Concentration of Elements in Marine Zooplankton from Coastal Waters of Boso Peninsula, Japan

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Concentrations were determined for 14 elements in zooplankters, which were collected along the Pacific coast of central Japan (Boso Peninsula) from October 1991 to August 1992. Analyses were conducted by inductively coupled plasma atomic emission spectrometry and inductively coupled plasma mass spectrometry on 5 separate species of Copepoda, 2 cladoceran species and other taxonomic groups including fish eggs. Less variable concentrations were noted for most elements (*e.g.* Ca, P and Sr) among different species or groups, except for cladocerans and fish eggs which had higher concentrations of some major elements (K, Mg and Na). Negative correlations were found between the concentration factors determined and oceanic residence time of elements.

Key words: elemental concentration, concentration factor, copepoda, cladocera, fish egg

Marine zooplankton, as secondary producers in the marine food chain, play a key role in cycling of elements in the aquatic ecosystem.¹⁾ Since they constitute major diets for fishes and organisms of higher trophic levels, it is important to have information on their element compositions in order to understand the accumulation of specific elements in these planktivorous animals.

Some reports have been published on element compositions of mixed zooplankton,^{2–5)} but works on single zooplankton species are scarce⁶⁾ due to the difficulty of obtaining samples of a single species in sufficient amounts to be analyzed.

The present study analyzed 14 elements in some separate zooplankton species and taxonomic groups, collected in neritic waters of the Pacific coast of central Japan.

Materials and Methods

Zooplankton samples were collected in waters 2 km off Onjuku Town, Chiba Prefecture (35°10'N, 140°20'E), on the Pacific coast of Boso Peninsula, on October 3 and December 24, 1991 and April 2, June 2 and August 19, 1992. A collecting net, 60 cm in diameter, 200 cm in length and with filtering gauze of 0.328 mm mesh size, was towed horizontally near the sea surface. Catches were fixed with a neutral 10% formalin seawater on board to prevent the outflows of elements when frozen samples were sorted. Individuals of selected zooplankton species or taxonomic groups which were abundant enough for subsequent chemical analyses were separated by observation with binocular microscope. Sorting procedure was completed within 24 hours after collection. The sorted samples were weighed after drying at 70°C in an oven.

Each sample was digested with 10 ml of mixed acid

(conc. HNO₃ and HClO₄) on a hot plate (*ca.* 150°C). Then 0.5 N HNO₃ solution was added to the dried digests to get a volume of 20 ml. Concentrations of 14 elements were measured using a Shimadzu ICPQ-1012W inductively coupled plasma atomic emission spectrometer and VG Elemental Plasma Quad PQ-II inductively coupled plasma mass spectrometer. Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, Sr and Zn were analyzed by inductively coupled plasma atomic emission spectrometry (ICP-AES). Ba, Co and Cs were determined by ICP-MS, because detection limits of ICP-MS for most elements are higher than those of ICP-AES⁷⁾. Prior to the application of ICP-AES and ICP-MS to the plankton samples, the accuracy of both techniques was examined using two standard reference materials (NBS-SRM-1566 Oyster tissue and NIES-CRM-6 Mussel). Multi-elements analyses by both techniques with yttrium internal standard were carried out using the same standard solutions (SPEX XSTC-13).

At the time of zooplankton collection, oceanographic data such as surface water temperature, salinity, nutrient (phosphate-P, nitrite-N and nitrate-N) concentrations and chlorophyll-a concentration were also determined. Nutrient concentrations were measured on seawater samples filtered through GFC filters according to Parsons *et al.*⁸⁾ Chlorophyll-a concentration was determined with a luminometer after filtration through GFC filters, thermal treatment at 450°C and extraction with dimethylformaldehyde.⁹⁾ Vertical tows of the zooplankton net were carried out from a depth of 3 m to the surface in order to elucidate the general zooplankton composition at the collection spot.

Results and Discussion

Environmental Conditions in Survey Area

Surface temperature and salinities were 14.0–24.1°C and 31.94–34.74, respectively. Nutrient concentrations were 0.06–0.52 $\mu\text{mol/l}$ ($\text{PO}_4\text{-P}$), 0.20–0.56 $\mu\text{mol/l}$ ($\text{NO}_2\text{-N}$) and 2.10–7.88 $\mu\text{mol/l}$ ($\text{NO}_3\text{-N}$). Chlorophyll-*a* concentrations were 1.98–6.98 $\mu\text{g/l}$. Wet-weights of net samples ranged from 12–59 mg/m^3 , and individual numbers of zooplankton ranged from 18–4277 individuals/ m^3 . Dominant species (percentage occurrence in number) were *Paracalanus parvus* (61%) and Oikopleuridae (19%) on December 24, 1991; *Paracalanus parvus* (30%), *Podon leuckarti* (29%), and *Acartia omorii* (22%) on April 2, 1992; and *Acartia omorii* (94%) on June 2, 1992. No dominant species were observed on October 3, 1991 and August 19, 1992 because of the small number of individuals collected.

Elemental Concentrations in Zooplankton Taxonomic Groups

As shown in Table 1, the concentrations of elements measured by ICP-AES and ICP-MS are in good agreement with the certified values of the reference materials except for iron by ICP-MS. The somewhat high concentration of iron (mass number = 57) measured by ICP-MS must result from the interference of molecular ions such as $^{40}\text{Ar}^{16}\text{O}^+\text{H}^+$, $^{40}\text{Ca}^{16}\text{O}^+\text{H}^+$ and $^{41}\text{K}^{16}\text{O}^+$. Therefore, we considered that the determination of iron by ICP-MS was not practical since large amounts of matrix elements such as Ca, K, Mg, Na and P are contained in marine organisms. From the data mentioned above, we concluded that the application of ICP-AES to Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, Sr and Zn, and of ICP-MS to Ba, Co and Cs is reasonable for the determination of elements in the marine organisms, including plankton, studied in this work.

As we used formalin-fixed zooplankton samples, effects of the formalin preservation on subsequent results of analyses had to be experimentally tested. Euphausiid *Euphausia pacifica* obtained from a local fishermen's cooperative was fixed with a neutral 10% formalin seawater and then

Table 2. Change of the concentrations of elements*¹ in *Euphausia pacifica* stored in a neutral 10% formalin-seawater solution

Element	0 day* ²	1 day	3 days	7 days
Ca	22800 ± 1120	21700 ± 1200	17600 ± 1300	11500 ± 1500
Cd	0.34 ± 0.09	0.36 ± 0.10	0.34 ± 0.09	0.31 ± 0.07
Cu	20.1 ± 1.1	19.2 ± 0.8	17.1 ± 0.8	18.1 ± 0.7
Fe	21.9 ± 2.1	22.5 ± 3.1	23.2 ± 2.6	24.7 ± 2.1
K	11000 ± 400	10420 ± 350	7740 ± 230	6500 ± 120
Mg	7360 ± 210	7840 ± 220	8790 ± 450	10700 ± 690
Mn	4.21 ± 0.41	4.02 ± 0.99	3.97 ± 0.54	3.88 ± 0.50
Na	38400 ± 2800	40700 ± 2980	48100 ± 3140	53000 ± 4300
Sr	300 ± 20	285 ± 50	260 ± 20	213 ± 30
P	13200 ± 490	12400 ± 470	10900 ± 200	8910 ± 180
Zn	67.6 ± 3.6	68.6 ± 7.7	69.8 ± 8.0	72.9 ± 3.0

*¹ Al, Co, and Cs were not examined in this experiment.

*² Non-preserved sample

analyzed by ICP-AES and ICP-MS. Daily changes of each element in formalin-fixed euphausiid *Euphausia pacifica* are shown in Table 2. No prominent changes were observed in the first day for each element compared to the non-preserved sample (0 day). However, concentrations varied for each element on and after three days. When compared with the results of analysis on the non-preserved sample, Mg and Na concentrations tended to increase with time, while those of Ca, K and P decreased during formalin preservation. Concentrations of trace elements were comparatively unaffected by this fixation treatment. The sorting of formalin-fixed zooplankton samples into species and groups and their refrigeration were carried out within 24 hours after collection. Concentration changes of these elements by the formalin treatment were estimated to be within 10% from comparison with non-preserved controls. From this result, we judged that only few effects occurred from formalin-fixation, if the sorting procedure was finished within 24 hours.

The concentrations of 14 elements analyzed in 14 zooplankton species or taxonomic groups, including two categories of fish egg, are summarized in Table 3. Copepods *Calanus sinicus* and *Paracalanus parvus* occurred abundantly for all sampling occasions except on June 2,

Table 1. Concentrations of elements in the marine standard reference materials measured by ICP-AES and ICP-MS

Elements* ¹	NBS-SRM-1566* ² Oyster tissue certified values ($\mu\text{g/g dry}$)	Values measured by ICP-AES ($\mu\text{g/g dry}$)	Values measured by ICP-MS ($\mu\text{g/g dry}$)	NIES-CRM-6* ³ Mussel certified values ($\mu\text{g/g dry}$)	Values measured by ICP-AES ($\mu\text{g/g dry}$)	Values measured by ICP-MS ($\mu\text{g/g dry}$)
Al	—	—	—	220	230	217
Ca	1500	1510	1520	1300	1320	1330
Co	0.4	ND* ⁴	0.39	0.37	ND	0.38
Cu	63.0	66.6	70.1	4.9	5.0	5.8
Fe	195	190	230	158	154	198
K	9690	9850	NA* ⁵	5400	5430	NA
Mg	1280	1270	1300	2100	2000	2100
Mn	17.5	16.5	17.4	16.3	14.8	17.3
Na	5100	5000	NA	10000	9700	NA
P	8100	7900	NA	7700	7500	NA
Sr	10.36	10.05	10.29	17	17	17
Zn	852	865	882	106	103	112

*¹ The certified value of Al for oyster tissue was not reported. No certified values of Ba and Cs for oyster tissue and mussel were reported.

*² 0.853 g of oyster tissue sample were digested and diluted to 100 ml.

*³ 0.978 g of mussel sample were digested and diluted to 100 ml.

*⁴ ND=not detected because of poor sensitivity of ICP-AES.

*⁵ NA=not analysed because the protection of an electron multiplier of the ICP-MS apparatus.

Table 3. Stable elements in twelve taxa zooplankters and four species of fish eggs collected from October 1991 to August 1999 collected on the Pacific coast of the Boso Peninsula

Organisms	Al	Ba	Ca	Co	Cs	Cu	Fe	K	Mg	Mn	Na	P	Sr	Zn
Sagittoidea														
<i>Sagitta</i> spp.														
91. 12. 24	162	0.275	8350	0.110	0.0540	38.0	253	1690	4750	49.0	15600	17900	94.6	92.6
Cladocera														
<i>Evadne nordmanni</i>														
92. 4. 2	229	3.85	28600	1.29	0.286	33.3	500	11100	12700	10.9	52400	34700	301	358
<i>Evadne tergestina</i>														
92. 6. 2	489	1.25	22300	0.433	0.0172	17.3	266	12400	11300	3.58	50800	22200	243	231
8. 19	452	2.43	24300	1.21	0.0307	12.7	701	15800	12300	4.40	64400	22000	275	167
Copepoda														
<i>Calanus sinicus</i>														
91. 10. 3	204	0.909	10700	0.412	0.0740	116	533	NA*1	5430	8.39	11800	15800	117	213
12. 24	NA	ND*2	13000	ND	ND	18.8	255	8380	7040	3.69	35400	15000	183	91.0
92. 4. 2	186	3.15	13200	ND	ND	10.0	295	NA	4640	NA	6260	20300	122	245
8. 19	815	1.92	19900	0.315	0.0343	19.1	1420	9000	8830	7.46	31800	25700	206	202
<i>Paracalanus parvus</i>														
91. 10. 3	533	0.190	17500	0.247	0.0480	46.1	734	9800	9170	7.91	37500	17000	202	114
12. 24	207	ND	14600	ND	ND	6.80	538	7900	6950	5.85	35700	14500	184	75.1
92. 4. 2	571	ND	33000	ND	ND	NA	1480	7700	11300	NA	11700	NA	280	464
8. 19	901	3.09	18900	0.459	0.0656	18.0	1120	NA	7270	0.900	18300	22900	173	19.3
<i>Eucalanus attenuatus</i>														
91. 12. 24	149	0.761	7480	0.0865	0.0480	14.1	241	4280	5300	1.16	24000	14200	111	34.8
<i>Acartia omorii</i>														
92. 4. 2	169	1.15	10600	ND	ND	NA	415	5500	4160	4.67	11000	16500	94.8	287
92. 6. 2	588	ND	14500	0.321	ND	25.7	835	1890	6330	4.70	8160	20500	137	179
<i>Temora</i> spp.														
91. 10. 3	1330	1.23	9240	0.534	0.141	53.7	1750	NA	4450	14.2	NA	22600	93.1	138
<i>Labidocera japonica</i>														
92. 8. 19	546	1.64	16400	0.336	0.0390	21.5	813	5150	7890	5.24	28700	20100	175	186
<i>Oncaea</i> spp.														
91. 12. 24	393	2.44	6950	0.177	ND	15.4	660	1980	3640	3.43	15500	9850	86.3	50.0
Decapoda														
<i>Brachyura</i> zoea														
91. 10. 3	588	0.365	9570	0.923	0.0660	101	838	922	5540	6.24	16400	37300	116	156
<i>Macrura mysis</i>														
92. 8. 19	362	1.74	17800	0.702	0.0261	29.5	709	4920	8360	3.82	30200	31000	178	253
Fish egg														
<i>Engraulis japonicus</i> egg														
92. 6. 2	194	ND	3180	ND	ND	NA	234	NA	6310	4.68	20100	6730	41.1	231
Unidentified spherical egg														
91. 12. 24	NA	ND	7190	ND	ND	34.0	146	4800	18100	3.00	137000	4580	116	56.7
92. 6. 2	NA	ND	6570	ND	ND	38.0	239	5250	18200	5.06	130000	7110	111	243
8. 19	335	1.85	8740	0.233	0.0190	33.7	555	4330	20900	2.54	103000	12400	128	116

Values are $\mu\text{g/g}$ on a dry weight basis.

*1 NA; not analyzed.

*2 ND; not detected.

1992. The copepod *Acartia omorii* was dominant among zooplankton on this date and on April 2, 1992. The cladoceran *Evadne tergestina* was collected in an amount sufficient for analysis on two occasions, but other species or taxonomic groups except for unidentified spherical fish eggs could be obtained only once out of 5 sampling times. When possible, analysis of a taxonomic group obtained at a certain sampling occasion was conducted in duplicate or more replicates.

Concentration ranges of the major elements in the copepod samples were: Ca, 7.0 to 33.0; K, 1.9 to 9.8; Mg, 3.6 to 11.3; Na, 6.3 to 37.5; and P, 9.9 to 25.7 mg/g dry wt. The decapod larvae also showed similar concentration ranges. These values were in agreement with those for *Calanus plumchrus* caught in the Sea of Japan in September 1984.⁶⁾ In the cladocerans, concentrations of K (11.1 to

15.8 mg/g dry wt), Mg (11.3 to 12.7 mg/g dry wt) and Na (50.8 to 64.4 mg/g dry wt) were comparatively higher than in the copepods.

Besides the crustaceans, on one occasion arrow worms (*Sagitta* spp.) were employed for chemical analyses, and their major element concentrations were nearly within the ranges for the copepods. Planktonic elliptic eggs of the anchovy *Engraulis japonicus*, and unidentified spherical fish eggs were also analyzed. The Mg and Na concentrations of the spherical eggs were 18 to 21 and 103 to 137 mg/g dry wt, respectively. These values were considerably higher than in the crustaceans.

Concentration ranges ($\mu\text{g/g}$ dry wt) of the trace elements in the copepods were: Al, 149 to 1330; Ba, N.D. to 3.15; Co, N.D. to 0.534; Cu, 6.8 to 116; Cs, N.D. to 0.141; Fe, 241 to 1750; Mn, 0.9 to 14.2; Sr, 86.3 to 280;

Zn, 19.3 to 464. These ranges were similar to those in the cladocerans and decapod larvae. When compared with the reported concentration of Fe (33 $\mu\text{g/g}$ dry wt) for *Calanus plumchrus* from the Sea of Japan,⁹ concentration ranges found in the present study were considerably higher (241 to 1750 $\mu\text{g/g}$ dry wt).

The trace element concentrations of our Sagittoidea and fish egg samples were also within the ranges of those of the crustaceans.

Generally speaking, the concentrations of most elements detected in our zooplankton samples did not differ greatly among the taxonomic groups, although the marine cladocerans *Evadne* spp., had higher concentrations of some of the major elements (K, Mg, and Na). However, the Fe concentrations of our crustacean samples were remarkably higher than those in the Sea of Japan samples reported by Masuzawa *et al.*⁶ We are not certain whether this reflects any differences in Fe concentration in the seawater or biological characteristics of the animals between the two regions.

Relationship between the Concentration Factors and the Oceanic Residence time

Marine organisms concentrate elements through seawater and foodstuffs. Elemental equilibrium is maintained between marine organisms and their environmental waters through concentration and excretion. The concentration factor is formulated for each element as follows:

$$CF = C_{PL} / C_{SW}$$

where *CF* is the concentration factor; *C_{PL}* is elemental concentration in zooplankton; and *C_{SW}* is elemental concentrations in seawater.¹⁰ Concentration factors ranged from 0.9 (Na) to 3.1×10^7 (Fe) (Table 4). Generally, *CF* values have been reported as being low in major elements and high in minor elements.¹ Yamamoto¹¹ found a log-log linear relationship between the concentration factor of zooplankton (*CF*) and the mean oceanic residence time

(*T*). Regarding the relation between the concentration factor and the mean oceanic residence time, Cherry *et al.*,¹² have given the following equation:

$$\log CF = a + b \log T$$

Mean oceanic residence time is defined as the average time an element spends in ocean water between its introduction into the ocean and its incorporation into the sediments,¹² and is defined as follows:

$$T = A / (dA/dT)$$

where *A* is the total amount of the element in seawater; *dA/dt* is the inflow element amount to the ocean or removal amount from ocean to sediment. Masuzawa *et al.*⁶ gave the following equation:

$$\tau_R = (C_{SW} \cdot V_{SW}) / (C_{RW} \cdot F_{RW})$$

where *C_{SW}* is the element content of seawater; *C_{RW}* is the mean element content of riverwater;¹³ *V_{SW}* is the total volume of seawater;¹⁴ and *F_{RW}* is river discharge to the ocean.¹⁵ A negative correlation on the relationship between log *CF*-log *T* has been reported for some marine organisms such as sea algae,¹⁶⁻¹⁸ phytoplankton¹⁷ and zooplankton.^{6,11} The same negative correlation was seen in this study (Fig. 1). The values of *a* and *b* were 7.38 to 8.87 and -0.75 to -1.00, respectively (Table 5). Generally, *a* is 6 to 9, and *b* is nearly -1.¹⁹

In the copepods *Calanus sinicus* and *Paracalanus parvus*, some elements were determined three or more different times during the year. Generally, concentrations of major elements analyzed were found to be less variable with sampling occasions. Among trace elements, some elements, such as Ba, Cu, Zn, seemed to change concentrations with sampling occasions.

In marine organisms with longer life spans, seasonal variations in element concentration have been investigated and related to growth, reproduction or other life cycle stages.²⁰⁻²⁵ Copepods in temperate waters, however, are

Table 4. Concentration factors of zooplankton and fish egg

Organisms	Al	Ba	Ca	Co	Cs	Cu	Fe	K	Mg	Mn	Na	P	Sr	Zn
Sagittoidea														
<i>Sagitta</i> spp.	3.0×10^5	19.6	20.4	9.2×10^4	1.9×10^2	1.5×10^5	4.5×10^6	4.2	3.7	1.8×10^6	1.4	2.5×10^5	12.0	2.3×10^5
Cladocera														
<i>Evadne nordmanni</i>	4.2×10^5	2.8×10^2	69.8	1.1×10^6	9.9×10^2	1.3×10^5	8.9×10^6	27.8	9.9	3.9×10^5	4.9	4.9×10^5	38.1	9.2×10^5
<i>Evadne tergestina</i>	8.7×10^5	1.3×10^2	56.8	6.9×10^5	82.8	5.8×10^4	8.6×10^6	35.3	9.2	1.4×10^5	5.3	3.1×10^5	32.8	5.1×10^5
Copepoda														
<i>Calanus sinicus</i>	7.4×10^5	1.4×10^2	34.6	3.0×10^5	1.9×10^2	1.6×10^5	1.1×10^7	21.7	5.1	2.3×10^5	2.0	2.7×10^5	19.9	4.8×10^5
<i>Paracalanus parvus</i>	1.0×10^6	1.2×10^2	51.2	2.9×10^5	2.0×10^2	9.1×10^4	1.7×10^7	21.2	6.8	1.7×10^5	2.4	2.5×10^5	26.6	4.3×10^5
<i>Eucalanus attenuatus</i>	2.8×10^5	54.4	18.2	7.2×10^4	1.7×10^2	5.4×10^4	4.3×10^6	10.7	4.1	4.1×10^4	2.2	2.0×10^5	14.1	8.9×10^4
<i>Acartia omorii</i>	7.0×10^5	82.1	30.7	2.7×10^5	—	9.9×10^4	1.1×10^7	9.3	4.1	1.7×10^5	0.9	2.6×10^5	14.7	6.0×10^5
<i>Temora</i> spp.	2.5×10^6	87.9	22.5	4.5×10^5	4.9×10^2	2.1×10^5	3.1×10^7	—	3.5	5.1×10^5	—	3.2×10^5	11.8	3.5×10^5
<i>Labidocera japonica</i>	1.0×10^6	1.2×10^2	40.0	2.8×10^5	1.3×10^2	8.3×10^4	1.5×10^7	12.9	6.2	1.9×10^5	2.7	2.8×10^5	22.2	4.8×10^5
<i>Oncaea</i> spp.	7.3×10^5	1.7×10^2	17.0	1.5×10^5	—	5.9×10^4	1.2×10^7	5.0	2.8	1.2×10^5	1.4	1.4×10^5	10.9	1.3×10^5
Decapoda														
Brachyura zoea	1.1×10^6	26.1	23.3	7.7×10^5	2.3×10^2	3.9×10^5	1.5×10^7	2.3	4.3	2.2×10^5	1.5	5.3×10^5	14.7	4.0×10^5
Macrura mysis	6.7×10^5	1.2×10^2	43.4	5.9×10^5	90.0	1.1×10^5	1.3×10^7	12.3	6.5	1.4×10^5	2.8	4.4×10^5	22.5	6.5×10^5
Fish egg														
<i>Engraulis japonicus</i> egg	3.6×10^5	—	7.8	—	—	—	4.2×10^6	—	4.9	1.7×10^5	1.9	9.5×10^4	5.2	5.9×10^5
Unidentified spherical egg	6.2×10^5	1.3×10^2	18.3	1.7×10^5	65.5	1.3×10^5	5.6×10^6	12.0	14.9	1.3×10^5	11.4	1.1×10^5	14.9	3.6×10^5

Fig. 1. Plots of $\log CF$ - $\log T$ for four copepods, *Calanus sinicus*, *Paracalanus parvus*, *Acartia omorii*, and *Labidocera japonica*, one cladoceran, *Evadne nordmanni*, and one decapod *Macrura mysis*.
T values are calculated from the river input data.

Table 5. Coefficients of a and b for zooplankton and fish egg in the following formula

Plankton species	a	b
<i>Sagitta</i> spp.	8.67	-0.99
<i>Evadne nordmanni</i>	7.88	-0.75
<i>Evadne tergestina</i>	8.15	-0.84
<i>Calanus sinicus</i>	8.40	-0.91
<i>Paracalanus parvus</i>	8.39	-0.90
<i>Acartia omorii</i>	7.98	-0.82
<i>Labidocera japonica</i>	8.42	-0.91
Brachyura zoea	8.87	-1.00
Macrura mysis	8.33	-0.89
<i>Engraulis japonica</i> egg	7.38	-0.79

$\log CF = a + b \log T$.

shorter-lived and repeat several generations within a year. Little has been reported as to whether the concentrations and compositions of elements vary depending on different generations, populations or stages of copepods. Accordingly, we are planning to clarify these subjects. By using ICP-AES and ICP-MS which have some analytical advantages such as satisfactory accuracy, high sensitivity and wide dynamic range, it would be possible to investigate seasonal and stage variations in future.

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