Radiomanganese Distribution to Female and Male of *Haliotis discus* at the Post-Spawning Stage

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Immediately after spawning had occurred in the early stage of exposure experiment, the female individuals exposed to ⁵⁴Mn concentrated more actively the nuclide than the males, and retained it in whole bodies at 1.70 times higher compared to the males in 100%-SW. Moreover, the biological half-life estimated from the excretion formula was 1.58 times longer in the females than in the males, i.e. ⁵⁴Mn in the females was eliminated more gradually than that in the males. These results differed clearly from those obtained on *H. discus* at the pre-spawning stage and furthermore, from stable-Mn distribution observed in females and males of *Mytilus edulis* at the post-spawning season presented previously elsewhere.

⁵⁴Mn distributed to various soft tissues in the females were apparently higher than those in the males at the end of the exposure in 100%-SW. The concentration factor for ovary of specimens in 100%-SW raised up to 990 at the end of the exposure, while that of spermary was only 146. The difference between that for the ovary and the spermary was of the highest value among tissues, and consequently a similar correlation existed in absolute amounts and concentrations. The distribution of ⁵⁴Mn to ovary exhibited similar trend at the pre- and the post-spawning stages, but its degree differed from each other and was enhanced in concentration at the latter stage.

The nuclide uptake, retention and excretion by the females were inhibited under depressed specific gravity condition, whereas the males were not affected at all.

Manganese is required biologically as one of essential trace elements in animals, and it is remarkably concentrated in ovaries of bivalve and gastropod molluscs, such as *Crassostrea virginica*,¹⁾ *Patinopecten* (*Mizuhopecten*) *yessoensis*,²⁾ *Batillus cornutus*,³⁾ *Haliotis discus*,^{4,5)} and probably *Mytilus edulis*,⁶⁾ compared to those spermaries according to their respective cycles of reproduction, especially at the pre-spawning stages bearing developed gonads. Ikuta⁴⁾ revealed that in *H. discus* at the pre-spawning stage, stable-Mn concentration in whole body included conch and soft part of females was equivalent to that of males, but the metal concentration in conchs was significantly higher in males than in females, and in soft parts the correlation was inverted. Moreover, in soft parts, distribution rates of stable-Mn in ovaries and feet differed greatly between both sexes, i.e. in the former the rate was higher in females than in males, while in the latter higher in males. The ⁵⁴Mn behaviour to females and males of black abalones was examined by Ikuta and Nakahara⁵⁾ at the pre-spawning stage, and they

demonstrated that the intersexual difference of the nuclide was recognized only in gonads, and the concentration was significantly higher in ovary than in spermary after short exposure period in laboratory condition.

In the present paper, at the post-spawning stage, ⁵⁴Mn behaviours to females and males of black abalones were examined individually under two different specific gravities, i.e. 100%-seawater (100%-SW) as seen for that of coastal water and extremely low 60%-seawater (60%-SW). The results obtained here were regressed to accumulation and excretion curves, and intersexual differences for ⁵⁴Mn behaviour to black abalones were discussed.

Materials and Methods

The abalones employed in this study had been raised during about two years from their larval stage immediately after spawning in the land installation at Seed Production Section, Ibaraki Prefectural Fisheries Experimental Station.

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Table 1. Sizes and weights of specimens, and rearing conditions

Group	Sex & number	S. L.	W. W.	Uptake		Excretion	
				W. T.	Sp. Gr.	W. T.	Sp. Gr.
100%-SW	F (17)	6.53±0.34	34.06±5.77	19.5±0.4	1.02536	19.5±0.4	1.02537
	M (18)	6.24±0.30	27.67±4.36	19.6±0.4		19.5±0.4	
60%-SW	F (12)	6.39±0.33	37.85±6.74	19.5±0.3	1.01552	19.4±0.4	1.01583
	M (12)	6.22±0.27	33.10±5.25	19.6±0.3		19.5±0.4	

S.L., W.W., W.T. and Sp. Gr. are abbreviated from shell length, whole weight, water temperature and specific gravity, and units are centimeter for the first, gramme for the second and centigrade for the third. Values of Sp. Gr. are converted to those at 15°C.

The gamma-emitting radioisotope ^{54}Mn (MnCl_2 in 0.5 M HCl, specific activity: 48.33 mCi/mg Mn; stock solution diluted with deionized water: 1.16 mCi/50 ml) was used in the experiment. Rearing seawaters (60 l) had previously been spiked with the nuclide at certain concentrations by addition of 1 ml of the stock solution to expose black abalones separated sexually. Seawaters of two specific gravities, 100%-SW and 60%-SW groups, were prepared to examine salinity effect on uptake, retention and excretion for the nuclide by abalones and the water temperature for rearing was almost nearly adjusted to that of natural environment at the season, as listed in Table 1.

During the exposure experiment, rearing seawaters were exchanged with fresh labeled seawaters to maintain relatively constant levels of the nuclide on the zero (the beginning of exposure), 3rd, 6th, 9th, 12th and 15th days, and those activities were regularly counted every morning. The measurements were converted to counts per minute per milliliter (cpm/ml) and averaged. Throughout 19 days' exposure period, intact individual specimens in each experimental group were removed from the labeled seawaters, counted individually by a whole body counter on the 1st, 3rd, 6th, 9th, 12th, 15th and 19th (the end of exposure) days and returned into each aquarium. The measurements obtained were expressed as counts per minute per gramme fresh weight (cpm/g fresh wt) and used to estimate concentration factors at steady state together with activities in rearing seawaters.

During the excretion experiment that specimens were held in non-labeled seawaters exchanged every other day over 25 days, residual radioactivities in whole bodies were individually counted on the zero (equal to the 6th day of exposure experiment), 1st, 2nd, 4th, 7th, 10th, 18th and 25th days.

Regression curves for accumulation were com-

puted using the following equation,

$$Y = A(1 - e^{-bt}), \quad (1)$$

in which 'Y' is concentration factor for whole body at time 't' in day, 'A' represents an asymptote, and 'b' is a regression coefficient as substituted for an excretion rate that is induced from the equation of excretion described below. Excretion curves were expressed as

$$Y = ae^{-bt}, \quad (2)$$

in which 'Y' is retention rate adjusted radioactivity on the zero day in excretion experiment to 100%, and 'a' represents an intercept cross the Y axis on zero day, and 'b' is a regression coefficient represented as an excretion rate.

At the end of exposure experiment, radioactivities in whole soft bodies (WSB), various tissues (*i.e.* foot (FT), mantle (ME), gill (GL), gonad (GD), digestive diverticulum (DD), buccal mass (BM) and remains (RS)) and whole bodies (WB) were counted, and those were altered to absolute amounts, absolute amount percents, and concentration factors.

Results

In the acclimation to experimental conditions and in the early stage within a few days after the beginning of exposure, all the members of black abalones kept in aquaria had spawned, in turn, at several times, by physical stimuli, *i.e.* a series of handling to count activity, such as taking off from substratum, exposure to air, wrapping with a sheet to prevent contamination, counting and returning to each aquarium, and thereafter, they had turned physiologically into spent condition.

There were no significant differences among initial levels of activities of rearing seawaters as changed over six times: 220 ± 3.5 and 219 ± 6.2 (cpm/ml) for females and males in 100%-SW

Table 2. Concentration factors for whole bodies of females and males in 100%-SW and 60%-SW groups during uptake experiments

Days	Concentration Factor			
	100%-SW		60%-SW	
	Female	Male	Female	Male
1st	15 ± 2.0	19 ± 2.8	10 ± 1.6	10 ± 1.4
3rd	38 ± 4.8	40 ± 2.5	22 ± 3.4	23 ± 2.8
6th	70 ± 8.6	56 ± 6.5	43 ± 6.9	43 ± 6.0
9th	87 ± 11	69 ± 3.5	55 ± 6.5	66 ± 5.7
12th	109 ± 18	84 ± 7.4	65 ± 7.2	88 ± 7.1
15th	132 ± 19	97 ± 11	86 ± 8.1	97 ± 13
19th	168 ± 23	119 ± 17	95 ± 6.7	113 ± 15

groups, respectively, 225 ± 4.1 and 219 ± 4.6 (cpm/ml) in 60%-SW group, respectively. Residual activities in rearing seawaters fluctuated with similar decreasing trends, and 196 ± 30.9 and 212 ± 10.9 (cpm/ml) for females and males of 60%-SW group were apparently higher than 175 ± 29.1 and 154 ± 27.1 (cpm/ml) for females and males of 100%-SW group, respectively. However, any significant relationships could not be found between activities in rearing seawaters.

The regression analysis to the equation (1) was applied to concentration factors calculated from ratios (activity in whole body/activity in seawater) listed in Table 2. In excretion experiment, retention rates (%) for residual activities in whole bodies were regressed to the equation (2). Regression coefficients in the equation (2) were applied to excretion rates in the equation (1). The predicted formulae for accumulation were given in

Table 3 with confidence limits (90, 95 and 99% levels) for regression coefficients and figures for 'A' and 'b' at each level. The figures on accumulation of the four lots were regressed to the equation (1) with good fitness at a confidence limit of 99.9%. The highest value for 'A' that represented an asymptote of an accumulation curve was found of females in 100%-SW group and raised up to 240, which corresponded to the concentration factor at steady state. In 100%-SW group, the ratio of concentration factor at steady state for females to those for males was given as 1.70. This demonstrates distinctly that accumulation and/or retention abilities for ⁵⁴Mn in females differ from those of males. Salinity effect occurred distinctively in females, i.e. the concentration factor at steady state for females in 60%-SW was depressed to 52.5%, when compared to that for 100%-SW females, while in males, the concentration factors for both groups were comparable to each other. Therefore, it is inferred that salinity effect on males could not occur. The concentration factor at steady state for 100%-SW females differed significantly ($p < 0.05$) from those of 100%-SW males, 60%-SW females and 60%-SW males which were comparable to one another.

On the 6th day in process of exposure phase, the half numbers of specimens were transferred to excretion phase, in which they kept in non-labeled seawaters for 25 days and during the period, the radioactivities in whole bodies were counted at intervals. The predicted curves for excretion had not good fitness to plotted dots at

Table 3. Predicted formulae, and A and b values estimated from confidence limit for regression coefficient

Group	Sex	Predicted formula	C. L.	Upper limit		Lower limit	
				A	b	A	b
100%-SW	<F>	$Y = 240(1 - e^{-0.055t})$	90	293	0.045	203	0.065
			95	314	0.042	194	0.063
			99	377	0.035	176	0.075
	<M>	$Y = 142(1 - e^{-0.087t})$	90	180	0.068	116	0.106
			95	198	0.062	110	0.112
			99	255	0.048	97	0.126
60%-SW	<F>	$Y = 126(1 - e^{-0.089t})$	90	153	0.057	107	0.081
			95	161	0.054	104	0.084
			99	189	0.046	95	0.094
	<M>	$Y = 153(1 - e^{-0.055t})$	90	174	0.065	136	0.073
			95	181	0.055	133	0.075
			99	202	0.049	123	0.081

Confidence limit is abbreviated to C.L. (%).

Table 4. Retention rates (%) of females and males in 100%-SW and 60%-SW, respectively, during excretion experiment

Days	100%-SW		60%-SW	
	F	M	F	M
Zero	100	100	100	100
1st	81.02	79.32	69.63	73.76
2nd	74.69	75.09	69.30	75.52
4th	62.51	54.06	60.65	60.12
7th	50.42	40.81	37.07	37.69
10th	38.82	27.55	31.41	29.90
14th	37.77	17.71	23.81	25.35
18th	29.08	14.99	20.36	23.30
25th	24.65	13.07	17.75	20.17

the early stage immediately after the beginning of excretion, and thereafter, the plotted dots superimposed on the predicted curves toward the end of experiment. In both groups, it can be suggested that losses of the nuclide were consisted of more than one component. Retention rates in excretion phase were listed in Table 4, in which a similar decreasing trend was seen on females and males in 60%-SW group. But, in 100%-SW group, those of females were about 10% or more higher than those of males toward after the 4th day, though they were similar to each other until the 2nd day. In any case, activities lost at the 1st day were highest by ca. 40% for 60%-SW and

Fig. 1. Accumulation and excretion curves of ^{54}Mn by whole bodies of female (open circle) and male (solid circle) reared in 100%-SW. Curves were fitted visually to plotted dots only in depuration time. Data used here are represented as count per minute per gramme of fresh weight. Vertical bar represents one standard deviation, and upper and lower figures attached to the X axis indicate exposure and depuration times, respectively.**Table 5.** Absolute amounts (AA, cpm/tissue) and absolute amount percents (AAP, %) of ^{54}Mn in various tissues and whole soft bodies, and weight percents (WP) of tissues in 100%-SW at the end of exposure

Tissue	Sex	AA	AAP	WP
FT	<F>	172,000 ± 32,200	35.31 ± 4.24	69.75 ± 2.35
	<M>	120,000 ± 15,700	43.88 ± 3.56	65.62 ± 2.60
ME	<F>	15,200 ± 2,760	3.12 ± 0.42	5.55 ± 0.62
	<M>	10,800 ± 1,420	4.01 ± 0.58	5.09 ± 0.45
GL	<F>	19,500 ± 3,410	4.02 ± 0.52	2.13 ± 0.08
	<M>	14,700 ± 1,530	5.41 ± 0.41	2.13 ± 0.08
GD	<F>	146,000 ± 95,000	26.10 ± 8.52	3.95 ± 0.79
	<M>	5,600 ± 5,000	1.92 ± 1.50	1.28 ± 0.42
DD	<F>	99,600 ± 26,500	20.12 ± 3.16	8.89 ± 0.85
	<M>	82,300 ± 20,000	29.69 ± 3.62	12.48 ± 1.78
BM	<F>	17,800 ± 2,020	3.76 ± 0.81	4.48 ± 0.57
	<M>	14,300 ± 818	5.38 ± 1.09	5.08 ± 0.35
RS	<F>	36,900 ± 6,840	7.57 ± 0.95	5.25 ± 0.91
	<M>	26,400 ± 2,170	9.72 ± 0.68	8.32 ± 0.94
WSB	<F>	507,000 ± 166,000	100	100
	<M>	274,000 ± 37,300	100	100

Note: Abbreviation for tissue name is explained in the text.

Table 6. ⁵⁴Mn concentrations (cpm/g fresh wt) in various tissues and whole soft bodies in 100%-SW group at the end of exposure

Tissue	Female	Male
FT	14,300± 1,740	12,100± 216
ME	15,900± 990	14,200± 1,110
GL	53,500± 6,920	46,100± 1,540
GD	193,000±69,000	25,100± 1,540
DD	65,900±17,000	44,500± 9,770
BM	23,400± 1,930	19,300± 3,830
RS	41,900± 8,270	21,300± 712
WSB	29,000± 6,640	18,200± 1,410

Table 7. Concentration factors of various tissues and whole soft bodies in 100%-SW and 60%-SW groups at the end of exposure

Tissue	100%-SW		60%-SW	
	F	M	F	M
FT	74± 8.9	71± 1.2	42± 5.7	61± 6.2
ME	81± 5.6	83± 6.3	42± 3.9	53± 2.0
GL	275± 36	269± 9.1	145±13	199±29
GD	990±354	146±90	189±69	214±26
DD	338± 87	259±57	187±36	213± 8.3
BM	120± 9.9	112±22	71± 4.3	88±11
RS	215± 43	124± 4.2	103±15	113± 5.4
WSB	149± 34	106± 8.1	66± 5.7	92± 4.3

ca. 20% for 100%-SW. Biological half lives from the predicted formulae for excretion were calculated to be 13 and 8 days for females and males in 100%-SW, respectively, and to be 11 days for both sexes in 60%-SW

In the early stages of exposure and depuration, accumulation and excretion curves expressed as cpm per fresh gramme weight are shown in Fig. 1. Those regressed patterns are similar to those explained above in concentration factor and retention rate.

The absolute amounts and concentrations of ⁵⁴Mn in all the tissues and whole soft bodies in 100%-SW at the end of exposure phase were apparently higher in females than in males (Tables 5 & 6), among which remarkable differences relating to sex were found on those in gonadal tissues with 26.07 and 7.69 times higher levels in ovary, respectively, and 1.85 and 1.59 times higher in whole soft body of females, respectively. The values of absolute amount percent of ⁵⁴Mn in all the tissues of males were enhanced on account of the difference for the absolute amounts in whole soft bodies between females and males (Table 5), and those of males except for gonads were signi-

ficantly higher than those of females. Almost a quarter of the absolute amount in whole soft body of females was distributed to ovary, whereas only about 2% in spermary. The highest value for concentration factor was found on that of ovary in 100%-SW, which was significantly higher than those of the other three lots, and than any other tissues (Table 7).

Discussion

Some papers are published relating to intersexual differences for heavy metal concentrations in bivalves and gastropods. For example, Walting and Walting⁶⁾ found that Mn, Cu and Zn were more concentrated in whole soft bodies of female *Cholomytilus meridionalis*, but that Pb and Bi were more concentrated in those of male, and that no significant differences were found in the concentrations of Cd, Fe, Co, Ag, Ni and Cr. Orren *et al.*⁷⁾ analyzed some heavy metals in whole soft bodies of the same species on two occasions, pre- and post-spawning seasons, and found that Mn, Fe, Cu and Zn were concentrated in those of female higher than male only before a major spawning event, and no significant difference were found after spawning had occurred. Latouche and Mix⁸⁾ demonstrated that Mn and Zn were more concentrated in female than in male gonadal tissues of *Mytilus edulis* and there were no significant difference between gonadal concentrations of Ni, Cu or Cd. They supposed that the two metals (Mn and Zn) may be related in a quantitative way to gametogenesis, and Ikuta²⁻⁴⁾ and Ikuta and Nakahara⁵⁾ revealed that the three species, one bivalve and two gastropods, exhibited intersexual differences for stable-Mn distribution and biokinetic behaviours of ⁵⁴Mn. Among these studies¹⁻⁸⁾ mentioned above, only Orren *et al.*⁷⁾ had conducted a comparison for stable heavy metal concentrations between pre- and post-spawning seasons. Except for the article on ⁵⁴Mn distribution to females and males of black abalones⁵⁾ some stable metals had been only analyzed in all the cases.^{1-4,6-8)} Therefore, biokinetic behaviours for metals could not enough be understood yet. In many cases, stable metals retained in soft bodies were analyzed only at pre-spawning season or certain stages of respective cycles of reproduction, and discussed intersexually.

At the pre-spawning stage, it was demonstrated in the previous paper⁵⁾ that black abalones took

up ^{54}Mn equivalently in those whole bodies (conchs and soft parts) irrespective of sex over the two weeks' exposure, but the intersexual difference for ^{54}Mn contents was clearly confirmed only in gonads with the ratios of radioactivities in ovary to those in spermary, 3.7 in concentrations (cpm/g fresh wt) and 6.10 in total activity (cpm/tissue), and there were no differences relating to sex for those in the other tissues. In the present study, at the post-spawning stage, but still, it was revealed that ^{54}Mn was accumulated more actively in whole bodies of females than in those of males, with the ratio of concentration factors at steady state between sexes reared in 100%-SW, 1.70. Moreover, the biological half life was longer in females than in males in 100%-SW.

These facts will manifest reasonably that the response to ^{54}Mn of black abalones at the pre-spawning stage differ extremely from those immediately after spawning, *i.e.* in the latter physiological condition females take up more actively, retain higher and eliminate slower ^{54}Mn than males do. Under depressed specific gravity condition, uptake, retention and excretion abilities of ^{54}Mn are inhibited in females down to those of males.

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