Nippon Suisan Gakkaishi 54(5), 811-816 (1988)

Inherent Differences in Some Heavy Metal Contents Among Ostreids, Mytilids and Acmaeids

Kunio Ikuta*

(Received August 3, 1987)

Multiple discriminant and discriminant analyses were applied to distinguish inherent differences for heavy metal contents among oysters/mussels/limpets and between oysters/mussels, oysters/limpets and mussels/limpets using the concentrations of Zn, Cu, Mn, Cr, Cd and Pb in the soft bodies of the three groups as variates.

The properties of the metal contents in limpets and mussels were perfectly distinguished from that in the oysters. The property of the metal contents in oysters was distinguished from that in the mussels *ca.* 70% and from that in the limpets by 80%; in mussels, from the oysters by 100% and from the limpets by *ca.* 36%; and in limpets, from the oysters by 100% and from the mussels by *ca.* 89%. The results on discriminations between the two groups were obtained as follows: the property of the metal contents in the oysters was distinguished from that in the mussels by *ca.* 75% with mussel-like character of *ca.* 25% and from that in the limpets by 80% with limpet-like character of 20%; and the properties of the metal contents between mussels and limpets were clearly separated each other without any mixture. In respective sampling sites, where more than two groups were collected, the properties of the metal contents in the three groups were completely distinguished from one another. The discrimination rates were decreased by the mixtures of the specimens grossly contaminated with heavy metals.

The orders of metal concentrations in the three groups were as follows: oysters>mussels>limpets for zinc; oysters>mussels>limpets for copper; mussels=oysters>limpets for manganese; mussels=oysters=limpets for chromium; oysters>mussels=limpets for cadmium; and limpets=mussels>oysters for lead.

Recently a number of articles[1-23] have been published in relation to heavy metal accumulation by marine bivalves and gastropods. In some investigations,[19-23] comparisons of heavy metal concentrations have been only carried out between species or sampling sites by the classical methods of statistics, notwithstanding multivariate analysis including several analytical methods could be applied to the treatments of the data obtained. There are only a few investigations which multivariate analysis has been applied to some heavy metal concentrations of marine bivalves, gastropods and other invertebrates, and environmental factors. Lumoa and Bryan[24] discussed environmental factors affecting heavy metal accumulation by the bivalves, *Scrobicularia plana* and the polychaete, *Nereis diversicolor* using multiple regression to estimate the body burdens of metals. Popham and D'Auria[25] applied principal component analysis to distinguish the unpolluted from the polluted mussels with some heavy metals.

Inherent species differences in heavy metal concentrations, especially zinc and copper were found incidentally in several investigations using two or more experimental specimens.[19-22] These results were no more than comparisons among several heavy metal concentrations in specimens. In general, oysters can accumulate copper up to higher levels than mussels and other bivalves, and also zinc levels in oysters are overwhelmingly higher than those in the other species and the other metals in oysters.[19-22] In gastropods, iron concentrations are much higher in herbivora than carnivora due to the difference of their feeding habits, and contrarily zinc, copper and manganese are much more concentrated in carnivora than herbivora.[23] Thus, it is suggested that there are differences in inherent response to heavy metals among molluscan species. However, it is not clear whether the similar phenomena exist or not in the other molluscan species.

Among the three families employed in this study, the species of limpets vary especially from

* Laboratory of Aquatic Environment and Ecology, Department of Fisheries, Faculty of Agriculture, Miyazaki University, Miyazaki 889-21, Japan (生田國雄: 宮崎大学農学部).

Table 1. Common and scientific names of shellfish specimens used

Taxonomic group		Species
Common name	Family	
Oysters	Ostreidae	*Crassostrea gigas, Saccostrea echinata, etc.*
Mussels	Mytilidae	*Mytilus edulis, Septifer (Mytilisepta) virgata, etc.*
Limpets	Acmaeidae	*Notoacmaea shrenckii, Patelloida (Chiazacmea) pygmaea, Collisella (Conoidacmaea) heroldi, Notoacmaea concinna, etc.* and *Cellana nigrolineata* (belonging to Patellidae)

Note: The capital letters of common names, oysters, mussels and limpets, were abbreiviated to "O", "M", and "L" throughout the paper.

site to site, and several species of limpets can be commonly seen at the zone of rocky shores. In the other two families, the similar replacements occur, but the number of species is much less than that of limpets. In order to demonstrate inherent differences in heavy metal contents of ostreids, mytilids and acmaeids inhabiting from sheltered sites to exposed sites in Beppu Bay, Ohita, Kyushu, multiple discriminant and discriminant analyses, together with the classical statistics, were applied to the data on the concentrations of some heavy metals in the soft bodies of the three taxonomic groups.

Materials and Methods

Taxonomic Groups of Specimens collected and Sampling Sea Area

The intertidal sessile forms, such as ostreids, mytilids and acmaeids, are commonly inhabiting on inter- or sub-tidal rocky shore substrata. They have been separately used as indicator species for heavy metal pollution in littoral sea areas and experimental specimens in laboratory, since they are directly influenced by environmental factors on account of their habits and heavy metals can be accumulated in their bodies via ambient sea waters and food materials.

On the basis of the preliminary observation for inhabitants on the intertidal zones along the shore line of Beppu Bay, ostreids, mytilids and acmaeids were chosen for experimental specimens as given in detail in Table 1 and an effort had been paid to ensure two or three kinds of specimens in each sampling site. The thirteen sites numbered in the order in Table 2 were chosen clockwise from the south-side end of its mouth to the west-side end along the intertidal shore line of Beppu Bay.

Constitution for Analytical Groups and Analytical Methods for Heavy Metals

In order to ensure samples as many as possible, 97 lots including 55 for oysters, 14 for mussels and 28 for limpets were prepared for analytical groups. Five individuals of soft bodies for oysters and mussels, and ten for limpets were pooled in each analytical group to reduce individual variations in heavy metal concentrations. The gathered samples of soft bodies were wet-digested with a mixture of nitric and perchloric acids. Subsequently, zinc, copper, manganese, chromium, cadmium and lead in the digested residues diluted in small volumes of 0.1 N hydrochloric acid were quantitatively determined by atomic absorption spectrophotometry.

Methodology to demonstrate Properties for Metal Contents of the Three Groups

Multiple discriminant (for three groups) and discriminant (for two groups) analyses[28] were applied to demonstrate the inherent differences in the heavy metal contents in the three taxonomic groups using the six variables consisting of zinc, copper, manganese, chromium, cadmium and lead concentrations in soft bodies. In order to analyze the data, the results on heavy metal concentrations were classified into the combinations of oysters/mussels/limpets, oysters/mussels, oysters/limpeis and mussels/limpets. In the multiple discriminant analysis, at first, all of the sample data from the thirteen sites were used. Subsequently, the analysis was applied again to the three groups after the high values of metal concentrations in the sample data were discarded, and also to the three groups from Beppu International Sightseeing Harbour. As for the discriminant analyses for the two groups collected for each sampling site, lead contents were excluded from variables on account of few instances of the sample data which are detected in the three groups from Saganoseki Inlet alone.

Results and Discussion

Characteristics of Heavy Metal Contents in Three Taxonomic Groups

Heavy metal concentrations in the specimens of the three taxonomic groups from the thirteen sites in Beppu Bay were given in Table 2 with averages and standard deviations. Names of sampling sites were represented as the Arabic numerals parenthesized in front of group names that were explained in the footnote of Table 2.

In the specimens from the three sites (1, 2 & 3) of Saganoseki Inlet, all of the six heavy metal concentrations were extremely higher than those in all of the specimens from the other sites with exception of lead in oysters from the site (1). Lead was not detected in all the specimens except

Table 2. Heavy metal concentrations (μg/g fresh wt) in specimens of the three taxonomic groups collected from the thirteen sites in Beppu Bay

Sampling site & taxonomic group		Zinc	Copper	Manganese	Chromium	Cadmium	Lead
(1)	Oysters (n=5)	1525±292	609±121	1.61±0.09	1.23±1.03	12.8±1.70	tr.
(2)	Oysters (n=3)	1730±165	849±379	12.9±7.50	1.83±0.37	10.5±1.13	4.29±0.57
	Mussels (n=3)	90.7±9.87	52.3±8.50	15.4±2.00	4.13±0.46	1.47±0.11	21.4±0.95
(3)	Oysters (n=2)	1056±226	1768±495	4.10±1.47	0.97±0.46	4.48±0.57	9.60±2.40
	Limpets (n=1)	30.5	35.5	3.89	1.34	1.99	24.8
(4)	Oysters (n=5)	488±67.4	20.7±1.35	7.50±3.37	1.02±0.02	0.52±0.23	tr.
	Mussels (n=5)	26.7±5.54	0.85±0.12	4.03±1.43	0.20±0.02	0.39±0.45	tr.
(5)	Oysters (n=5)	1489±803	52.3±35.7	10.4±7.12	1.26±0.91	0.49±0.21	tr.
	Limpets (n=1)	22.4	1.47	2.13	0.42	0.45	tr.
(6)	Limpets (n=1)	18.4	0.97	1.40	0.70	0.37	tr.
(7)	Oysters (n=5)	794±142	505±191	13.4±2.12	tr.	0.65±0.08	tr.
	Mussels (n=1)	33.4	9.69	3.87	tr.	0.45	tr.
(8)	Oysters (n=5)	649±144	22.5±6.61	1.17±0.15	0.16±0.04	1.12±0.54	tr.
	Limpets (n=5)	10.3±0.62	1.05±0.19	2.01±0.35	0.21±0.04	0.34±0.04	tr.
(9)	Oysters (n=5)	1077±86.1	50.1±5.52	5.87±2.15	0.24±0.07	0.64±0.17	tr.
	Mussels (n=5)	44.5±12.2	1.09±0.16	6.33±1.47	0.20±0.04	0.36±0.03	tr.
	Limpets (n=5)	14.6±1.00	1.61±0.28	4.03±0.91	0.27±0.02	0.42±0.05	tr.
(10)	Oysters (n=5)	445±68.7	39.8±4.64	6.79±0.25	0.39±0.23	0.45±0.04	tr.
	Mussels (n=5)	11.3±0.93	1.72±0.71	3.72±0.28	0.33±0.13	0.39±0.14	tr.
(11)	Oysters (n=5)	764±20.9	40.7±4.19	6.31±0.88	0.20±0.04	0.49±0.01	tr.
(12)	Oysters (n=5)	856±67.4	41.6±3.86	2.11±0.35	0.44±0.41	1.55±0.11	tr.
	Limpets (n=5)	8.15±2.93	0.83±0.06	2.72±1.21	0.22±0.03	0.75±0.03	tr.
(13)	Oysters (n=5)	655±43.3	34.0±3.28	1.96±0.17	0.15±0.03	1.03±0.08	tr.
	Limpets (n=5)	12.5±2.13	1.01±0.08	2.60±0.27	0.22±0.05	0.81±0.06	tr.

Note: The parenthesized figures in front of group name represent sampling sites as follows: (1), Saganoseki (the mouth of inlet); (2), Saganoseki (the middle part of inlet); (3), Saganoseki (the inner part of inlet); (4), Hoso; (5), 5th reclaimed ground; (6), Nishi-nihon Electric Wire Factory; (7), Nishiohita Anchorage; (8), Hamawaki; (9), Beppu International Sightseeing Harbour; (10), Toyooka; (11), Hiji; (12), Fukae; (13), Minousaki. The letter and figure after group name parenthesized represent the number of analytical group prepared.

Table 3. Heavy metal concentrations (m.±s.d.) of the three taxonomic groups and results of t-test

Group	Zn	Cu	Mn	Cr	Cd	Pb
Oysters (n=55)	927±468	239±423	6.04±4.92	0.60±0.67	2.53±4.06	4.29±0.57[a] 9.60±2.40[b]
	***O>M	***O>M	O=M	O=M	**O>M	O<M***
Mussels (n=14)	47.2±26.2 ***M>L	12.6±21.9 ***M>L	7.27±4.72 **M>L	1.01±1.70 M=L	0.62±0.53 M=L	21.4±0.95[c] (M=L)
Limpets (n=28)	12.7±4.97 ***O>L	2.47±6.49 ***O>L	2.96±1.02 **O>L	0.30±0.24 **O>L	0.58±0.34 **O>L	24.8[d] (O<L)

Note: Lead was detected in the specimens from the two sites of Saganoseki, and a, b, c, d superscripted represent the number of analytical groups: a=3; b=2; c=3, d=1. The number of asterisk represents significant levels as follows: *** ($P<0.001$); ** ($p<0.01$).

those from the two sites (2 & 3) in Saganoseki Inlet. In the specimens from Nishiohita Anchorage (7) and its around sites (5 & 6), relatively high copper and manganese concentrations were found. From these facts, it was clarified that heavy metal pollutions occurred locally in Beppu Bay. This was, as a result, advantageous to discriminant inherent differences in heavy metal contents of the three taxonomic groups exposed to a wide variety of polluted and unpolluted environmental conditions.

Affinities of Respective Groups to Various Heavy Metals

From comparisons among heavy metal concentrations of the three taxonomic groups (Tables 2 & 3), it was proved that oysters were liable to take up zinc, copper and cadmium to higher levels and lead to lower levels than the other two, and that mussels accumulated chromium to apparently higher levels than the other two, with the large coefficient of variation, and that manganese was taken up equally by oysters and mussels but in limpets, it was almost equivalent to half of those in the former two groups. Taking heavy metal concentrations in the specimens from the three sites (1, 2 & 3) in Saganoseki Inlet into account, it could be said that oysters, mussels and limpets may highly concentrate simultaneously multi-elements in the polluted waters irrespective of the taxonomic groups.

Discrimination of Properties for Heavy Metal Contents of the Three Groups

Using all of the sample data for the three groups from the thirteen sites, multiple discriminant and discriminant analyses were applied to the four combinations, such as oysters/mussels/limpets, oysters/mussels, oysters/limpets and mussels/limpets, with the six variates of zinc, copper, manganese, chromium, cadmium and lead concentrations. In the combinations between the two groups for respective sampling sites, the data on lead were discarded from the reason described before. The discriminated rates (%) of the respective combinations are given in Tables 4–6.

As for the combination of oysters/mussels/limpets including all of the sample data (Table 4), the property of the metal contents in oysters was discriminated from that in mussels by *ca.* 69% with *ca.* 31% of the mingled rate for mussel-like character, and also from that in limpets by 80% with 20% of the mingled rate in limpet-like character. Mussels were perfectly discriminated from oysters by 100%, but between mussels and limpets the discriminated rate for mussels was the lowest among the rates obtained by *ca.* 36% with *ca.* 64% of limpet-like character. Limpets were perfectly discriminated from oysters by 100%, and from mussels with relatively high rate of *ca.* 89%.

Subsequently, discriminant analyses were conducted between two groups, such as oysters/mussels, oysters/limpets and mussels/limpets. From the results in Table 5, the rates were somewhat raised in the combinations of the two groups compared with the rates in Table 4. Furthermore, in the respective sampling sites, the discriminant analyses were conducted for the nine combinations given in Table 6. All of the results indicated clear discriminations between the two groups. Especially, the three taxonomic groups from Beppu International Sightseeing Harbour (9) were perfectly discriminated from one another. Also, the other sampling sites, each taxonomic group was perfectly discriminated from the respective opposite group as shown in Table 6.

Table 4. Discriminated rates (%) by multiple discriminant analysis applied to the three taxonomic groups

Combination	Group	Discriminated Rate (%) between O and M	O and L	M and L
Oysters/	O	69.09	80.00	
Mussels/	M	100.00		35.71
Limpets	L		100.00	89.29

Note: Variables composed of Zn, Cu, Mn, Cr, Cd and Pb concentrations were used for the analysis.

Table 5. Discriminated rates (%) obtained by discriminant analyses between the two groups, such as oysters/mussels, oysters/limpets, and mussels/limpets

Combination	Group	Discriminated Rate (%) Oysters	Mussels	Limpets
Oysters/	O	74.55	25.45	—
Mussels	M	0.00	100.00	—
Oysters/	O	80.00	—	20.00
Limpets	L	0.00	—	100.00
Mussels/	M	—	100.00	0.00
Limpets	L	—	0.00	100.00

Note: Variables used in the analyses are the same as the footnote of Table 4.

Table 6. Discriminated rates (%) obtained by discriminant analyses between the two groups, such as oysters/mussels, oysters/limpets and mussels/limpets from respective sampling sites

Sampling site	Combination	Discriminated Rate (%)		
		Oysters	Mussels	Limpets
Saganoseki (2)	Oysters/Mussels	100.00	0.00	
		0.00	100.00	
Hoso (4)	Oysters/Mussels	100.00	0.00	
		0.00	100.00	
Hamawaki (8)	Oysters/Limpets	100.00		0.00
		0.00		100.00
Beppu International	Oysters/Mussels	100.00	0.00	
Sightseeing		0.00	100.00	
Harbour (9)	Oysters/Limpets	100.00		0.00
		0.00		100.00
	Mussels/Limpets		100.00	0.00
			0.00	100.00
Toyooka (10)	Oysters/Limpets	100.00		0.00
		0.00		100.00
Fukae (12)	Oysters/Limpets	100.00		0.00
		0.00		100.00
Minousaki (13)	Oysters/Limpets	100.00		0.00
		0.00		100.00

Note: Variables were composed of the five metal concentrations except lead.

Significance of Variation in Discriminated Rate due to Combinations of Taxonomic Groups and Rearrangement of Sample Data

In each single site of sampling, it was clearly demonstrated that a group was completely discriminated from the other in any combination as shown in Table 6. This indicated that the three taxonomic groups in each single site had their inherent properties for heavy metal accumulation without regard to the environmental conditions. It can be inferred from the results obtained in the rearrangements of sample data as shown in Tables 4–6 that the sample data including the contaminated specimens, especially in the combinations of oysters/mussels and mussels/limpets, decreased the values of discriminated rates since the data from the contaminated specimens can be overlapped in feet of the sample data distributions in the respective two groups. Then, multiple discriminant analyses were conducted after the deletions of sample data of the three taxonomic groups from the sites, (1), (2), (3), (5), (7) and (9) which were seemed to be grossly contaminated judging from the fact that the zinc concentration was more than 1000 μg/g or the copper concentration was more than 500 μg/g. When the sample data were rearranged in accordance with the treatments mentioned above, the property for heavy metal contents was completely discriminated among three groups and between the two groups as given in Tables 4–6.

Namely, it is concluded that the specimens of the three taxonomic groups inhabiting in sea waters unpolluted and polluted by heavy metals possess their inherent properties with regard to natural contents and abnormal accumulations of some heavy metals.

References

1) M. H. Wong, C. K. Choy, W. M. Lau, and Y. H. Cheung: *Envir. Res.*, **25**, 302–309 (1981).
2) T. I. Waker: *Aust. J. Mar. Freshwater Res.*, **33**, 552–560 (1982).
3) D. A. Ritz, R. Swain, and N. G. Elliot: *Aust. J. Mar. Freshwater Res.*, **33**, 491–506 (1982).
4) M. Wooton and A. K. Lye: *Aust. J. Mar. Freshwater Res.*, **33**, 363–367 (1982).
5) J. D. Thomson: *Mar. Biol.*, **67**, 135–142 (1982).
6) D. H. J. Phillips, C. T. Ho, and L. H. Ng: *Arch. Envir. Contam. Toxicol.*, **11**, 533–537 (1982).
7) D. A. Nelson, A. Calabrese, R. A. Greig, P. P. Yevich, and S. Chang: *Mar. Ecol. Prog. Ser.*, **12**, 155–165 (1983).
8) G. E. Zaroogian and M. Johnson: *Mar. Ecol. Prog. Ser.*, **12**, 157–173 (1983).
9) G. E. Zaroogian and M. Johnson: *Arch. Envir. Contam. Toxicol.*, **12**, 127–133 (1983).
10) N. G. Elliot, R. Swain, and D. A. Ritz: *Mar. Envir. Res.*, **15**, 17–30 (1985).

11) D. J. Cain and N. Lumoa: *Mar. Envir. Res.*, **15**, 115–135 (1985).
12) G. J. Ramelow: *Mar. Envir. Res.*, **16**, 243–253 (1985).
13) D. Johnson and T. J. Lack: *Mar. Envir. Res.*, **17**, 277–280 (1985).
14) V. Talbot: *Aust. J. Freshwater Res.*, **36**, 169–175 (1985).
15) D. A. Wright, J. A. Mihursky, and H. L. Phelp: *Mar. Envir. Res.*, **16**, 181–197 (1985).
16) D. F. Robert, M. Elliot, and P. A. Read: *Mar. Envir. Res.*, **18**, 165–183 (1986).
17) J. B. Luten, W. Bouquet, M. M. Burggraaf, A. B. Rauchbaar, and J. Rus: *Bull. Environ. Contam. Toxicol.*, **36**, 770–777 (1986).
18) J. M. Marcus and A. M. Thompson: *Bull. Environ. Contam. Toxicol.*, **36**, 587–594 (1986).
19) D. Martincic, H. W. Nurnberg, and M. Branica: *Mar. Chem.*, **18**, 299–319 (1986).
20) V. Talbot and A. Chegwidden: *Aust. J. Mar. Freshwater Res.*, **33**, 779–788 (1982).
21) D. Martincic, H. W. Nurnberg, M. Stoeppler, and M. Branica: *Mar. Biol.*, **81**, 177–188 (1984).
22) D. H. J. Phillips and K. Muttarasin: *Mar. Envir. Res.*, **15**, 215–234 (1985).
23) K. Ikuta: *Bull. Fac. Agr., Miyazaki Univ.*, **32**, 79–84 (1985).
24) N. Lumoa and G. W. Bryan: *Estua. Coast. Shelf Sci.*, **15**, 95–108 (1982).
25) J. D. Popham and J. M. D'Auria: *Environ. Sci. Techno.*, **17**, 576–582 (1983).
26) P. G. N. Digby and R. A. Kempton: Multivariate Analysis of Ecological Communities, Chapman and Hall, London, 1987, pp. 1–206.

Nippon Suisan Gakkaishi : Formerly ***Bull. Japan. Soc. Sci. Fish.***