Seasonal Variations of Some Heavy Metal Concentrations in a Venus Clam *Meretrix lusoria*

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Venus clams were collected, over four periods of time: May, August, October/November 1984 and March 1985, from Wama tidal flat situated in the south-western end of Seto Inland Sea. Manganese, copper, zinc and iron in whole soft bodies were quantitatively determined. Seasonal variations of the four metal concentrations are discussed in relation to the reproductive cycle.

The concentration levels of four metals were as follows: iron > zinc > copper > manganese with seasonally considerable fluctuations in iron and manganese concentrations. This order, however, was consistently maintained throughout the year irrespective of sampling times. Manganese concentration in August decreased to about one-half of those in May, October/November and March, and was the lowest in the year. It is reasonably inferred that this reduction of manganese was induced by spawnings, particularly the discharging of ovarian substances, since the timing of reduction coincided with the final phase of spawning season and there were sexual differences in the metal contents between ovaries (high) and spermaries (low).

The results of discriminant analyses were obtained by relatively high rates of the proper characters in the four sampling times, with the four variates of manganese, copper, zinc and iron concentrations. Relatively low discrimination rate (77.27%) was found in the sample data of May, since the five sample data among 22 analytical groups obtained in May exhibited the proper character of August.

The concentration levels of three metals except copper in venus clams dwelling in the sheltered tidal flat were nearly equal to those in the wedge clams *Latona cuneata* inhabiting in the exposed sand beaches. Among 11 species of bivalves (7) and gastropods (4) the four metal concentrations in venus clams ranged at medium levels.

Fishery grounds for venus clams which are extending to tidal flats and shallow bottoms in embayments have been greatly decreased by reclaiming land from shallow waters along the coast of Japanese Islands. Therefore, the resources of venus clam that have been highly used for one among various sea foods have been reduced to a state just prior to being ruined.

There are few available articles on heavy metal concentrations in venus clams. As a matter of course, their metal levels in soft bodies have not been known for a long time. In general, their habitats are sheltered and calm sea areas extending to tidal zones which are easily influenced anthropogenically by different factors. Currently the productions of venus clams more than 100 tons per year in Japan are restricted to the sea areas of Kumamoto, Ibaraki, Mie and Ohita Prefec-

tures.*^{2,3}

In this paper, seasonal variations in concentrations of manganese, copper, zinc and iron, particularly of manganese, in venus clams *Meretrix lusoria* are discussed in relation to their reproductive cycles as determined histologically. Moreover, metal concentration levels of venus clams were compared to those of several bivalves and gastropods documented previously by the same author.

Materials and Methods

Collection Site, Sampling Time, Number of Analytical Lot and Others

The collection site, sampling tool, pre-treatments and preservations of specimens were the same as previously reported by Ikuta.¹⁾ Specimens were

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*^{2,3} Y. Kamijou, *et al.*: Research Report on Technical Development for Resources Cultivation of a Venus Clam *M. lusoria* (in Japanese), Ohita Prefectural Shallow Water Fisheries Experimental Station, pp. 1-54 (1985), and his personal communication.

sampled over four times, in May, August and October/November 1984 and March 1985 as shown in Table 1. Analytical groups for metals were comprised of 22, 20, 18 and 20 lots in respective sampling times according to size ranges of 2 mm intervals of shell length. Twenty individuals or more were gathered in respective lots in each range for metal analyses. Metals in whole soft bodies were quantitatively determined by atomic absorption spectrophotometry.

Specimens for Histological Examination of Gonads

Specimens for histological examination were collected over five times from March 1985 to March 1986. They were grouped by the shell length of 2 mm intervals and shell valves of two individuals in each group were shucked off and subsequently, gonads were dissected out from soft bodies and preserved in 10% formalin-seawater. Small blocks of gonad tissues were handled by standard methods of histological examination. Preparations were observed photomicroscopically and developing stages of gonads were determined.

Statistical Analyses for Comparisons of Metal Concentrations

In specimens collected in each sampling time and all specimens, metal concentration data were average and standard deviations and coefficients of variation were calculated. Differences in concentrations were tested by t-test between specimens of two sampling times, being combined as shown in Table 2. Correlation coefficients between

metal concentrations were calculated for specimens of each sampling time and all specimens. Discriminant analyses were conducted for six combinations, such as May and August, May and October/November, May and March, August and October/November, August and March, and October/November and March (Table 5).

Results

Concentration Levels of Four Metals in Whole Soft Bodies

In each sampling time, concentration levels were as follows: iron > zinc > copper > manganese, as shown in Table 1. Concentration ratios of iron, zinc and copper to manganese varied greatly on account of low manganese concentration in August and the variation in iron concentrations throughout the year. Ratios of maximum to minimum concentrations in total specimens of all sampling times were about 4 for iron, zinc and manganese, while that of copper was suppressed to almost 2.

Manganese concentrations in August differed significantly from those in the other three sampling times as shown in Table 2. In copper concentrations, except the two combinations of Oct/Nov and May, and March and May, there were significant differences in the other combinations as shown in Table 2. However, differences in copper concentrations among sampling times were much less than those in manganese. Zinc concentrations showed no significant differences in any combina-

Table 1. Metal concentrations in soft bodies collected over four times from May 1984 to March 1985

Sampling time and date		Metal			
		Fe	Zn ($\mu\text{g/g dry wt}$)	Cu	Mn
May 12-16, 1984	N=22	195 $\pm$ 41.2 (21.13%)	91.4 $\pm$ 28.4 (31.06%)	12.9 $\pm$ 2.27 (17.62%)	6.09 $\pm$ 1.48 (24.30%)
Aug. 10-14, 1984	N=20	220 $\pm$ 55.3 (25.17%)	96.0 $\pm$ 30.7 (32.01%)	11.3 $\pm$ 0.77 (6.84%)	3.29 $\pm$ 0.86 (26.14%)
Oct. 29-Nov. 2, 1984	N=18	395 $\pm$ 61.3 (15.52%)	83.5 $\pm$ 18.5 (22.20%)	12.2 $\pm$ 0.63 (5.16%)	5.76 $\pm$ 1.16 (20.14%)
Mar. 11-15, 1985	N=20	277 $\pm$ 80.3 (28.96%)	81.7 $\pm$ 21.6 (26.46%)	12.9 $\pm$ 1.07 (8.32%)	5.54 $\pm$ 1.06 (19.13%)
Total	N=80	267 $\pm$ 96.4 (36.14%)	88.3 $\pm$ 25.7 (29.13%)	12.3 $\pm$ 1.52 (12.34%)	5.18 $\pm$ 1.60 (30.89%)
Maximum		513	192	15.9	9.45
Minimum		128	48.4	7.50	2.19
Ratio (Max/Min)		4.00	3.97	2.12	4.32

Note: Parenthesized figures represent coefficient of variation. N indicates numbers of analytical groups.

Table 2. Matrices of significant differences between metal concentrations of respective sampling times

Sampling time	May	August	Oct/Nov	March
May	—	Mn $P < 0.001$ Aug < May	Mn ns	Mn ns
August	Cu $p < 0.01$ Aug < May	—	Mn $p < 0.001$ Aug < O/N	Mn $p < 0.001$ Aug < Mar
Oct/Nov	Cu ns	Cu $p < 0.001$ Aug < O/N	—	Mn ns
March	Cu ns	Cu $p < 0.001$ Aug < Mar	Cu $p < 0.05$ O/N < Mar	—

Sampling time	May	August	Oct/Nov	March
May	—	Zn ns	Zn ns	Zn ns
August	Fe ns	—	Zn ns	Zn ns
Oct/Nov	Fe $p < 0.001$ May < O < N	Fe $p < 0.001$ Aug < O/N	—	Zn ns
March	Fe $p < 0.001$ May < Mar	Fe $p < 0.05$ Aug < Mar	Fe $p < 0.001$ Mar < O/N	—

Note: "ns" represents not significant in concentration difference.

tions of sampling times (Tables 1 and 2). Iron concentration in May was equivalent to that in August and was apparently the lowest throughout the year. The seasonal variation pattern of iron differed greatly from those of manganese and copper.

Correlation coefficients between metal concentrations were calculated in specimens of sampling times and in total specimens (Table 3). Between manganese and copper concentrations, the highest value of correlation coefficient was found in the May specimens. Values of the coefficient decreased in successive sampling times and were minimal in October/November. Thereafter, the value of coefficient suddenly increased in March. In all specimens of the four sampling times, only the coefficient between manganese and copper was significant with 99% confidence limit.

Individual Numbers classified into Different Stages of Reproductive Cycles

Reproductive cycles for female and male were divided, on the basis of histological observations, into six stages, *i.e.* recovery for female or resting for male, early growing, late growing, maturing, breeding, spawning and spent stages. Individual

Table 3. Matrices for coefficients of correlation between different metal concentrations at each sampling time, and for those between those of all specimens irrespective of sampling times

Metal	Manganese	Copper	Zinc	Iron
Manganese	—	0.7263 ^a	−0.0030 ^a	0.3896 ^a
		0.3170 ^b	0.0060 ^b	0.2090 ^b
		0.0857 ^c	0.1096 ^c	0.3180 ^c
		0.5146 ^d	0.0419 ^d	0.3790 ^d
Copper	0.6301 ^a	—	0.0479 ^a	0.2131 ^a
			0.4377 ^b	0.0221 ^b
			0.1824 ^c	0.4004 ^c
			0.0939 ^d	0.2470 ^d
Zinc	−0.0829 ^a	0.0490 ^a	—	0.3869 ^a
				−0.2406 ^b
				0.0243 ^c
Iron	0.2808 ^a	0.0918 ^a	−0.1816 ^a	−0.2669 ^d
				—

Note: Superscripted a, b, c, and d represent sampling times: a (May), b (August), c (October/November), and d (March); and e for all specimens is superscripted.

numbers of specimens classified in different stages were listed in Table 4. In August both females and males developed gonads to the maturing and breeding stages, and subsequently in October the specimens turned to recovery or resting stage.

Table 4. Individual numbers classified into different stages of reproductive cycles

Sampling time	Sex	Stage of reproductive cycle					
		RY/RG	EG	LG	MG	BG	ST
Mar '85	F	8	17				
	M	27					
May '85	F	3	2	18			
	M	2	31	2			
Aug '85	F	2	2	2	3	13	6
	M	2		4	10	17	1
Oct '85	F	16					3
	M	10				2	
Mar '86	F	16	16				
	M	21					

Note: Abbreviations of stage names are as follows: RY (Recovery stage) for female, RG (Resting) for male, EG (Early growing), LG (Late growing), MG (Maturing), BG (Breeding), and ST (Spent).

Table 5. Discrimination rates (%) obtained by discriminant analyses between specimens of two sampling times with variates of Mn, Cu, Zn and Fe concentrations

Combination	Discrimination rate (%) proper character			
	May N=22	August N=20	Oct/Nov N=18	March N=20
May/Aug	77.27	90.00	—	—
May/O/N	95.45	—	100.00	—
May/Mar	90.01	—	—	85.00
Aug/O/N	—	95.00	100.00	—
Aug/Mar	—	100.00	—	95.00
O/N/Mar	—	—	100.00	85.00

Note: Respective figures in percentage represent proper characters for metal concentrations in sampling times.

Discrimination Rate (%) between Two Sampling Times

The discriminant analysis can comprehensively distinguished individually a sample datum with a proper character for metal concentrations similar to another group from a group to which the sample datum belongs.

Sample data with four variates of manganese, copper, zinc and iron concentrations were 22, 20, 18 and 20 analytical lots at four sampling times, respectively (Table 1). The mixed numbers of sample data were 5 for May and 2 for August between May and August, and successively, between May and October/November 1 for May and 0 for October/November, between May and March 2 for May and 3 for March, between August and October/November 1 for August and 0 for October/November, between August and March 0 for August and 1 for March, and between October/November and March 0 for October/November and 3 for March. These results given as dis-

crimination rates for proper character are shown in Table 5. As seen in Table 5, sample data in each sampling time exhibited, in general, respective proper characters with high discrimination rates. Namely, it was demonstrated that sample data in each sampling time possessed respective properties, being discrimination with the four variates of manganese, copper, zinc and iron concentrations. However, the mixture rate was the highest for the sample data in May, since 5 individuals bearing August-like property appeared in the sample data of May. This discriminant analysis is useful for distinguishing divergent sample data from a group constructed under the same conditions.

Discussion

Potential Factors influencing Seasonal Variation in Manganese Concentrations

In an early comparative study on metal concentrations in an American oyster, *Crassostrea virginica*, Galtsoff³⁾ documented that the distribution of manganese in the oyster body was related to the female reproductive cycle, because the concentration of this element in fully developed ovaries was 15 times that of the spermaries and its total concentration materially decreased after discharge of eggs. No such relationship was apparent for the other three metals, iron, copper and zinc. He pointed out that manganese could play some functions during the rapid propagation of ovocytes. Ikuta,³⁻⁴⁾ Ikuta and Nakahara,⁵⁻⁸⁾ Ikuta and Abe,¹⁰⁾ and Greig *et al.*¹¹⁾ documented that female individuals of *Batillus cornutus*, *Patinopecten (Mizuhopecten) yessoensis*, *Haliotis discus*, *Volutharpa ampullacea perryi*, and *Placopecten magellanicus* were higher in manganese

Table 6. Comparison of metal concentrations of *M. lusoria* obtained here with those of the same and other species

Species	Iron	Zinc	Copper	Manganese
<i>M. lusoria</i>	267±96.4	88.3±25.7	12.3±1.52	5.18±1.60
(1)				
<i>M. lusoria</i>	251±47.0	97.5±23.8	13.6±2.40	6.62±0.70
(13)				
<i>Latona</i>	231±25.5	113±6.33	27.2±2.72	6.74±0.48
<i>cuneata</i>	257±39.9	152±26.6	30.2±1.54	7.90±1.20
(14)				
<i>Pinctada fucata</i>				
<i>martensii</i>	243±43.0	883±146	5.04±0.30	14.2±3.14
(15)				
<i>Patinopecten</i>	16.4±0.74	33.6±1.53	1.89±0.06	1.48±0.10
(<i>Mizuhopecten</i>)	17.0±0.96	30.6±0.84	1.77±0.20	0.74±0.04
<i>yessoensis</i>				
(14)				
<i>Mytilus edulis</i>	133±15.9	156±13.8	5.67±0.19	6.54±0.24
(14)				
<i>Mytilus corsucus</i>	201±6.69	49.5±6.33	8.43±0.92	12.9±2.25
(14)				
<i>Atrina pectinata</i>	1010±262	2830±1690	7.84±0.58	35.7±10.9
(14)				
<i>Barbatia</i>				
(<i>Abrabatia</i>)	443±48.1	114±24.5	8.18±0.82	34.1±10.7
<i>decussata</i>				
(16)				
<i>Batillus</i>	574±91	49.7±4.50	7.69±0.51	1.75±0.24
<i>cornutus</i>	578±43	45.8±2.90	7.94±0.71	1.70±0.11
(17)				
<i>Haliotis</i>	62.4±5.4	14.9±1.8	9.94±2.29	0.98±0.16
<i>discus</i>	58.0±3.3	15.4±1.5	9.10±1.37	1.40±0.29
(18)				
<i>Babylonia japonica</i>	151±23.5	266±5.35	117±7.64	28.9±0.94
(19)				
<i>Volutharpa</i>	160±14.5	459±30.1	132±28.6	3.10±0.15
<i>ampullacea perryi</i>	185±9.37	458±34.2	203±25.3	1.46±0.12

Note: Parenthesized figures above species names represent numerical orders of references.

concentrations in ovaries or female whole soft bodies than those in spermaries or male whole soft bodies. Marui and Orland¹²⁾ demonstrated that manganese concentrations in a wedge clam, *Donax trunculus* increased during the gonadal development process for female individuals.

The manganese concentration of venus clams in August decreased to about one-half of those in May, October/November and March, and was the lowest in the year. It was reasonably inferred that this reduction of manganese was induced by spawnings, particularly discharging of ovarian substances, since the timing of reduction coincided with the final phase of the spawning season and there were sexual differences in the metal concentrations, i.e. ovaries with higher levels than

those in spermaries, as mentioned above. Taking the results²⁻¹²⁾ of manganese distributions in molluscan soft bodies into account, manganese concentrations in ovary of venus clams could decrease possibly to the same level as those in spermaries after spawning, since they are sex-separate. This experiment did not make clear directly the effect of sexuality on manganese concentration of venus clams because of the difficulty in separation of female and male from their mixture by external morphology and coloration of the gonads.

Comparisons of Metal Concentration Levels in Venus Clams to Those in Other Species

The concentrations of iron, zinc, copper and

manganese in several species of marine bivalves and gastropods are listed in Table 6. They were selected using as a criterion, this author's determinations of the metal concentrations. These data are not for specimens from the same sea area; therefore circumstances of sampling sites were taken into account, and data deemed abnormal were not included in Table 6.

The concentration levels of iron, zinc and manganese in venus clams, but not copper, were nearly equal to those in wedge clams *Latona cuneata* inhabiting exposed sand beaches and also the concentrations orders were the same in the both species. Except for *L. cuneata*, there were no similar concentration levels and order of metal concentrations as those of venus clams. It is likely that the specimens listed in Table 6 exhibit proper species-specificities for metal concentrations that are not influenced by environmental factors. Among 11 species of bivalves (7) and gastropods (4) the four metal concentrations in venus clams ranged at medium levels.

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