

# Trace element accumulations of Antarctic krill, *Euphausia superba*, in Areas III, IV, V and VI from the Antarctic Ocean during 1989-1999

GENTA YASUNAGA\*, YOSIHIRO FUJISE\* AND KATSUHISA HONDA\*#

\* *The Institute of Cetacean Research, 4-18, Toyomi-cho, Chuo-ku, Tokyo 104-0055 Japan.*

\*# *Department of Environmental Science for Industry, Ehime University, 3-5-7, Tarumi, Matsuyama-city, Ehime, 790-8566, Japan.*

## SUMMARY

Concentrations of Mn, Fe, Ni, Cu, Zn, Cd, Hg and Pb were determined in Antarctic krill, *Euphausia superba*, collected from stomach contents of Antarctic minke whales from Antarctic Ocean Areas III, IV, V and VI during 1989/90 and 1998/99. An interaction among Cd-Ni-Mn-Zn was observed in *E. superba*. These elements have the same distribution in the sea. Levels of Mn, Fe, Cu, Zn and Cd in each of three body length groups in Antarctic krill were significantly different. The results were not consistent with the previous study, suggesting that trace element levels in Antarctic krill may be affected by other life history factors rather than the body length alone. Seasonal change of trace elements in *E. superba* was observed only in Cd, suggesting that it is affected by the migration pattern. Temporal trends of the toxic elements Cd and Hg concentrations in *E. superba* were not observed during 1989 and 1999. Trace element, except for Cd, concentrations found in *E. superba* were almost the same to those levels previously reported in krill from the Japan Sea, the western North Pacific and the western North Atlantic, while Cd concentrations in *E. superba* reported here were higher than those in the other regions.

KEY WORDS: ANTARCTIC MINKE WHALE; TRACE ELEMENT; BIOACCUMULATION, ANTARCTIC KRILL

## Introduction

In 1992, the International Whaling Commission (IWC) decided to establish a regular agenda item for research on effects of environmental change on cetaceans in the IWC Scientific Committee (SC) (IWC, 1993). Given this, "The elucidation of the effect of environmental changes on cetaceans" was added to the research objectives from the 1995/96 JARPA research. Furthermore, we had collected samples and monitored pollutant levels in the tissue and stomach contents of Antarctic minke whales from 1987/88 research.

For monitoring of trace elements such as Cd and Hg in the aquatic environment, some organisms have been used as bioindicators (Philips, 1980; Schubert, 1985). Invertebrates such as bivalves and krill are especially useful for monitoring trace elements in the marine environment, because trace elements are directly taken up and accumulated in their body from solution and/or plankton. Trace elements include essential elements such as Fe, Cu and Zn and toxic elements (or nonessential elements) such as Hg, Cd and Pb. Essential elements are necessary to maintain normal metabolism and physiological functions in living organisms. Toxic elements induce adverse effects through bioaccumulation.

There are reports of trace element levels of krill in the Japan Sea (Masuzawa *et al.*, 1988), the Atlantic Ocean (Ridout *et al.*, 1989), the Mediterranean (Fossi *et al.*, 2002) and the Antarctic (Yamamoto *et al.*, 1987). There are few studies on monitoring of trace elements in krill, and research on temporal changes is scarce.

The object of this report is to reveal temporal and spatial trends of trace elements, especially toxic elements, in Antarctic krill obtained as stomach contents of Antarctic minke whales.

## Materials and Methods

Antarctic minke whales were collected from Areas IIE (35-70E) and IV (70-130E), and V (130E-170W) and VIW (170W-145W), in alternate years in JARPA. *Euphausia superba* samples were obtained from stomach contents of

100 Antarctic minke whales sampled from the Antarctic Areas III, IV, V and VI during 1989/90 and 1998/99. *E. pacifica* was obtained from stomach contents of 10 common minke whales sampled from western North Pacific and examined for trace element analysis (Table 1). All samples were stored in polyethylene bags at -20 °C until analysis.

For the analysis of Mn, Fe, Ni, Cu, Zn, Cd, Hg and Pb, the tissues were homogenized and digested in microwave (Milestone General: MLS 1200 mega) using nitric acid in a PTFE (Teflon) vessel (Okamoto, 1994). Mn, Fe, Ni, Cu, Zn, Cd and Pb were measured by inductively coupled plasma atomic emission spectrometry (Seiko Instruments Inc., SPS 1700R) and Hg was measured by cold vapour / atomic absorption spectrometry (Nippon Instruments Co. RA-2A) using external standard method. Concentrations of trace elements in krill are given on dry weight basis. Accuracy and precision of the methods were confirmed using 'bovine liver' (BCR-CRM No. 185) and 'pig kidney' (BCR-CRM No. 186). Chemical analyses were performed by the Miura Institute of Environmental Science.

A matrix of the 7 trace elements studied, which is the logarithmic conversion of Mn, Fe, Ni, Cu, Zn, Cd and Hg concentrations from 99 krill samples was statistically analyzed for intercorrelations by cluster analysis. The differences between sexes and areas were assessed by Mann-Whitney U test and the relationships between concentrations of trace elements and age were assessed by Spearman rank correlation (Zar, 1999). To analyze factors for trace element levels in Antarctic minke whales, we used the stepwise multiple regression analysis. A single sample was found to contain high concentrations of Fe (420 ppm) which was about 3 times higher than the second highest concentration in the sample population of 100. This value qualified as an outlier using the Smirnov test, and was omitted in the statistical analysis. These statistical analyses were executed by SPSS ver.11 for Windows (SPSS Co. Ltd.).

## Results and Discussion

Table 2 shows the concentrations of trace elements in Antarctic krill contained in the first stomach of Antarctic minke whales from the Antarctic Areas III, IV, V and VI during 1989/90 and 1998/99 seasons. Mn, Fe, Cu, Zn, Cd and Hg were detected in almost all the krill samples. Ni was also detected, except for two samples. Pb concentrations in all the krill samples were almost less than the lower level of determination limit.

### *In vivo interactions*

The 693 data in the 99 (krill) × 7 (elements: Mn, Fe, Ni, Cu, Zn, Cd and Hg) data set were statistically examined for elemental interaction by cluster analysis. Pb was excluded from this analysis because almost levels were below the determination limit. Figure 1 represents the dendrogram of a significant amount of cluster formation. Major correlation among Cd, Ni, Mn and Zn was observed, while Fe, Cu and Hg were not correlated with the other elements.

The appearance of a Cd-Ni-Mn-Zn correlation is of interest. From the standpoint of distribution in the ocean, trace elements are categorized into 3 types, i.e., recycle type (Cd, Ni, Zn) which is low in sea-surface and higher in deep-sea, scavenge type (Mn, Hg) which is higher in sea-surface and decreases with the depth, and mixed type (Fe, Cu) (Broecker, 1974). Also, the chemical properties of Mn and Zn are similar within the body, because their ligand field stabilization energy is the same (Lippard and Berg, 1994). This correlation might be attributable to the similarities of their chemical properties.

### *Body length effect*

The life span of *E. superba* is in the range of 4 to 6 years; spawning generally starts at age 2 years, and its maximum body length is over 60 mm (Siegel, 2000). Ichii and Kato (1991) reported that two size modes of Antarctic krill (25-28 mm in body length (1 years) and 41-48 mm (3-4 years)) are mainly contained in stomach contents of Antarctic minke whales. To examine the relationships between the body length and trace element levels in Antarctic krill, all individuals were classified into three body length groups (S < 40 mm, 40mm < M < 50 mm, L > 50 mm).

Levels of Mn, Fe, Cu, Zn and Cd in each body length group in Antarctic krill were significantly difference tested with Kruskal-Wallis test (Table 3). Figure 2 represents trace element levels in each body length group and significant differences among the groups tested with Scheffe's test were found: 1) Mn, Fe, and Zn levels of L (>50 mm) size were higher than those of M (40-50 mm) size, 2) Cd level of L (>50 mm) size was higher than

that of S (<40 mm) size, 3) Cu levels of M (40-50 mm) and L (>50 mm) sizes were higher than those of S (<40 mm) size.

Yamamoto *et al.* (1987) reported that significant correlation between Cu, Zn, Fe, Mn, Ni, Cd, Pb, Co and Hg levels in *E. superba* and the body length were not observed. Locarnini and Presley (1995) reported a negative correlation between Hg levels in *E. superba* and the body length. These results were not consistent with our data. It is suggested that trace element levels in Antarctic krill may be affected by other life history factors as well as the body length.

#### *Seasonal changes*

Figure 3 represents plots of trace element levels in Antarctic krill versus sampling day. Mn, Zn and Hg levels decreased with elapsed days. Cd levels in Antarctic krill increased from late November to middle January and decreased from middle January to late March in Areas III, IV, V and VI. Seasonal change of Cd is consistent with the study of Antarctic krill during 1984/85 and 1985/86 in Area V (Yamamoto *et al.*, 1987).

Yamamoto *et al.* (1987) suggested that this seasonal change would be caused by changes of water contents, however concentrations of krill are given on dry weight basis in their study. Antarctic krill migrate from ice-edge to offshore during January and February through Antarctic intermediate water (Ichii and Kato, 1991). This water has higher Cd levels, because this is a scavenge element (Lass *et al.*, 2001).

#### *Spatial and temporal differences:*

Table 4 shows significant differences in trace element levels in Antarctic krill between Areas III-IV and V-VI. Only Mn levels of krill in Area III-IV were higher than those of Area V-VI ( $p < 0.01$ ). Temporal differences of trace element levels in Antarctic krill between first and last researches in Areas III-IV (1989/90 and 1997/98) and V-VI (1990/91 and 1998/99) were tested with Mann-Whitney U test (Table 5). Mn, Ni and Zn levels in Antarctic krill significantly decreased in Area III-IV and Cu levels in Antarctic krill significantly increased in Area V-VI. The results were not consistent between these areas and these elements are essential elements. The results indicate that these trends may be affected by physical processes rather than temporal changes of trace elements. Furthermore, temporal trends of the toxic elements Cd and Hg were not observed in Antarctic krill.

#### *Comparison of trace element levels in krill around world*

Table 6 shows trace element levels in krill from other ocean areas as previously mentioned for comparison with our data. Mn, Fe, Zn and Cd levels of *E. superba* in this study were twice higher than those of *E. superba* during 1984 and 1986 (Yamamoto *et al.*, 1988), while Cu and Hg levels in this study were similar to other areas. Mn, Ni and Cd levels of *E. superba* in this study were higher than those of *E. superba* in the western Antarctic Peninsula (Locarnini and Presley, 1995), while Cu level in this study was lower than that in the western Antarctic. Ni, Cu and Cd levels in Antarctic krill were higher than those concentrations of krill in the North Pacific, however those of Mn, Fe, Zn and Hg were lower. In accumulation features of *E. superba* in the Antarctica, their Cd levels were approximately 1 order of magnitude higher than krill from the Northeast Pacific, the Japan Sea and the Northeast Atlantic, while their Hg levels were 1/2 lower than those from the Northwest Pacific.

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Table 1 Sample list of Antarctic minke and common minke whales

Species ( <i>Academic name</i> )	Year	Area	<i>n</i> Sex	Body length (m) Ave. (min.-max.)
Antarctic minke whale ( <i>Balaenoptera bonaerensis</i> )	1989/90	Antarctic Area IV	9 males	7.69 (5.50-8.80)
			1 female	9.70
	1990/91	Antarctic Area V	3 males	8.57 (8.30-8.90)
			7 females	8.80 (8.30-9.10)
	1991/92	Antarctic Area IV	7 males	8.55 (7.94-9.18)
			3 females	9.00 (8.66-8.27)
	1992/93	Antarctic Area V	6 males	8.61 (8.30-8.88)
			4 females	8.70 (8.68-8.72)
	1993/94	Antarctic Area IV	4 males	8.17 (7.87-8.29)
			6 females	9.01 (8.83-9.24)
	1994/95	Antarctic Area V	7 males	8.45 (7.99-9.03)
			3 females	8.73 (8.28-9.10)
1995/96	Antarctic Area III	10 males	8.09 (6.28-9.00)	
1996/97	Antarctic Areas V & VI	8 males	8.11 (7.30-8.74)	
		2 female	7.79(7.01-8.56)	
1997/98	Antarctic Areas III & IV	6 males	7.99 (5.69-9.12)	
		4 females	9.08 (8.57-9.68)	
1998/99	Antarctic Area V	7 males	8.58 (7.59-9.01)	
		3 females	9.25 (9.11-9.35)	
Common minke whale ( <i>Balaenoptera acutorostrata</i> )	1994-1995	NW Pacific (Off shore)	4 males	6.75(4.54-8.09)
	1996	NW Pacific (Coastal)	5 males	6.20(4.66-7.73)

Table 2 Trace element concentrations ( $\mu\text{g/g}$  dry wt) of *Euphausia superba* from Antarctic and *E. pacifica* from western North Pacific

Species	Area	year	n	Mn	Fe	Ni	Cu	Zn	Cd	Hg	Pb	
<i>E. superba</i>	Area-III, IV	1989/90	10	3.5 ± 0.7 ( 2.5 - 4.6 )	28 ± 27 ( 11 - 100 )	1.5 ± 0.5 ( 0.8 - 2.3 )	47 ± 23 ( 16 - 85 )	54 ± 5 ( 44 - 60 )	3.2 ± 2.1 ( 1.4 - 8.3 )	0.029 ± 0.011 ( 0.018 - 0.051 )	<2.1 ( - )	
		1991/92	10	3.2 ± 0.7 ( 1.9 - 4.3 )	72 ± 130 ( 4.9 - 420 )	1.3 ± 0.4 ( 0.9 - 2.2 )	43 ± 13 ( 25 - 58 )	49 ± 8 ( 38 - 58 )	3.0 ± 1.7 ( 0.42 - 5.5 )	0.020 ± 0.007 ( 0.01 - 0.031 )	<2.1 ( - )	
		1993/94	10	2.8 ± 0.8 ( 1.9 - 4.5 )	21 ± 24 ( 7.6 - 89 )	1.4 ± 0.3 ( 1.0 - 2.1 )	51 ± 16 ( 26 - 77 )	47 ± 7 ( 36 - 56 )	1.6 ± 0.7 ( 0.51 - 2.7 )	0.025 ± 0.010 ( 0.014 - 0.048 )	<2.1 ( - )	
		1995/96	10	2.8 ± 0.3 ( 2.5 - 3.3 )	11 ± 3 ( 8.5 - 18 )	0.7 ± 0.1 ( 0.6 - 0.9 )	50 ± 23 ( 20 - 76 )	46 ± 3 ( 39 - 50 )	0.62 ± 0.4 ( 0.32 - 1.6 )	0.028 ± 0.005 ( 0.021 - 0.037 )	<2.1 ( - )	
		1997/98	10	2.8 ± 0.3 ( 2.4 - 3.3 )	18 ± 8 ( 9.0 - 31 )	1 ± 0.2 ( 0.6 - 1.3 )	60 ± 13 ( 46 - 82 )	48 ± 7 ( 40 - 65 )	1.7 ± 1.2 ( 0.71 - 4.4 )	0.037 ± 0.009 ( 0.026 - 0.054 )	<2.1 ( - )	
	Area-V, VI	1990/91	10	2.5 ± 0.7 ( 1.3 - 3.4 )	25 ± 22 ( 11 - 73 )	1.4 (n=9) ( <0.5 - 2.4 )	39 ± 22 ( 1.6 - 63 )	48 ± 10 ( 40 - 74 )	2.5 ± 2.3 ( 0.20 - 7.1 )	0.037 ± 0.016 ( 0.018 - 0.066 )	<2.1 ( - )	
		1992/93	10	2.8 ± 0.4 ( 2.1 - 3.7 )	31 ± 25 ( 9.6 - 79 )	1.0 ± 0.3 ( 0.7 - 1.5 )	42 ± 12 ( 23 - 55 )	44 ± 4 ( 38 - 52 )	2.2 ± 2.3 ( 0.47 - 7.5 )	0.029 ± 0.008 ( 0.02 - 0.043 )	<2.1 ( - )	
		1994/95	10	2.2 ± 0.4 ( 1.5 - 2.9 )	26 ± 29 ( 5.2 - 100 )	0.9 (n=9) ( <0.5 - 1.4 )	35 ± 21 ( 12 - 86 )	42 ± 7 ( 28 - 49 )	1.1 ± 0.9 ( 0.15 - 2.6 )	0.027 ± 0.006 ( 0.018 - 0.041 )	<2.1 ( - )	
		1996/97	10	2.5 ± 0.4 ( 1.8 - 2.9 )	17 ± 17 ( 5.5 - 64 )	1.3 ± 0.4 ( 0.7 - 2.0 )	42 ± 24 ( 22 - 83 )	45 ± 7 ( 34 - 55 )	1.6 ± 1.2 ( 0.30 - 4.2 )	0.026 ± 0.008 ( 0.017 - 0.045 )	<2.1 ( - )	
		1998/99	10	2.8 ± 0.5 ( 2.4 - 3.7 )	17 ± 11 ( 7.9 - 43 )	1.4 ± 0.5 ( 0.8 - 2.3 )	66 ± 22 ( 20 - 98 )	45 ± 6 ( 37 - 54 )	2.1 ± 1.2 ( 0.78 - 4.3 )	0.025 ± 0.011 ( 0.014 - 0.046 )	<2.1 ( - )	
	total	1989-1999	100	2.8 ± 0.6 ( 1.3 - 4.6 )	26 ± 46 ( 4.9 - 420 )	1.2(n=98) ( <0.5 - 2.4 )	47 ± 21 ( 1.6 - 98 )	47 ± 7 ( 28 - 74 )	2.0 ± 1.6 ( 0.15 - 8.3 )	0.028 ± 0.010 ( 0.010 - 0.066 )	<2.1 ( - )	
	<i>E. pacifica</i>	Off shore	1994-1995	4	3.5 ± 1.7 ( 2.2 - 6.0 )	72 ± 88 ( 9.3 - 200 )	<0.5 ( - )	22 ± 9 ( 12 - 32 )	66 ± 13 ( 49 - 79 )	0.41 ± 0.2 ( 0.26 - 0.8 )	0.047 ± 0.02 ( 0.024 - 0.060 )	3.3(n=1) ( <2.1 - 3 )
		Coastal	1996	5	4.2 ± 0.8 ( 3.0 - 4.9 )	24 ± 11 ( 8.6 - 34 )	0.8(n=1) ( <0.5 - 0.8 )	34 ± 13 ( 18 - 51 )	92 ± 19 ( 71 - 120 )	0.74 ± 0.2 ( 0.41 - 0.9 )	0.042 ± 0.016 ( 0.028 - 0.070 )	<2.1 ( - )
		total	1994-1996	9	3.9 ± 1.3 ( 2.2 - 6.0 )	46 ± 60 ( 8.6 - 200 )	0.8(n=1) ( <0.5 - 0.8 )	29 ± 12 ( 12 - 51 )	81 ± 21 ( 49 - 120 )	0.60 ± 0.3 ( 0.26 - 0.9 )	0.044 ± 0.015 ( 0.025 - 0.070 )	3.3(n=1) ( <2.1 - 3 )

Table 3 Trace element concentrations ( $\mu\text{g/g}$  dry wt) in each body size group of *Euphausia superba* from Antarctic

Body Length	<i>n</i>	Mn	Fe	Ni	Cu	Zn	Cd	Hg
S <40mm	25	2.7	34	1.1	31	45	1.2	0.029
		(1.9-4.3)	(5.2-420)	(0.6-2.4)	(16-61)	(37-58)	(0.15-4.6)	(0.016-0.066)
M 40-50mm	12	2.5	14	1.3	60	42	1.8	0.027
		(1.8-2.8)	(5.5-64)	(0.7-2.0)	(25-98)	(34-47)	(0.78-3.1)	(0.017-0.043)
L >50mm	47	3.0	29	1.3	54	48	2.5	0.026
		(1.5-4.6)	(4.9-140)	(0.3-2.4)	(12-86)	(28-60)	(0.21-8.3)	(0.010-0.054)
Probability in Kruskal-Wallis test		*	*		***	**	**	

Table 4 Significant spatial difference of trace element concentrations of Antarctic krill in Areas III, IV and V, VI during 1988 and 1998 using Mann-Whitney U test ( $p < 0.05$ )

	Mn	Fe	Ni	Cu	Zn	Cd	Hg
Significance ( $p$ )	0.002						
	III,IV > V, VI						

Table 5 Significant temporal difference of trace element concentrations of Antarctic krill in Areas III, IV between '89/90 and '97/98, and V, VI between '90/91 and '98/99 using Mann-Whitney U test ( $p < 0.05$ )

	Mn	Fe	Ni	Cu	Zn	Cd	Hg
Area-III,IV	**		**		*		
	'89/90 > '97/98		'89/90 > '97/98		'89/90 > '97/98		
Area-V,VI				*			
				'90/91 < '98/99			

\*: $p < 0.05$ , \*\*:  $p < 0.001$



Table 6 Trace element concentrations ( $\mu\text{g/g}$  dry wt) of *Euphausia superba* from Antarctic and *Euphausia pacifica* from western North Pacific

Species	Area	year	n	Mn	Fe	Ni	Cu	Zn	Cd	Hg	Pb	Ref.
<i>Euphausia superba</i>	Antarctic Ocean	1989-99	100	2.8	26	1.2	47	47	2.0	0.028	<2.1	1)
	W Antarctic Peninsula			(1.3-4.6)	(4.9-420)	(<0.5-2.4)	(1.6-98)	(28-74)	(0.15-8.3)	(0.01-0.066)		
<i>Euphausia superba</i>	Antarctic Ocean	1984-86	76	1.8	9.2	1.2	33	25	1.1	0.021	0.10	2)
	Area -			(0.87-3.33)	(3.3-25)	(0.23-4.6)	(4.1-80)	(13-38)	(0.10-4.4)	(0.01-0.059)	(0.026-0.26)	
<i>Euphausia superba</i>	Antarctic Ocean	1993	70	1.98	28	0.54	80.5	43.5	0.29	0.0245	0.22	3)
	Area -			(0.03-3.94)	(8.5-108)	(0.10-1.5)	(37.8-140)	(35.2-51.3)	(0.13-0.75)	(0.013-0.049)	(0.15-1.8)	
<i>Euphausia pacifica</i>	NW Pacific	1994-96	9	3.9	46	0.8	29	81	0.60	0.044		1)
				(2.2-6.0)	(8.6-200)	(<0.5-0.8)	(12-51)	(49-120)	(0.26-0.88)	(0.025-0.07)		
<i>Thysanoessa longipes</i>	Sea of Japan	1984		1.8	110	NA	NA	175	NA	NA	NA	4)
<i>Thysanopoda microphthalma</i>	NE Atlantic	1985	4	1.7	24.3	0.57	48.6	56.8	0.3			5)
<i>Meganctiphanes norvegica</i>	NE Atlantic	1985	5	2.9	25.6	0.80	71.9	96.5	0.39			5)
<i>Nematoscelis megalops</i>	NE Atlantic	1985	7	2.4	26.3	0.91	42.0	44.0	0.2			5)
<i>Nematobrachion boopis</i>	NE Atlantic	1985	5	1.9	NA	0.76	44.9	47.7	0.5			5)
<i>Meganctiphanes norvegica</i>	NE Atlantic		8					104	0.25		0.26	6)

Cited from: 1) this study; 2) Yamamoto *et al.*, 1988; 3) Locarnini and Presley, 1995; 4) Masuzawa *et al.*, 1988; 5) Ridout *et al.*, 1989; 6) Leatherland *et al.*, 1973.

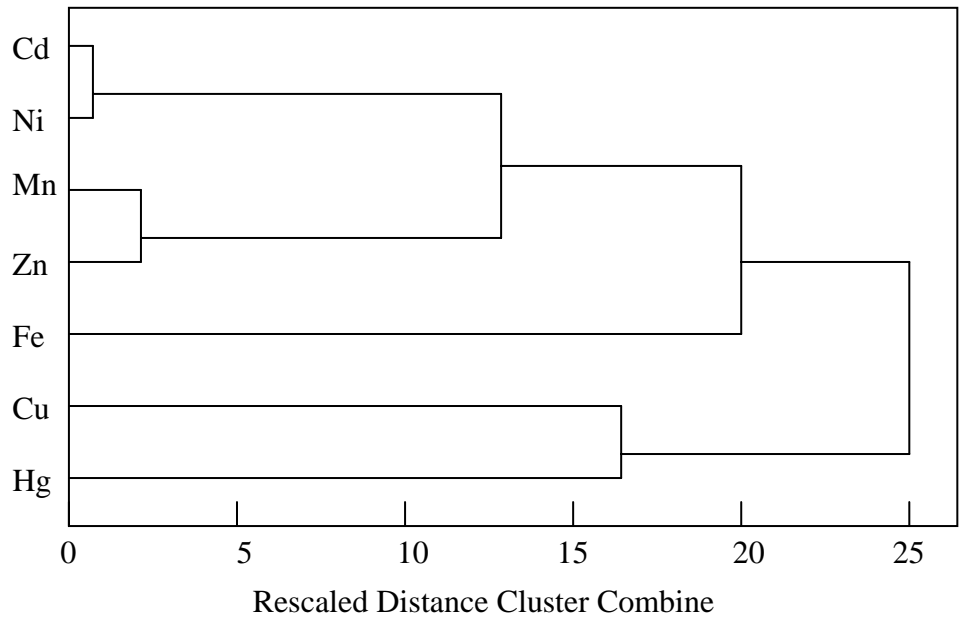


Fig. 1 Hierarchical Cluster analysis of trace element concentrations of Antarctic krill. Dendrogram using average linkage between groups

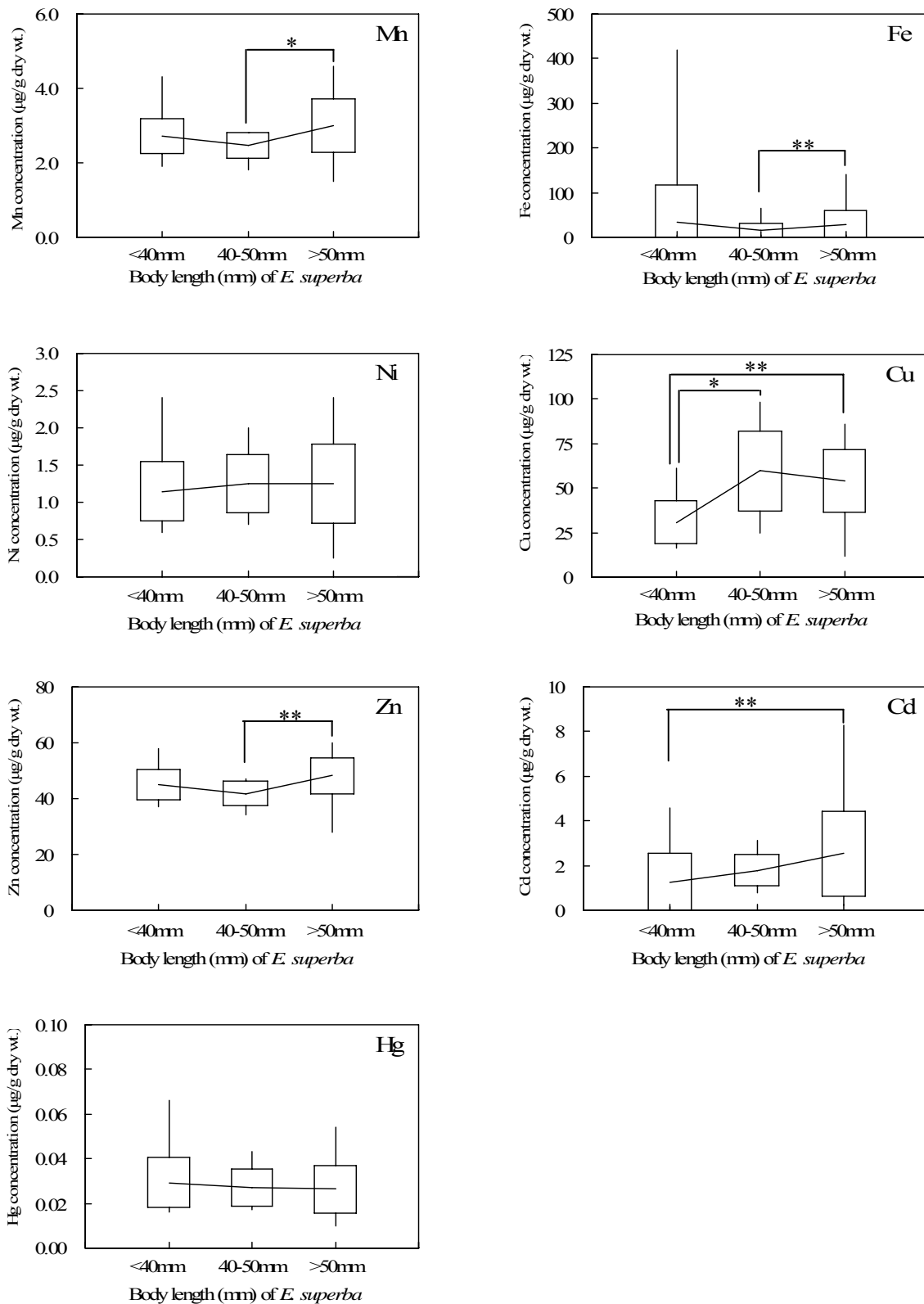


Figure 2 Comparison of trace element concentrations (ppm dry wt.) in three body size groups of *E. superba*

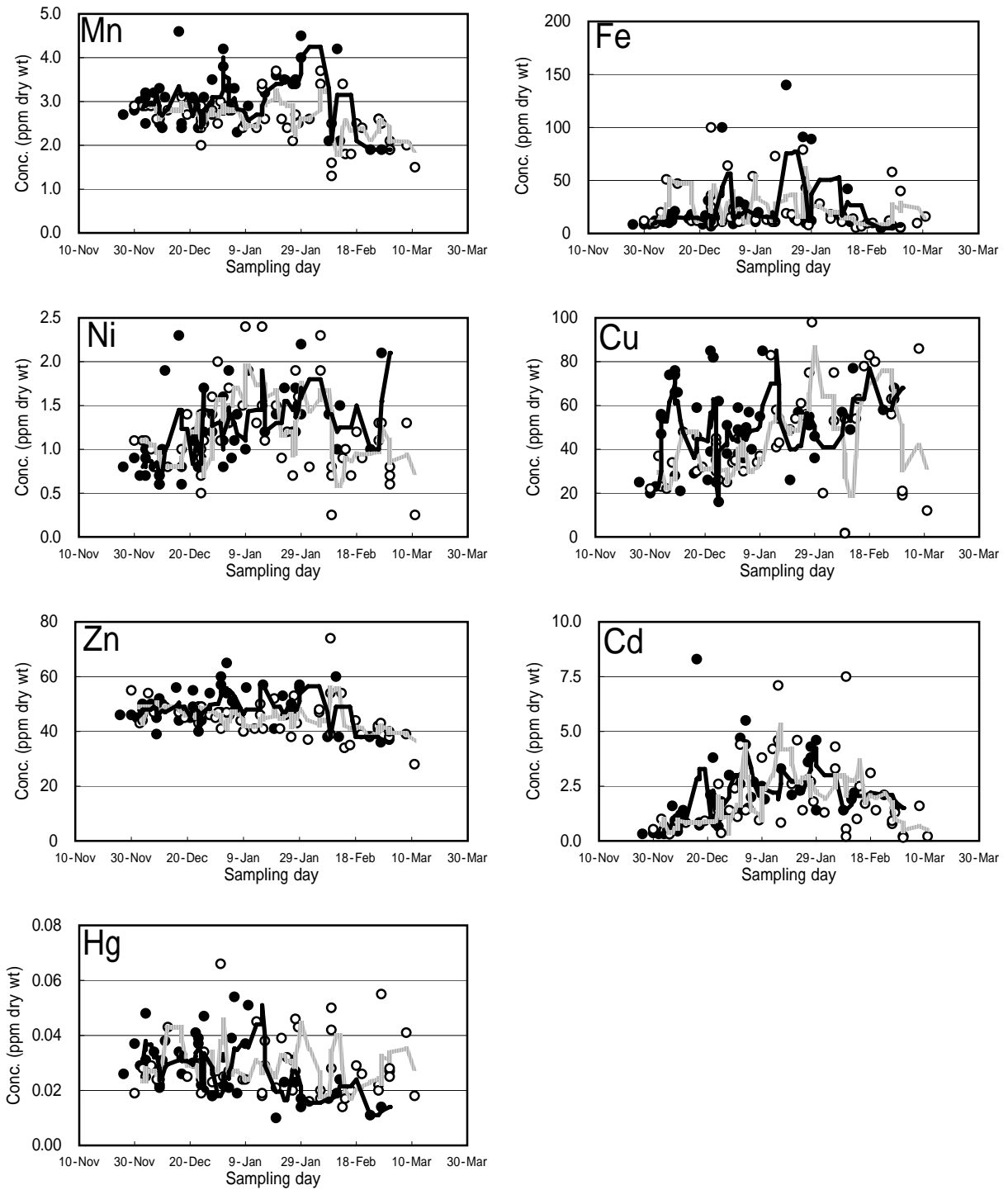


Fig. 3 Seasonal change of trace element concentrations in Antarctic krill  
 : Area III and IV, : Area V and VI