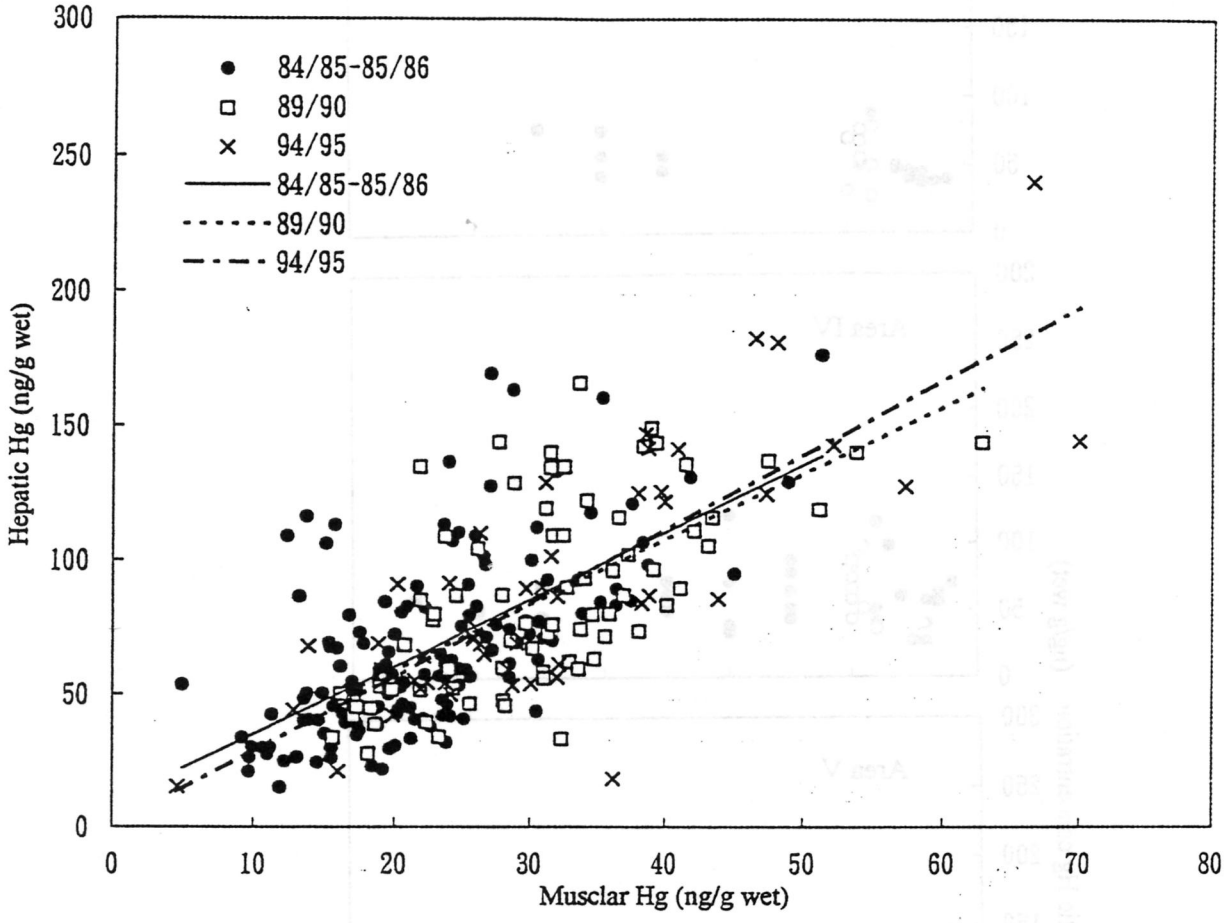


Fig. 2. Relationship of muscular and hepatic Hg concentrations in each season.

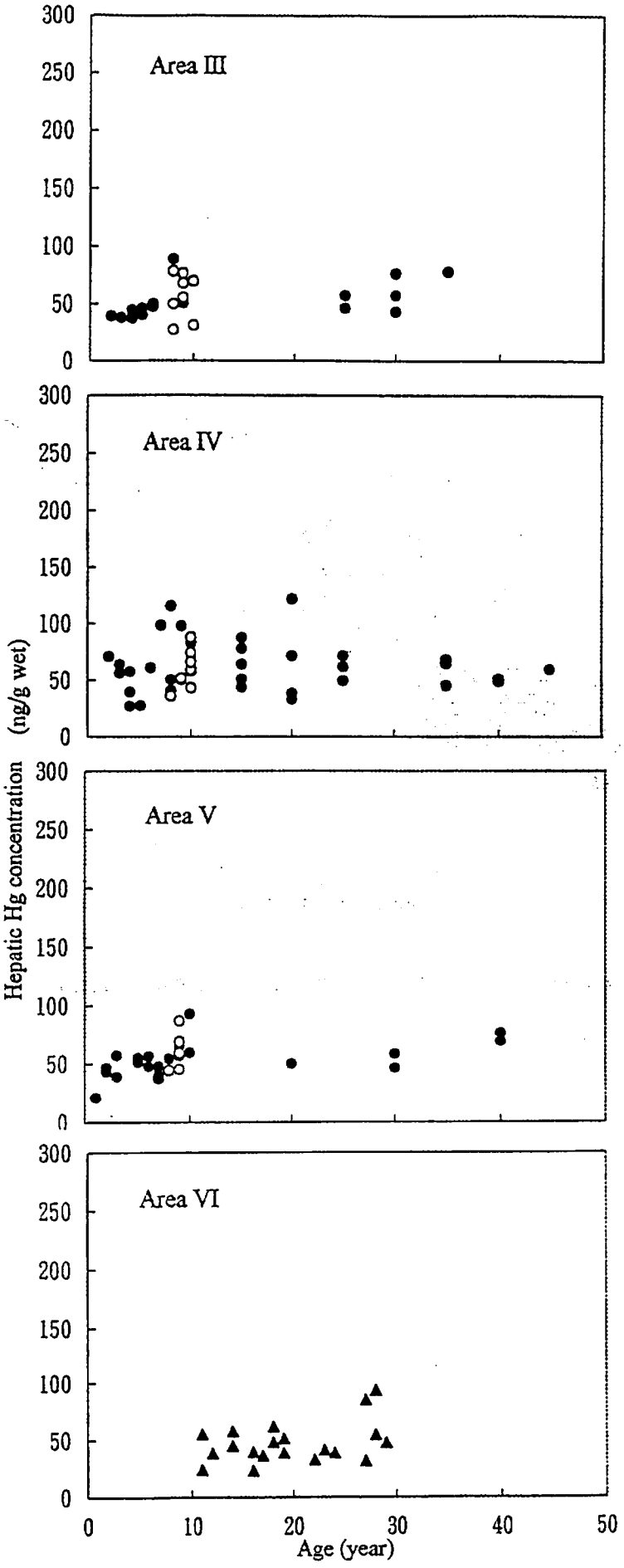


Fig.3. Comparison of age trends of hepatic Hg concentrations by Area in minke whales collected in 1980/81-1981/82 seasons.
Closed and open circles indicate males and females in 1980/81 season, respectively, and closed triangles indicate males in 1981/82 season.

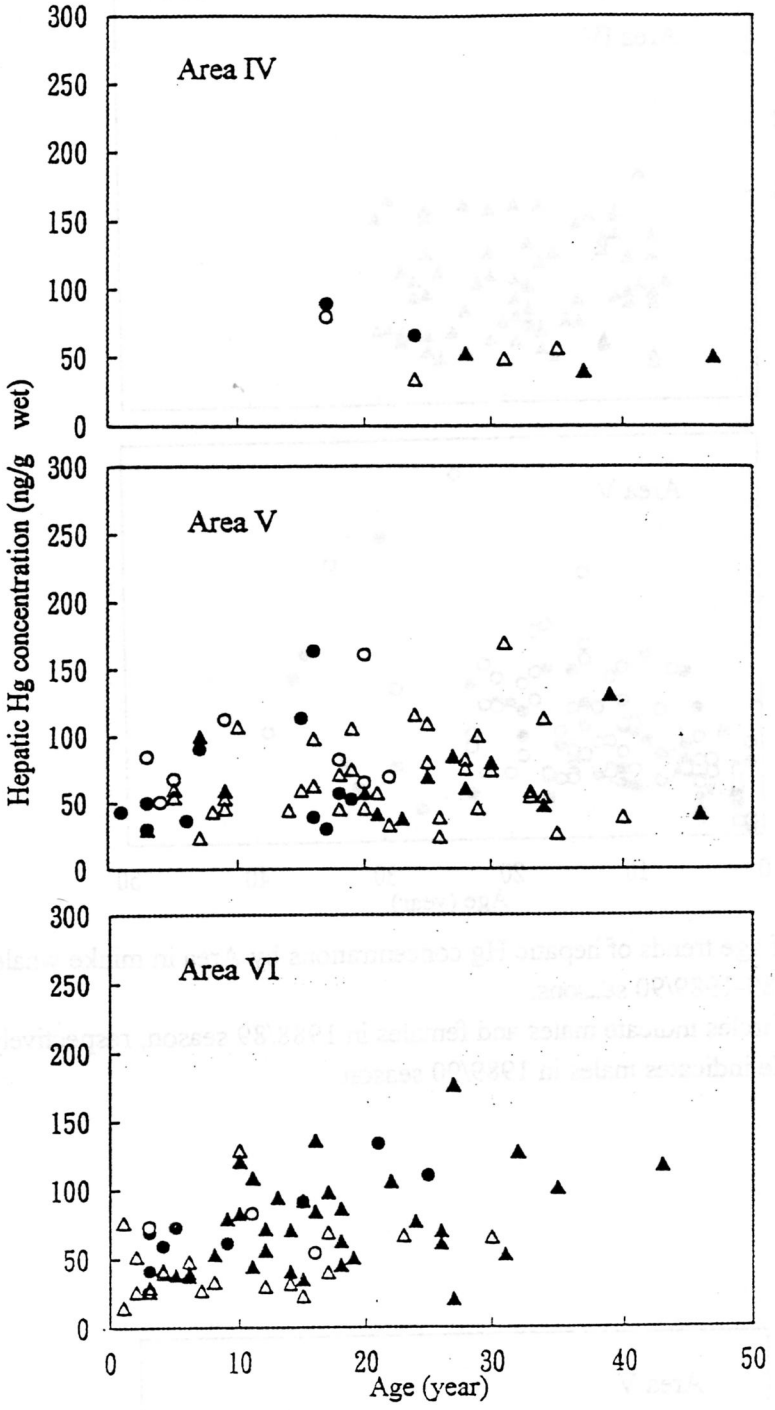


Fig.4. Comparison of age trends of hepatic Hg concentrations by Area in minke whales collected in 1984/85-1985/86 seasons.
Closed and open circles indicate males and females in 1984/85 season, respectively, and closed and open triangles indicate males and females in 1985/86 season.

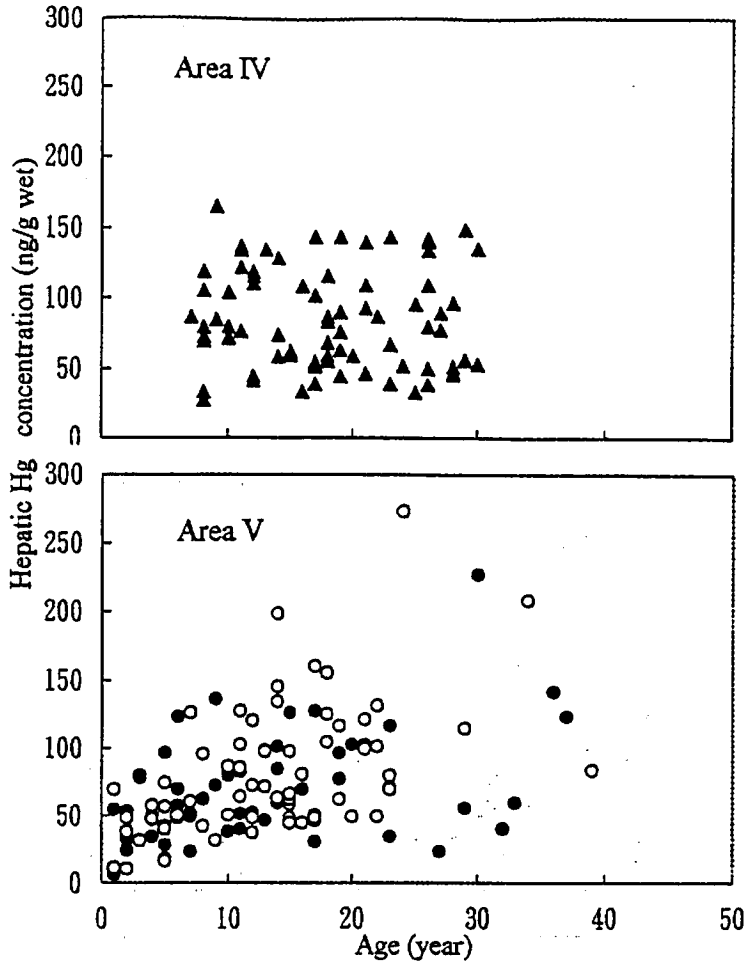


Fig.5. Comparison of age trends of hepatic Hg concentrations by Area in minke whales collected in 1988/89-1989/90 seasons.
Closed and open circles indicate males and females in 1988/89 season, respectively, and closed triangle indicates males in 1989/90 season.

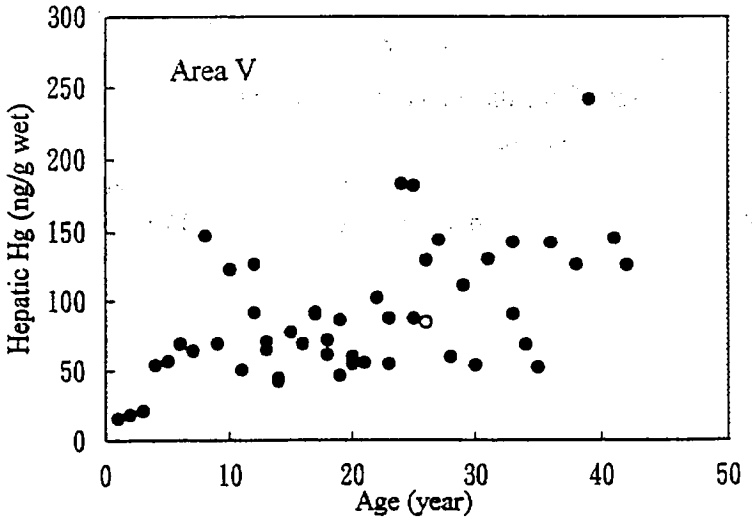


Fig.6. Age trend of hepatic Hg concentrations of minke whales collected in 1994/95 season. Closed circle indicates males and open circle female.

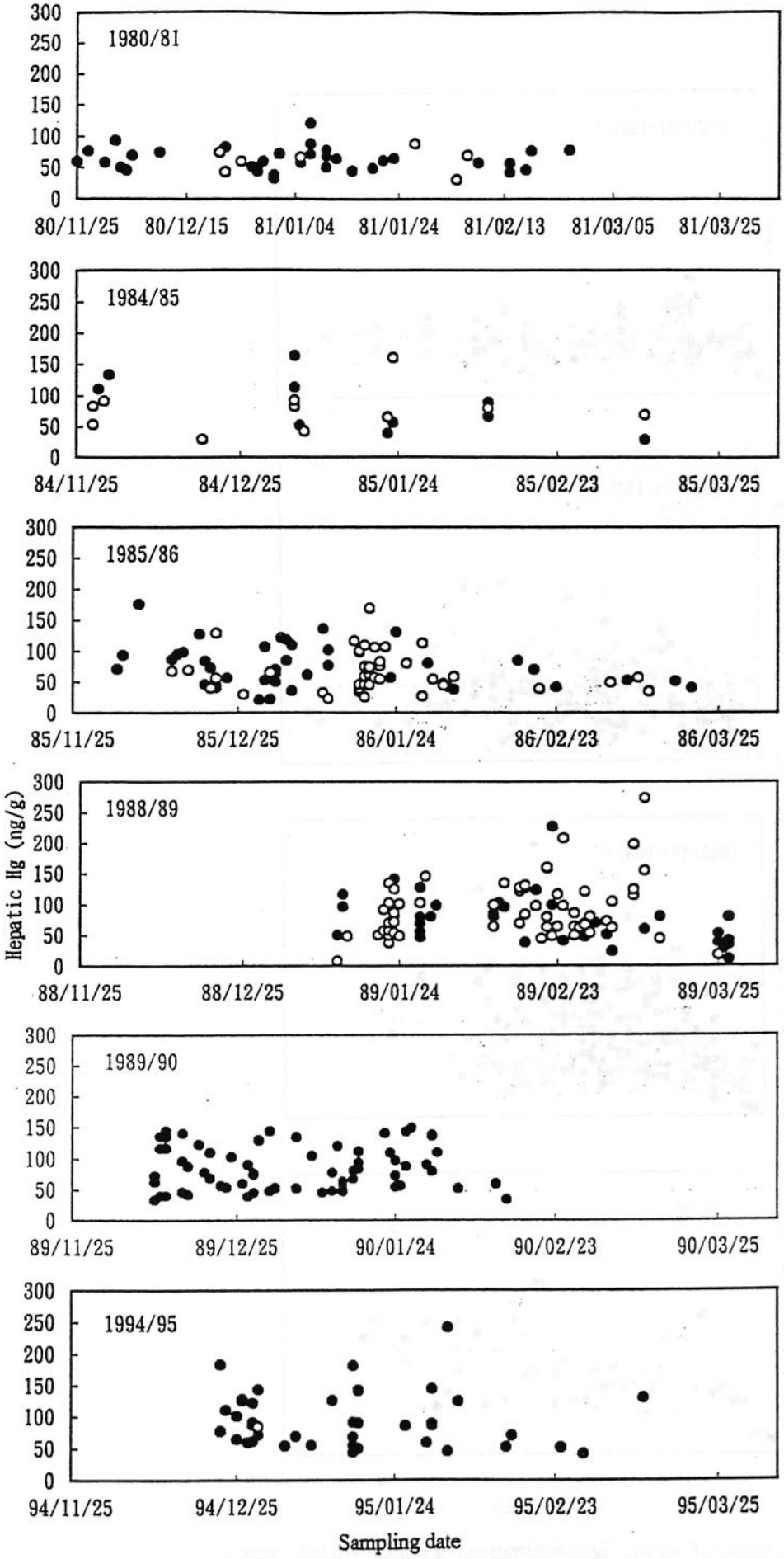


Fig.7. Seasonal changes of Hepatic Hg concentrations of minke whales aged 10 years or more. Closed and open circles indicate male and female, respectively.

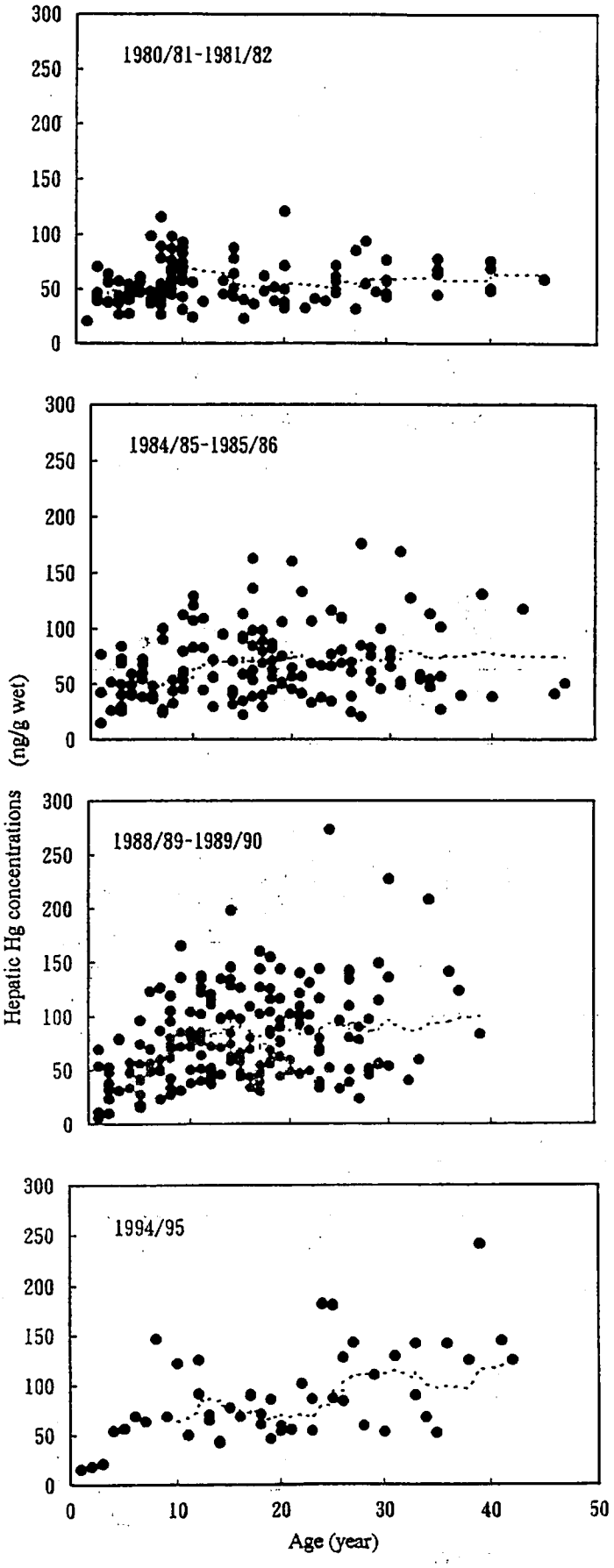


Fig. 8. Age trends of hepatic Hg concentrations of minke whale by survey season. Broken line was drown by running mean method.

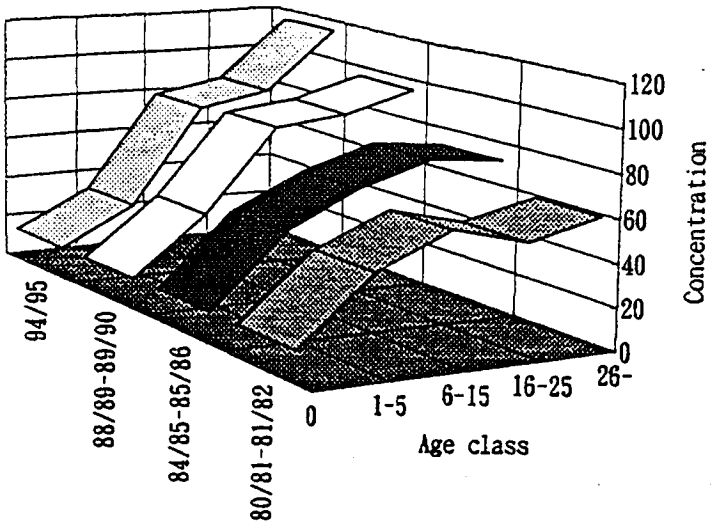


Fig.9. Comparison of age trends of hepatic Hg concentrations of minke whales

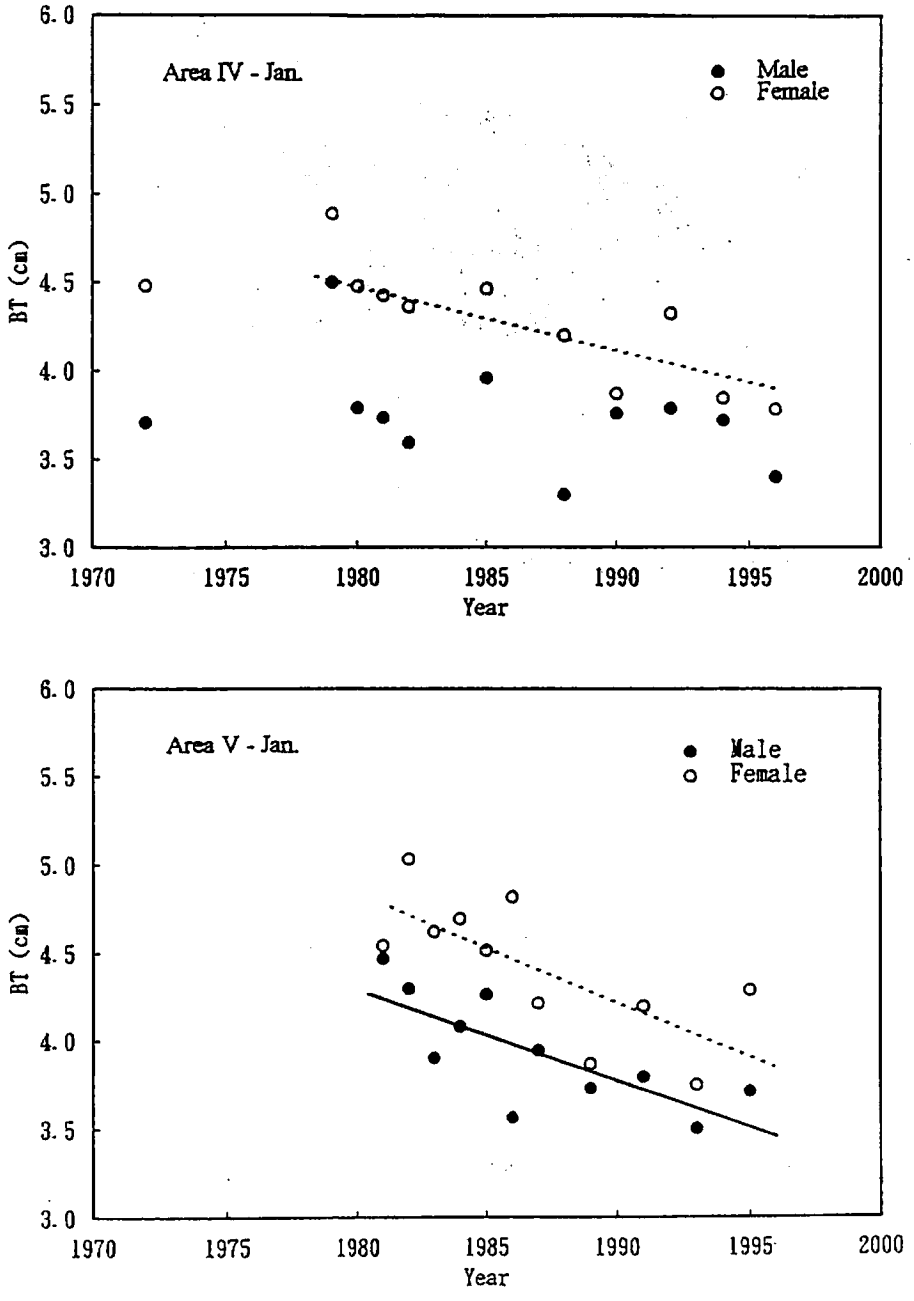


Fig. 10. Inter-annual changes of mean blubber thickness (cm) of male and female minke whales in Areas IV (upper) and V (bottom). Data used only whales which their ages are 20 years old or more, in area of 60 degrees south or more, in January to avoid the variation of other fluctuation factors (growth, migration pattern, etc.).