Proc. NIPR Symp. Polar Biol., 3, 57-63, 1990

SEX- AND MATURITY-RELATED HEAVY METAL ACCUMULATIONS IN THE ANTARCTIC KRILL *EUPHAUSIA SUPERBA*

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Abstract: Concentrations of heavy metals and their chemical forms were analyzed in the Antarctic krill (*Euphausia superba*) collected from the Scotia Sea in December, 1987, and the results were discussed in relation to sex and maturity stages. The whole body metal concentrations were in the order of Zn≈Cu>Fe>Mn>Ni>Cd. The values of Fe, Mn and Zn were higher in females than in males. The values of Cu, Fe, Ni and Cd in adult females were highest in stage IIIA. Also, the values for Cu was higher in juveniles than in adults.

Concentrations of the metals were generally higher in cephalothorax than in abdomen. In particular, a majority of Cu burden in the cephalothorax existed as Cu-binding proteins, which were likely to be mainly hemocyanin and metallothionein. We speculate that changes in amounts of Cu-binding proteins by physiological conditions might be important causes for the variations of Cu concentration between sexes and between maturity stages of the Antarctic krill.

1. Introduction

Heavy metal accumulations in marine zooplankton have been reported by several workers (HENNING *et al.*, 1985; MARTIN and KNAUER, 1973; RIDOUT *et al.*, 1985). However, information on variations of heavy metal accumulations related to the environmental and physiological conditions of zooplankton is very scarce.

Among marine zooplankters, the Antarctic krill is an abundant and widely distributed member of the Antarctic marine biota (MARR, 1962; MAKAROV *et al.*, 1970), and also a staple food source for higher trophic animals such as whales, seals, birds, squids and fishes (LAWS, 1977; KNOX, 1970; BENGTSON, 1985). Thus detailed information about heavy metal accumulation in the krill is essential for understanding the bioaccumulation processes of heavy metals through food web in the Antarctic Ocean.

We have already reported spatial and temporal variations of metal concentrations

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in the Antarctic krill (YAMAMOTO *et al.*, 1987). However, details about variations of heavy metal concentrations related to sex and maturity stages in the Antarctic krill have not yet been elucidated. This report deals with the heavy metal (Zn, Cu, Fe, Mn, Ni, and Cd) accumulations in male and female Antarctic krill at various maturity stages.

2. Materials and Methods

2.1. *Antarctic krill*

Krill samples were collected by a rectangular trawl net (3×3 m, 3.4 mm mesh aperture, named KYMT net) in the southwestern Scotia Sea on December 9, 22, and 23, 1987 during the 5th Antarctic cruise of R.V. KAIYO MARU (Table 1). The stages of sexual maturity of the krill were determined based on the system of MAKAROV and DENYS (1980). All samples were stored in polyethylene bags and frozen at below −40°C until analysis.

Table 1. Sampling data of the Antarctic krill during the 5th Antarctic survey cruise of R.V. KAIYO MARU in 1987–1988.

Sex and maturity stage*	Location	Date
I. Juvenile	60°59.9′S, 50°00.2′W	Dec. 23, 1987
II. Sub-adult		
Male IIA	59°59.9′S, 55°00.1′W	Dec. 19, 1987
Female IIB	59°59.9′S, 55°00.1′W	Dec. 19, 1987
III. Adult		
Male IIIB	59°59.9′S, 55°00.1′W	Dec. 19, 1987
Female IIIA	59°59.9′S, 55°00.1′W	Dec. 19, 1987
IIIB, C	59°59.9′S, 55°00.1′W	Dec. 19, 1987
IIID	59°00.1′S, 50°00.1′W	Dec. 22, 1987
Mixed sample	59°59.9′S, 50°00.2′W	Dec. 23, 1987

* MAKAROV and DENYS (1980).

2.2. *Metal analysis*

Three to fourteen individuals for each maturity stage of the krill were pooled, dried for 12h at 80°C and then their moisture contents were determined. Dried samples of 0.5 g each were digested to a transparent solution in a mixture of nitric and perchloric acid. The resultant solutions were diluted to 50 m*l* with deionized water and transferred to acid-washed test tubes with Teflon screw-caps. The concentrations of Zn, Cu, Fe, and Mn were directly measured by atomic absorption spectrophotometry. Ni and Cd were measured after methyl isobutyl ketone-diethyl dithiocarbamate treatment (HONDA *et al.*, 1982). The accuracy of this method has been determined in this laboratory by spiking bones and other tissues of dolphin with known amounts of Zn, Cu, Fe, Mn, Ni and Cd at recovery rates of 90.0 to 99.9%.

2.3. *Metal-binding protein*

Krill samples used for this analysis were not sorted into male and female. Individual krill was cut into cephalothorax and abdomen, and the pooled cephalothoraxes

(4 g wet weight) were homogenized with twice volume of 0.1 M Tris-HCl buffer, pH 7.4; and centrifuged at 105000 g for 60 min. The supernatant fraction was applied to a column (2.6 × 80 cm) of Sephadex G-75 and eluted with Tris-HCl buffer (pH 8.6, 0.01 M) at a flow rate of 40 ml/h. Absorbances at 254 nm and 280 nm and concentrations of the metals were measured in all of the eluted column fractions.

3. Results

3.1. *Metal concentrations in the Antarctic krill*

Metal concentrations in each sexual maturity stage of the krill are given in Table 2. The metal concentrations were in the order of Zn $\approx$ Cu > Fe > Mn > Ni > Cd. The concentrations of Zn, Fe and Mn were significantly higher in females than in males ($p<0.05$, Wilcoxon-Mann-Whitney test).

Table 2. The metal concentrations (μg/dry g) and water content (%) in the Antarctic krill.

Stage *	Zn	Cu	Fe	Mn	Ni	Cd	Water (%)
Juvenile	66.6	71.9	32.2	3.91	1.68	0.51	82.1
Male IIA	62.3	47.2	27.5	4.20	1.70	0.40	80.5
Male IIIB	52.9	62.2	29.0	3.00	1.24	0.54	80.9
Female IIB	70.7	37.7	34.3	4.83	1.24	0.53	82.1
Femlae IIIA	69.4	59.5	41.1	4.62	1.51	0.92	81.5
Female IIIB, C	66.5	47.4	33.1	4.50	1.16	0.55	81.0
Female IIID	72.0	45.4	30.4	4.79	0.87	0.41	76.4

* see Table 1.

There were also some differences in metal concentrations between sexual maturity stages of the female krill. The values of Cu were higher in juveniles than in adults. In adult females, the values of Cu, Fe, Ni and Cd were highest in the maturity stage IIIA, with no spermatophores attached to the thelycum, followed by stages IIIB, C and IIID.

3.2. *Metal-binding proteins*

Distributions of the heavy metal concentrations in the krill's tissues are given in Table 3. The concentrations, irrespective of the size of krill, were higher in the ceph-

Table 3. Concentrations (μg/wet g) of the heavy metals in the cephalothorax (A) and abdomen (B) of small-size (<4 cm) and large-size (≧4 cm) Antarctic krill.

	Small size		Large size	
	A	B	A	B
Zn	85.4	58.6	108	54.2
Cu	73.9	23.5	83.7	41.0
Fe	76.9	29.6	86.9	27.8
Mn	7.20	2.84	7.23	2.95
Ni	1.40	1.19	2.00	0.65
Cd	0.88	0.26	0.91	0.33

alothorax than in the abdomen, and this was most pronounced for Cd. When the metal burdens for a whole animal were calculated from the weights of tissues and their metal concentrations, 60–80% of the whole body burdens existed in the cephalothorax. Among the metals examined, the subcellular distributions of Cu and Zn in the cephalothorax are shown in Fig. 1, which shows 60–75% of Cu and Zn burdens in the cephalothorax existed in the supernatant fraction.

Elution profile of the supernatant fraction on Sephadex G-75 (Fig. 2) indicates that there are at least three Cu-binding proteins, and their major components (peaks I and II) contain the same percentage, *i.e.* 40%, of Cu content in the supernatant fraction. Judging from the elution volume of proteins on Sephadex G-75 column, the molecular weights of peaks I and II were estimated to be more than 35000 and about 10000 dalton, respectively. In contrast, molecular weights of Zn-binding proteins, peaks I and III, were more than 35000 and less than 7000 dalton, respectively. Absorbances of 254 and

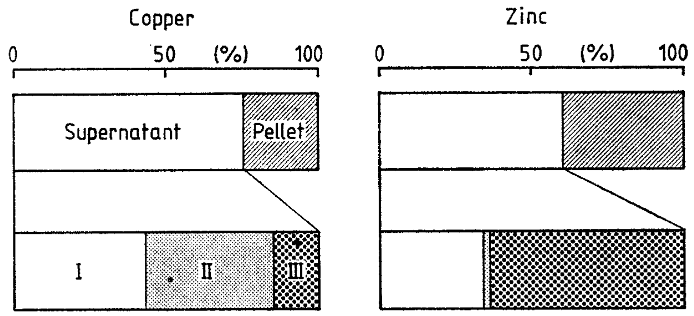

Fig. 1. Distributions of copper and zinc in the cephalothorax of the Antarctic krill. Upper graph shows the subcellular distribution of the metals in the cephalothorax which was given by centrifugation at 105000 g × 60 min. Lower graph shows the distribution of the metals in the supernatant fraction (I: MW>35000 dalton, II: 7000 dalton<MW<35000 dalton, III: MW<7000 dalton).

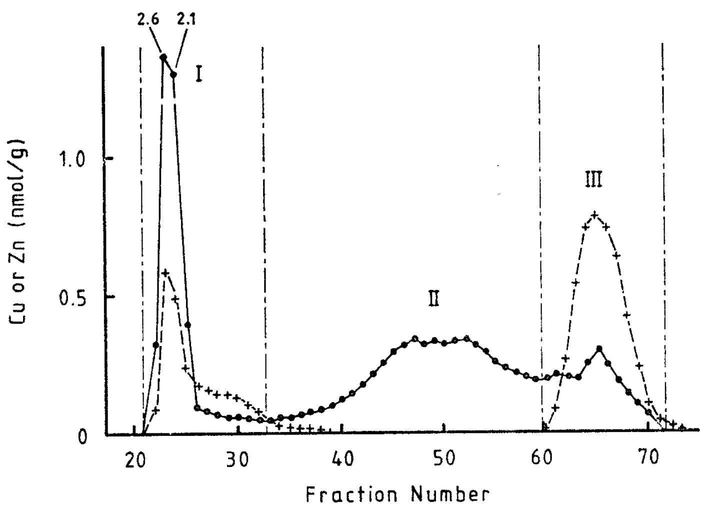

Fig. 2. Elution profiles of supernatant fraction of the Antarctic krill cephalothorax on Sephadex G-75 column.
●——● Cu, +---+ Zn.

280 nm showed the same pattern with maximum absorptions in fraction numbers 23 and 31.

4. Discussion

The concentration levels of Zn, Cu, Fe, Mn, Ni and Cd in the Antarctic krill were 52.9–72.0, 37.7–71.9, 27.5–41.1, 3.00–4.83, 0.87–1.70 and 0.40–0.92 μg/g wet weight respectively. In some cases, metal concentrations in the krill were variable in relation to the sex, growth, and maturity stage of the adult females, but their variations appeared to be relatively small when compared with the ranges of the seasonal and geographical variations in metal concentrations as reported by YAMAMOTO *et al.* (1987). The values of Cu, Ni and Cd in this study were similar to the results previously reported for the same species from the Scotia Sea (STOEPPLER and BRANDT, 1979).

Although the effect of maturity stage-related metal concentrations was not very clear, Cu was consistently higher in juveniles than in adults. Cu is an essential metal for crustaceans, and its concentration is not affected by the metal concentrations in ambient water: according to WHITE and RAINBOW (1982), the whole body concentration of Cu in a decapod crustacean *Palaemon elegans* remained constant even when they were exposed to various dissolved metal concentrations up to 100 μg/*l*.

It is well known that large amounts of Cu in crustaceans exist in the hepatopancreas and this organ synthesizes the Cu-containing respiratory protein, hemocyanin (ENGEL and BROUWER, 1986; WHITE and RAINBOW, 1986; SENKBEIL and WRISTON, 1981). The level of hemocyanin seem to be affected by physiological conditions such as hypoxic stress, molting cycle and feeding conditions (DJANGMAH, 1970; HAGERMAN, 1983). Also, metallothionein, which is an important protein in crustaceans, may act as a Cu donar to apohemocyanin (BROUWER *et al.*, 1986) and its concentration in hepatopancreas varies during the molting cycle (ENGEL, 1987; ENGEL and BROUWER, 1987).

The two components, peaks I and II, on the elution profile of Sephadex G-75 column were composed of large amounts of Cu in the krill (Fig. 2). Judging from molecular weights of the Cu-binding proteins, peaks I and II were considerable to include hemocyanin and metallothionein, respectively. However, peak II was broader, compared to the elution pattern of metallothionein (about 6000 dalton) ever published for a crustacean (OVERNELL, 1986). Also, It is known that Cu-metallothionein absorbes strongly at 280 nm (HARTMANN and WESER, 1977), but peak II found in this study did not have a light absorption at 280 nm. These results might be the reason that Cu-metallothionein was subject to uncontrolled oxidation under examinations and leading to disulfide-bridged polymeric species. Indeed, OVERNELL (1982) reported that the polydisperse Cu-binding component found on Sephadex G-75 chromatograms of the soluble fraction in hepatopancreas of *Cancer pagurus* was unstable, and had only a small light absorption at 280 nm.

Although further studies are needed for chemical forms of Cu in the krill, it can be speculated that possible differences in physiological conditions as mentioned above might cause the variations of hemocyanin and metallothionein-like proteins, and result in the variations of Cu concentration between sexes and between maturity stages of the Antarctic krill.

Acknowledgments

We are grateful to the captain and crew of R.V. KAIYO MARU for their cooperation during sampling. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (Project No. 63790368) and the Nissan Science Foundation.

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(Received May 11, 1989; Revised manuscript received August 23, 1989)