Bull. Fac. Fish. Hokkaido Univ.
32(4), 376-387. 1981.

Effects of Formalin Preservation on Heavy Metal Concentration in Zooplankton*

Tsuneyasu HAMANAKA**

Abstract

Cadmium and zinc concentrations were determined for 3% formalin-preserved samples of euphausiids, copepods and amphipods collected in the North Pacific Ocean and the adjacent seas. The cadmium and zinc levels in formalin samples were higher (1–79%) than the frozen samples. The effects of preservation on metal concentration depend upon the storage time. For euphausiid preserved in formalin for 2 months, the weight loss due to fixation seemed to be the main contributing factor in increased metal levels. The preservation effect and contamination problem in zooplankton are briefly discussed.

Introduction

Recently, heavy metal concentrations have been studied for zooplankton as well as fishes and other marine biota because of their importance in the food web of marine ecosystem and in the cycling and transprot of elements in water column.[1)2)] Cadmium and zinc concentrations in single species of North Pacific zooplankton have previously been examined.[3)4)]

Most of the zooplankton samples are usually preserved in aqueous formalin solution in order to avoid deterioration of organisms. However, procedures using preservatives such as formalin is believed to be unsuitable for an analysis of biochemical and toxic elements, because of the lack of reliable information about the effects of fixation process on the concentation and about the contamination problem.

Some investigators have studied the effects of preservation on trace metals in fishes.[5)6)] No research efforts, however, have been paid to zooplankton, because of the difficulty in obtaining sufficient quantities of the samples.

The objective of this study is to elucidate the extent of the effect of formalin preservation on cadmium and zinc concentrations in planktonic organisms and to estimate the applicability of its preservation method for heavy metal concentration study. Zooplankton samples were taken from the northern North Pacific, the Bering Sea, the Okhotsk Sea, and off the Hokkaido coast during 1974–1978.

Materials and Methods

Sample collection and preliminary procedures

During the summers of 1974–1978, zooplankton were collected in the northern North Pacific, the Bering Sea, the Okhotsk Sea, and off the Hokkaido coast (Tables

* *Contribution No. 142 from the Research Institute of North Pacific Fisheries, Faculty of Fisheries, Hokkaido University.*

** *Research Institute of North Pacific Fisheries, Faculty of Fisheries, Hokkaido University, Hakodate, Hokkaido 041, Japan.*
(北海道大学水産学部附属北洋水産研究施設)

Table 1. *Details of sampling station of zooplankton.*

Group	Species	Sample No.	Station No.	Sea Area	Date	Position	
						Lat.	Lond.
Euphausiids	*E. pacifica*	1	Os 74006	North Pacific	6/13/74	51-20N	179-49E
		2	Os 74010	Hokkaido	9/ 9/74	41-48N	142-49E
		3a/3b	Os 75001	North Pacific	6/ 8/75	43-02N	158-52E
		9/9b	Os 76002	Hokkaido	9/ 3/76	42-39N	144-13E
		10	MOs 76002	Hokkaido	9/ 3/76	42-39N	144-13E
		11	Os 76003	Hokkaido	9/ 6/76	42-22N	143-46E
	Th. longipes	4	Os 75008	Bering Sea	6/15/75	52-30N	178-22W
		5	Os 75011	Bering Sea	6/19/75	52-54N	176-07W
		6	Os 75012	Bering Sea	6/22/75	53-26N	176-33W
		7a, b, c	Os 75013	Bering Sea	6/23/75	53-30N	176-42W
		8	Os 75016	Bering Sea	6/26/75	56-21N	174-48W
Copepods	*C. plumchrus*	1	Os 74006	North Pacific	6/13/74	51-20N	179-49E
		2a/2b	Os 75001	North Pacific	6/ 8/75	43-02N	158-52E
		3	Os 75002	North Pacific	6/ 9/75	44-41N	164-17E
		5	Os 75011	Bering Sea	6/19/75	52-54N	176-07W
		6	Os 75025	North Pacific	7/ 6/75	55-33N	156-15W
		7	MOs 75025	Bering Sea	6/26/75	56-01N	176-19W
		12	Oy 75024	Okhotsk Sea	9/ 8/75	52-00N	151-00E
		13	Oy 75025	Okhotsk Sea	9/ 7/75	52-00N	149-00E
		15	Oy 75020	Okhotsk Sea	9/ 7/75	52-08N	149-00E
		16	Oy 74017	Okhotsk Sea	9/ 5/74	52-00N	153-00E
		17	Oy 74023	Okhotsk Sea	9/ 9/74	52-00N	145-00E
	C. cristatus	4	Os 75004	North Pacific	6/11/75	47-33N	174-38E
		9	MOs 75038	Bering Sea	6/30/75	54-40N	172-21W
	C. glacialis	10	Oy 75021	Okhotsk Sea	8/20/75	58-30N	152-30E
		11	Oy 75022	Okhotsk Sea	8/21/75	58-30N	150-30E
		14	Oy 75003	Okhotsk Sea	8/19/75	57-30N	152-30E
	E. bungii b.	8	MOs 75038	Bering Sea	6/30/75	54-40N	172-21W
Amphipods	*P. pacifica*	1	Os 74006	North Pacific	6/13/74	51-20N	179-49E
		4	Os 75005	North Pacific	6/12/75	49-59N	178-49E
		5	Os 75008	Bering Sea	6/15/75	52-30N	178-22W
		6	Os 75013	Bering Sea	6/23/75	53-30N	176-42W
		10a/b	Os 75025	North Pacific	7/ 6/75	55-33N	156-15W
		11	Os 75027	North Pacific	7/13/75	53-10N	158-46W
	P. libellula	2a/b	Os 74019	Bering Sea	6/25/74	57-00N	175-30W
		3	Os 74032	Bering Sea	6/10/74	57-09N	163-29W
		9	Os 75023	Bering Sea	7/ 3/75	57-00N	165-00W
		14	Hb 74069	Bering Sea	7/ 3/74	59-18N	176-40E
		—	Os 75018	Bering Sea	6/28/75	58-32N	170-08W
		—	Os 78054	Bering Sea	6/29/78	57-45N	163-59W
	P. japonica	12	Os 76003	Hokkaido	9/ 6/76	42-22N	143-45E
		13	Oy 75022	Okhotsk Sea	8/21/75	58-30N	150-30E
		15	Oy 75018	Okhotsk Sea	8/30/75	56-15N	141-30E
		16	Oy 75020	Okhotsk Sea	9/ 7/75	52-08N	149-00E
		17	Oy 75021	Okhotsk Sea	8/31/75	55-00N	142-00E
		18	Oy 75028	Okhotsk Sea	9/13/75	48-00N	146-00E
	H. medusarum	7	Os 75015	Bering Sea	6/25/75	56-38N	179-45W

1, 6 and Fig. 1) by a larval net (130 cm in diameter, 450 cm in length, 3 mm aperture and 0.35 mm aperture mesh at codend) in the cruises of T/S *Oshoro Maru* of Hokkaido University and R/V *Habomai Maru No.* 21, and R/V *Oyashio Maru.*

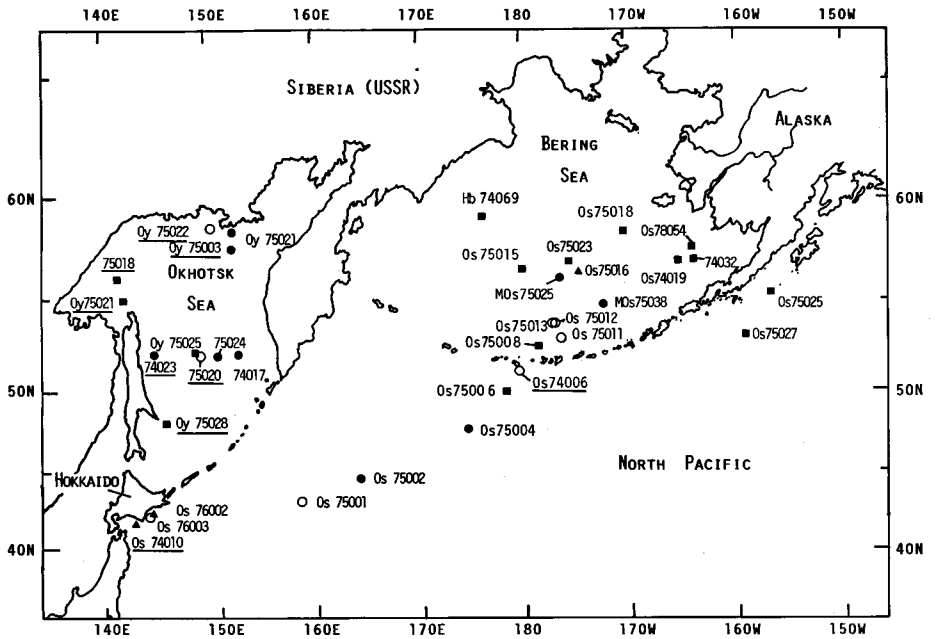

Fig. 1. Map showing the location where zooplankton were collected. Underline denotes formalin-preserved samples. (▲); Euphausiids, (●); Copepods, (■); Amphipods, (○); Euphausiids and/or copepods and amphipods. Figures denote the Station No.: Os; Larval net station of T/S *Oshoro Maru*, MOs; MTD net station of T/S *Oshoro Maru*, Hb; Larval net station of R/V *Habomai Maru* No. 21, Oy; Larval net sation of R/V *Oyashio Maru.*

A larval net was towed horizontally twice daily at one hour after sunset at 2 knot speed.

After net sampling, the collected zooplankton were immediately sorted, on board, into taxonomic groups with a plastic spatula and a glass tick, and only predominant species were rinsed quickly on the 0.35 mm mesh strainer with deionized water in order to remove salt from the animal surface. All experimental instruments were washed with diluted acid and deionized water after every procedure. Then the samples were stored in a plastic box at −20°C. Of the collected samples, only a large quantity of euphausiid samples (Station No. Os 75001; Os 75013, see Table 2) were divided into two subsamples; frozen and formalin-preserved (in 3% formalin for two months) samples. Two samples of copepod (Station No. Os 75011) and amphipod (No. Os 75025) were sorted into the species after fixation in 3% formalin for 1–3 hours. Large-sized amphipod, *Parathemisto libellula* were grouped to several subsamples by the body size (No. Os 74019, Os 74032, Hb 74069, Os 75018, Os 75023 and Os 7854A). A small part of each sample

was preserved in 5% formalin in order for further identification of species in the laboratory.

In the laboratory, the frozen and formalin-preserved samples were weighed after removing other species or contaminants such as paints, rust chip particles, etc., by a plastic coated pincette and by passing a Teflon-coated magnet over the sample on a filter paper.[2)4)]. They were then dried in an oven at 58–60°C for at least 48 hours, and stored in a plastic box until analysis.

Analytical procedures

Sample weighing 0.1–5.0 g were left to pre-digest overnight at room temperature and subsequently digested on a hot plate in a mixture of nitric- and sulfuric acid. For zinc assay, the digests were brought to a known volume with deionized water. To determine the cadmium concentration, samples were chelated with sodium diethyldithiocarbamate (DDTC), extracted with methyl isobutylketone (MIBK), then the extract was gradually evaporated on a hot plate and diluted to 10 ml with nitric acid (1:20 v/v). Zinc and cadmium concentrations were measured on a Hitachi flame atomic absorption spectrophotometer.[7)] To confirm the accuracy of the present analytical method, standard materials prepared by the U.S. National Bureau of Standards and the Japanese National Institute of Environmental Studies were simultaneously analyzed. Our data were reasonably close to both cerified values.[3)]

We analyzed two different commercial brands of formaldehyde (37%) according to the method of Gibbs et al. (1974). Undiluted formaldehyde, after first being evaporated to dryness in a water bath at 60°C, was then redissolved in a nitric acid and the heavy metal concentrations were determined.

Results

The heavy metal concentrations in euphausiids, copepods, and amphipods (*Parathemisto pacifica*, *P. japonica* and *Hyperia medusarum*) of the formalin-preserved sample and the frozen sample[4)] are shown in Tables 2–4. The mean metal concentrations of zooplankton groups and *Parathemisto libellula* are presented in Tables 5 and 6, respectively.

The metal concentrations of formalin-preserved samples were higher than those in frozen samples with the exception of zinc in copepods. The mean cadmium contents in copepods and amphipods were 49% and 1% higher repsectively than the frozen samples. Zinc in formalin-preserved euphausiids and amphipods had 42% and 7.5% increased levels respectively as compared to the frozen samples. Similarly, the higher cadmium and zinc concentrations in formalin-preserved lantern fishes as compared to the frozen samples have been also observed by Gibbs et al. (1974). Further, euphausiids, *Euphausia pacifica* (Sample No. 3a; 3b) and *Thysanoessa longipes* (No. 7b; 7c) preserved in formalin for 2 months had 2.5 times and 1.3 times higher zinc concentrations respectively than the same sample preserved by the freezing method (Table 2). In contrast, copepod (*Calanus plumchrus*; No. 5) and amphipod (*Parathemisto pacifica*; No. 10b) which were sorted in 3% formalin for 1–3 hours after thawing gave normal levels for cadmium and zinc in duplicate analyses (Tables 3 and 4).

Table 2. *Euphausiids. Cadmium and zinc concentrations in euphausiids whih have been preserved in freezing method and in formalin solution.*

Preservation methods	Sample No.	Species	Station No.	Sea area	Fixation period[f]	Cd[b]			Zn[b]		
						Mean	SD	n	Mean	SD	n
Frozen sample[a]	3a	*Eupahusia pacifica*	Os 75001	North Pacific	—	0.79	0.11	2	96	1	2
	9	*E. pacifica*	Os 76002	Hokkaido	—	2.17	0.78	4	195	50	5
	9b	*E. pacifica*	Os 76002	Hokkaido	—	1.46	0.54	2	156	5	2
	10	*E. pacifica*	MOs 76002	Hokkaido	—	4.22[c]		1	298[c]		1
	11	*E. pacifica*	Os 76003	Hokkaido	—	1.54		1	95		1
	4	*Thysanoessa longipes*	Os 75008	Bering Sea	—	0.76	0.02	2	73	1	2
	5	*Th. longipes*	Os 75011	Bering Sea	—	0.36		1	83		1
	6	*Th. longipes*	Os 75012	Bering Sea	—	0.85		1	72		1
	7a	*Th. longipes*[d]	Os 75013	Bering Sea	—	0.70	0.09	2	59	1	2
	7b	*Th. longipes*[e]	Os 75013	Bering Sea	—	0.79		1	88		1
	8	*Th. longipes*	Os 75016	Bering Sea	—	1.14		1	87		1
				Overall Mean=1.16±0.53 (10)					100±42 (10)		
Formalin sample	1	*E. pacifica*	Os 74006	North Pacific	1 yr	2.17	0.41	2	153	5	2
	2	*E. pacifica*	Os 74010	Hokkaido	1 yr	1.98	0.09	2	172	13	2
	3b	*E. pacifica*	Os 75001	North Pacific	2 mo	1.87	0.21	2	118	7	2
	7c	*Th. longipes*	Os 75012	Bering Sea	2 mo	2.04		1	123		1
				Overall Mean=2.02±0.13 (4)					142±26 (4)		

a) Hamanaka and Tsujita (1981).
b) Mean±standard deviation (No. of replicates) in μg/g dry weight.
c) These values were not included in the mean because of white and rust chips found in the sample (see text).
d) This sample contained *Th. spinifera* (ca., 30% of total number).
e) This sample contained *Th. inermis* (ca., 10% in total number).
f) yr; year, mo; month.

Table 3. *Copepods. Cadmium and zinc concentrations in copepods whih have been preserved in freezing method and in formalin solution.*

Preservation methods	Sample No.	Species	Station No.	Sea area	Fixation period	Cd[b]			Zn[b]		
						Mean	SD	n	Mean	SD	n
Frozen sample[a]	2a	*Calanus plumchrus*	Os 75001	North Pacific	—	3.09	0.47	2	104	30	2
	2b	*C. plumchrus*	Os 75001	North Pacific	—	8.53	0.45	2	151	5	2
	3	*C. plumchrus*[d]	Os 75002	North Pacific	—	9.11	0.12	2	111	1	2
	6	*C. plumchrus*	Os 75025	North Pacific	—	14.55	0.35	2	160	5	2
	5	*C. pumchrus*[e]	Os 75011	Bering Sea	—	1.66	0.36	3	61	5	3
	7	*C. plumchrus*	MOs 75025	Bering Sea	—	12.69	0.48	2	111	0.1	2
	12	*C. plumchrus*	Oy 75024	Okhotsk Sea	—	4.67	0.65	2	72	8	2
	13	*C. plumchrus*	Oy 75025	Okhotsk Sea	—	5.21		1	141		1
	4	*Calanus cristatus*	Os 75004	North Pacific	—	2.35	0.22	2	121	4	2
	9	*C. cristatus*	MOs 75038	Bering Sea	—	4.74[c]	0.11	2	261[c]	5	2
	10	*Calanus glacilis*	Oy 75021	Ohotsk Sea	—	4.40		1	96		1
	8	*Eucalanus bungii bungii*	MOs 75038	Bering Sea	—	6.62	0.36	2	152	27	2
				Overall Mean=6.63±4.19 (11)					116±33 (11)		
Formalin sample	1	*C. pluchrus*[f]	Os 74006	North Pacific	1 yr	19.50	0.53	2	135	1	2
	15	*C. plumchrus*	Oy 75020	Okhotsk Sea	2 mo	9.47		1	102		1
	16	*C. plumchrus*	Oy 74017	Okhotsk Sea	1 yr	3.89		1	72		1
	17	*C. plumchrus*	Oy 74023	Okhotsk Sea	1 yr	1.60		1	42		1
	11	*C. glacialis*	Oy 75022	Okhotsk Sea	2 mo	15.88		1	111		1
	14	*C. glacialis*	Oy 75003	Okhotsk Sea	2 mo	8.90		1	77		1
				Overall Mean=9.87±6.84 (6)					90±33 (6)		

a) Hamanaka and Tsujita (1981).
b) Mean±standard deviation (No. of replicates). in μg/g dry weight.
c) These values were not included in the mean because of white paint and rust chips found in the samples (see text).
d) This sample contained *C. cristatus* (ca., 5% in total number).
e) This sample was sorted in 3% formalin for 1–3 hours.
f) This sample contained *C. cristatus* (ca., 10% in total number) and *E. bungii bungii* (ca., 45%). Other abbreviations are the same as those in Table 2.

Table 4. *Amphipods. Cadmium and zinc concentrations in amphipods which have been preserved in freezing method and in formalin solution.*

Presavation methods	Sample No.	Species	Station No.	Sea area	Fixation period	Cd[b]			Zn[b]		
						Mean	SD	n	Mean	SD	n
Frozen sample[a]	4	*Parathemisto pacifica*	Os 75005	North Pacific	—	13.23	0.77	2	122	2	2
	10a	*P. pacifica*	Os 75025	North Pacific	—	14.50	0.37	2	137	4	2
	10b	*P. pacifica*[c]	Os 75025	North Pacific	—	4.16	1.22	2	97	4	2
	11	*P. pacifica*	Os 75027	North Pacific	—	24.08[d]	4.31	2	134[d]	10	2
	5	*P. pacifica*	Os 75008	Bering Sea	—	6.72		1	90		1
	12	*P. japonica*	Os 76003	Hokkaido	—	19.63[d]	0.21	2	289[d]	7	2
	13	*P. japonica*	Oy 75022	Okhotsk Sea	—	2.83		1	81	2	2
	7	*Hyperia medusarum*	Oy 75015	Bering Sea	—	11.43	0.41	2	116	2	2
				Overall Mean=8.81±4.91 (6)					107±21 (6)		
Formalin sample	1	*P. pacifica*	Os 74006	North Pacific	1 yr	20.08		1	145	4	2
	6	*P. pacifica*	Os 75013	Bering Sea	2 mo	7.05	0.27	2	105	1	2
	15	*P. japonica*	Oy 75018	Okhotsk Sea	2 mo	6.14		1	120		1
	16	*P. japonica*	Oy 75020	Okhotsk Sea	2 mo	4.77		1	80		1
	17	*P. japonica*	Oy 75021	Okhotsk Sea	2 mo	6.41		1	126		1
	18	*P. japonica*	Oy 75028	Okhotsk Sea	2 mo	14.46[d]		1	216[d]		1
				Overall Mean=8.89±6.31 (5)					115±24 (5)		

a) Hamanaka and Tsujita (1981).
b) Mean±standard deviation (No. of replicates) in μg/g dry weight.
c) This sample was sorted in 3% formalin for 1–3 hours.
d) These values were not included in the means because of white paint and/or rust chips found in the samples (see text).
Other abbreviations are the same as those in Table 2.

Table 5. *Comparison of heavy metal concentrations (μg/g dry weight) between frozen and formalin samples. The concentrations are overall mean.*

Zooplankton Group	Cd		Zn	
	frozen sample	formalin sample	frozen sample	formalin sample
Euphausiids	1.16±0.53(10)[a]	2.02*±0.13(4)	100±42(10)	142±26(4)
Copepods	6.63±4.19(11)	9.87 ±6.84(6)	116±33(11)	90±33(6)
Amphipods[b]	8.81±4.91(6)	8.89 ±6.31(5)	107±21(6)	115±24(5)
(*P. pacifica*)				
(*P. japonica*)				
(*H. medusarum*)				

a) Mean±SD (No. of sample analyzed).
*) The cadmium concentrations of formalin-preserved euphausiid was significantly higher than that of frozen sample ($P<0.05$; Mann-Whiteny U-test).
b) *Parathemisto libellula* sample was not included in these amphipods sample because of Cd in *P. libellula* was related to the age of animal (see text).

Table 6. *Parathemisto libellula: Comparison of heavy metal concentrations between frozen and formalin samples. Data are mean±SD (No.).*

Sample No. Date Location	Preservation methods	Body length	Body weight	Cd[a]	Zn[a]
Os 7854A	Frozen sample	5-10 mm	4.1 mg	3.35	162
1978. 6. 29		10-14 mm	5.0 mg	6.47	119
57-45N,		15-20 mm	13.2 mg	4.69±0.83(3)	80±16(3)
163-59W		21-25 mm	15.7 mg	4.37±1.05(2)	78±23(3)
	Formalin sample	10-14 mm	—	7.11±2.09(2)	60
		15-20 mm	—	5.15	79
		21-25 mm	—	5.59	84
Os 74019[b]	Frozen sample	20 mm	13.2 mg	6.49±1.35(3)	98±28(3)
Os 74032		(mostly <10 mm)	(<7.5 mg)		
Hb74069		20-30 mm	27.7 mg	24.54	97
Os 75018		30 mm	42.2 mg	33.40	99
Os 75023					
	Formalin sample	20 mm	13.2 mg	11.61±2.17(3)	123±32(3)
		(mostly <10 mm)	(<7.5 mg)		

a) μg/g dry weight.
b) Details of date and location are in Table 1.

As far as *Parathemisto libellula* is concerned, simple comparison cannot be exercised because the cadmium concentration in this species increased with the body size.[8] Comparison in the same size class indicated that mean metal concentrations in formalin (fixation in 3% formalin for 1–5 hours) were 10–79% higher for camdium and 1–25% higher for zinc than the frozen samples with the exception of zinc in 10–14 mm size class (Table 6).

Discussion

There are four major reasons which could alter the metal concentrations; (1) contamination from impurities such as paints, rust chip particles etc., from

vessels, (2) contamination from other planktonic organisms coexisting in the same sample, (3) contamination from preservatives such as formalin, and (4) weight loss due to dehydration.

In general, impurities such as rust chips from hydrowires and vessel-related paints are recognized as sources of contamination.[2)9)10)11)12)]

In this study, paints and/or rust chips were removed by a plastic-coated pincette or passing a Teflon-coated magnet over the materials on the filter paper according to the method of Martin and Knauer (1973)[2)]. In practice, it is impossible to avoid contamination from small particles of inert materials which remain in the sample after removing the impurities above mentioned. However, it seems that the contamination from these impurities tends to be detected in the analytical results, mostly in zinc concentration. For example, our results showed that three of five samples in which paints and/or rust chip particles were found had anomalously high zinc concentrations (over 260 μg/g dry wt; see footnotes of Tables 2–4). Extremely high zinc concentration occurs in rust chip particles and rubber and slightly higher in antifouling paints.[2)9)11)] Cadmium is also contained in certain paints.[12)] Thus, abnormaly increased zinc concentrations in several samples in this study may be due to the contamination from the impurities found.

The second factor, potentially significant source of contamination, depend upon the composition of plankton species coexisting in the same net sample, the metal contents of the other organisms, contact time (i.e., time of chemical interaction), and storage time. In addition, it is possible that factors (1) and (2) reveal various extents of contamination and hence these seem to affect the frequency of distribution of metal concentrations and variability (e.g., standard deviation). Therefore, a reliability in data seems to increase with an increase in the sample size.[13)]

The copepod, *Calanus pulmchrus* (Sample No. 5) and amphipod, *Parathemisto pacifica* (No. 10b) which were sorted in 3% formalin within 1–3 hours fell within the normal range of metal concentration in duplicate analyses (Tables 3 and 4). The formalin reagent (37% formaldehyde) of two commercial brands had the following ranges; 1.5–3.2 ng Cd/ml (mean: 2.4 ng Cd/ml; 7 samples); 6.3–14 ng Zn/ml (mean: 9.0 ng Zn/ml; 7 samples). Therefore, a possible contamination from the formalin solution is expected to be a negligible or lesser one, at least in short-time fixation or in a sense of direct contamination, because of substantially lower heavy metal concentrations in formalin. However, heavy metal levels in *Parathemisto libellula* sorted in 3% formalin for 1–5 hours were slightly higher than those in frozen samples. A possible reason for this discrepancy is difficult to interprete. In addition, although time dependence of metal level change during (after) preservation could not be made in this study, one should bear in mind the difference of fixation effect between species, because the weight loss process of organisms due to fixation is very rapid.[14)15)16)].

The increased metal levels in euphausiids (*Euphausia pacifica* and *Thysanoessa longipes*) preserved in 3% formalin for 2 months seems to associate primarily with the fourth factor (i.e., weight loss during preservation) rather than the other contributors. According to Lasker (1966)[17)], formalin-preserved euphausiid, *E. pacifica* loses considerable body weight (about 50% of fresh weight) and this may

be due to dehydration and leaching of organic materials from the body. This is also indicate by the lower ash content of formalin-preserved animals[18] (mean: 6.20% of dry weight; range: 4.35–7.51%, Table 7), as compared to the value of fresh (frozen) animals (mean: 12.7% range: 9.90–16.20%).

Table 7. *Water and ash contents in zooplankton.*

Zooplankton Group	Water content[a]		Ash content[b]	
	frozen	formalin	frozen	formalin
Euphausiids	82.93±4.17(4)	85.07±0.55(3)	12.67±2.26(5)	6.20±1.35(4)
Copepods	73.63±4.63(4)	88.50 (1)	6.63±3.80(10)	5.98±2.32(3)
Amphipods	82.98±2.31(5)	85.43±0.69(4)	—	11.39 (1)
P. libellula	84.87±2.27(10)	85.75±0.92(2)	24.36 (1)	—

a) % of wet weight.
b) % of dry weight.

If one assumes that only weight loss is responsible for the contributing to increased metal concentrations in specimens, and euphausiids lose 20–80% of its original weight during fixation of 2 months, then the conversion of the observed metal concentration to its fresh-weight equivalent was accomplished by multiplying the observed values by 0.2–0.8. Thus, the corrected metal concentrations using these conversion factors in *E. pacifica* (Sample No. 3a) and *Th. longipes* (No. 7c) are 0.75–1.50 μg Cd/g; 50–94 μg Zn/g and 0.82–1.63 μg Cd/g; 49–98 μg Zn/g, respectively. On the other hand, the observed metal concentrations (frozen sample) in *E. pacifica* and *Th. longipes* are 0.79 μg Cd/g; 96 μg Zn/g and 0.79 μg Cd/g; 88 μg Zn/g, respectively (Table 2). The contrast between cadmium and zinc in these corrected metal concentrations apparently indicates the difference of the behavior of these heavy metals during the fixation process. A possible explanation for this difference could be that cadmium may be easier to leach than zinc from euphausiid tissue. For example, the different subcellular distribution between cadmium and zinc was previously reported in the experimental studies.[19][20][21] This seems to suggest the possibility of difference in degradation, leaching and transporting and affinity to binding site of the metals concerned.

In summary, cadmium and zinc concentrations in zooplankton preserved in 3% formalin over two months were higher than the frozen samples. No effect on the metal concentrations were found in the case of short term fixation within 1–3 hours for copepod and amphipod. However, *Parathemisto libellula* sorted in formalin in a short period gave slightly higher metal concentrations. These findings seem to indicate that the effect of preservation on metal concentration depends upon a variety of factors which, taken together, make the effect rather difficult to estimate. However, it must be noted that in the case of euphausiid preserved for 2 months, the weight loss of animal during the fixation seems to be the main contributing factor causing increased metal concentrations.

Acknowledgements

I would like to thank Mr. M. Kamba of The Japan Atomio Power Co. and Mr. H. Semura of The Simane Prefectural Fisheries Experimental Station, for their identification of the zooplankton species, and Captain T. Fujii, Messrs. N. Masuda, G. Kobayashi, G. Anma, T. Kubodera and crew members of T/S *Oshoro Maru*, for their helping the sampling on board. I am indebted to Dr. H. Ogi, Hokkaido University, who supplied zooplankton samples and provided helpful advice, and Dr. K. Shimazaki and Mr. T. Takanashi, Hokkaido University, who graciously provided samples. I am also very grateful to Prof. S. Mishima for his encouragement throughout this study.

References

1) Fowler, S.W., Small, L.F. and Dean, J.M. (1971). Experimental studies on elimination of zinc-65, cesium-137, and cerium-144 by euphausiids. *Mar. Biol.* **8**, 224–231.
2) Martin, J.H. and Knauer, G.A. (1973). The elemental composition of plankton. *Geochim. Cosmochim. Acta.* **37**, 1639–1653.
3) Hamanaka, T. and Mishima, S. (1981). Cadmium and zinc concentrations in marine organisms in the northern North Pacific Ocean. *Res. Inst. North Pacific Fish. Hokkaido Univ. Special Vol.* p. 191–206.
4) Hamanaka, T. and Tsujita, T. (1981). Cadmium and zinc concentrations in zooplankton in the subarctic region of North Pacific. *J. Oceangor. Soc. Japan.* **37**, 166–178.
5) Gibbs, R.H. Jr., Jarosewich, E. and Windom, H.L. (1974). Heavy metal concentrations in museum fish specimens: Effects of preservatives and time. *Science*, **184**, 475–477.
6) Kelly, T.M., Jones, J.D. and Smith, G.R. (1975). Historical changes in mercury contamination in Michigan walleyes (*Stizostedion vitreum vitreum*) *J. Fish. Res. Bd. Canada*, **32**, 1745–1754.
7) Hamanaka, T., Kato, H. and Tsujita, T. (1977). Cadmium and zinc in ribbon seal, *Histriophoca fasciata*, in the Okhotsk Sea. *Res. Inst. North Pacific Fish., Hokkaido Univ. Special Vol.* p. 547–561.
8) Hamanaka, T. (in preparation). Cadmium and zinc contents in hyperiid amphipod, *Parathemisto libellula* in the Bering Sea. *Bull. Fac. Fish., Hokkaido Univ.*,
9) Robertson, D.E. (1968). Role of contamination in trace element analysis of seawater. *Anal. Chem.* **40**, 1067–1072.
10) Martin, J.H. and Borenkow, W.W. (1975). Cadmium in plankton: Elevated concentrations off Baja California. *Science*, **190**, 884–885.
11) Bernhard, M. (1977). Chemical contamination of culture media Assessment, avoidance and control, p. 1460–1499. In Kinne, O. (ed.). *Marine Ecology*, Chapter 7, Volume III, Part 3, John Wiley & Sons, N.Y.
12) Young, D.R., Alexander, G.V. and McDermotte-Ehrlich, D. (1979). Vessel-related contamination of southern California harbours by copper and other metals. *Mar. Pollut. Bull.* **10**, 50–56.
13) Giesy, J.P.Jr. and Wiener, J.G. (1977). Frequency distribution of trace metal concentrations in five freshwater fishes. *Trans. Am. Fish. Soc.* **106**, 393–403.
14) Lockwood, S.J. (1973). Weight and length changes of O-group plaice (*Pleuronectes platessa* L.) after preservation in 4% neutral formalin. *J. Cons. int. Explor. Mer.* **35**, 100–101.
15) Lockwood, S.J. and Daly, C. De B. (1975). Further observations on the effects of preservation in 4% formalin on the length and weight of O-group flatfish. *J. Cons. int. Explor. Mer.* **36**, 170–175.

16) Schnack, D. and Rosenthal, H. (1978). Shrinkage of Pacific herring larvae due to formalin fixation and preservation. *Meeresforsch*, **26**, 222–226.
17) Lasker, R. (1966). Feeding, growth, respiration and carbon utilization of a euphausiid crustacean. *J. Fish. Res. Bd. Canada.* **23**, 1291–1317.
18) Fudge, H. (1972). Biochemical analysis of preserved zooplankton, p. 51–52. In *Biology of Plankton*, Library of Congress Cataloging in Publication Data MSS Information Corporation.
19) Marafante, E. (1976). Binding of mercury and zinc to cadmium-binding protein in liver and kidney of goldfish (*Carassius auratus* L.). *Experimentia*, **32**, 149–150.
20) Oh, S.H., Nakaue, H., Deagen, J.T., Whanger, P.D. and Arscott, G.H. (1979). Accumulation and depletion of zinc in chick tissue metallothioneins. *J. Nutri.* **109**, 1720–1729.
21) Cooms, T.L. (1979). Cadmim in aquatic organisms. In Webb, M. (ed.) *The Chemistry, Biochemistry and Biology of Cadmium*, p. 93–139. Chapter 3m Elsevier/North-Holland Biomedical Press.