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A Preliminary Study on Heavy Metals in the Antarctic Limpet, *Nacella concinna* (Strebel, 1908) (Gastropoda: Patellidae) in an Intertidal Habitat on King George Island

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ABSTRACT.- Concentrations of five metals (Cu, Cd, Mn, Zn, Fe) were determined in the tissues of the Antarctic limpet *Nacella concinna*. Limpets were collected from intertidal zones close to the King Sejong Station (Monitoring Site) and at a remote site 1.4 km away from the station which was considered to have been least affected by human activities (Reference Site). Tissue concentrations of these metals in *N. concinna* were relatively high compared to those of other limpets in temperate waters, and decreased with the increase in body size. Fe and Mn concentrations were higher in the limpets from the monitoring site, while Cd was higher in those from the reference site. This seems to be associated with regional variations in input of ice-melt water laden with heavy metals which have originated from erosion of terrestrial volcanic rocks, and the results of this study indicate higher input of ice-melt water into the monitoring site. The results of the present study, combined with the fact that *N. concinna* is abundant along the Antarctic Peninsula and the adjacent islands and easily collective, suggest that this limpet species can be used as a useful indicator for metal pollution monitoring in the Antarctic intertial areas. However, regional variation in geochemical processes and size-effect should be taken into consideration in establishing a framework for an environmental monitoring program.

Key Words: Antarctic, Heavy metal, Limpet, Nacella concinna

Introduction

The nearshore environments on King George Island where eight countries have been operating their research stations for years, have been exposed to contamination by human activities such as liquid waste disposal, oil spill, and fuel combustion. Bivalves such as mussels and oysters have been used as indicators for long-term contaminant monitoring in coastal waters world-widely under the design of 'Mussel Watch' (Farrington *et al.*, 1983; Goldberg *et al.*, 1983; Lauenstein *et al.*, 1990). In the Antarctic waters, however, there are no mussels or oysters, and heavy metal pollution monitoring has started in the Korean Station, *King Sejong* using the Antarctic clam, *Laternula elliptica* collected from 20-30 m water depths at sites close to the King Sejong Station (Ahn *et al.*, 1996). Furthermore, in the Antarctic attention has rarely been paid to intertidal zones despite the fact that they are most likely subject to input of anthropogenic contaminants, probably because of physical instability of the substrates due to ice impacts and the lack of large-sized life forms.

The Antarctic limpet, *Nacella concinna* (Strebel 1908, Patellidae) is the most conspicuous and usually the only large invertebrate found in the ice-scouring intertidal zones. *N. concinna* occurs in dense patches in intertidal and shallow subtidal zones around the Antarctic Peninsula and the adjacent islands, and its density tends to decrease with increasing water depth (Picken, 1980). Its ecology and physiology have been studied in several areas (Picken, 1980; Clarke, 1989; Clarke, 1990; Nolan,

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1991; Beaumont and Wei, 1991; Kennicutt *et al.*, 1992; Brêthes *et al.*, 1994; Peck *et al.*, 1996). This species was previously used to monitor a fuel spill in Arthur Harbor (Kennicutt *et al.*, 1990; Kennicutt *et al.*, 1991; Kennicutt *et al.*, 1992). Kennicut *et al.* (1992) reported that the intertidal and subtidal limpets in Arthur Harbor were found to contain elevated levels of polynuclear aromatic hydrocarbons near the station in the Arthur Harbor with the highest contaminant levels in the intertidal specimens, indicating the utility of this limpet species for an environmental monitoring program in the Antarctic.

The present study was undertaken to evaluate the suitability using *N. concinna* as an indicator species for monitoring heavy metal contamination in the Antarctic intertidal areas. Five selected metals (Cu, Cd, Mn, Zn, Fe) were determined in the tissues of *Nacella concinna*, and the tissue levels of these metals were compared with those of other limpets from temperate coastal waters. Influence of local geochemical process on the tissue concentrations was also discussed.

Materials and Methods

Sample collection and preparation for analysis

Nacella concinna were collected from intertidal zones close to the King Sejong Station which was likely to be subject to input of anthropogenic contaminants (Monitoring Site) and from a remote site 1.4 km away from the station which was considered to have been least affected by human activities (Reference Site) (Fig. 1). Limpets were hand-collected near the low water level during ebb tide on the 13th of December, 1996 at the monitoring site and on the 14th of December at the reference site. After sampled, limpets were frozen to -20°C for the transportation to Korea. Transported samples were stored at -70°C until analyzed. For the metal analysis, thirty-five limpets larger than 20 mm in shell lenth were randomly selected from each group. Frozen limpets were partially thawed and shell was detached from each of the limpets. Height, width and length of each shell were determined to the nearest 0.01 mm.

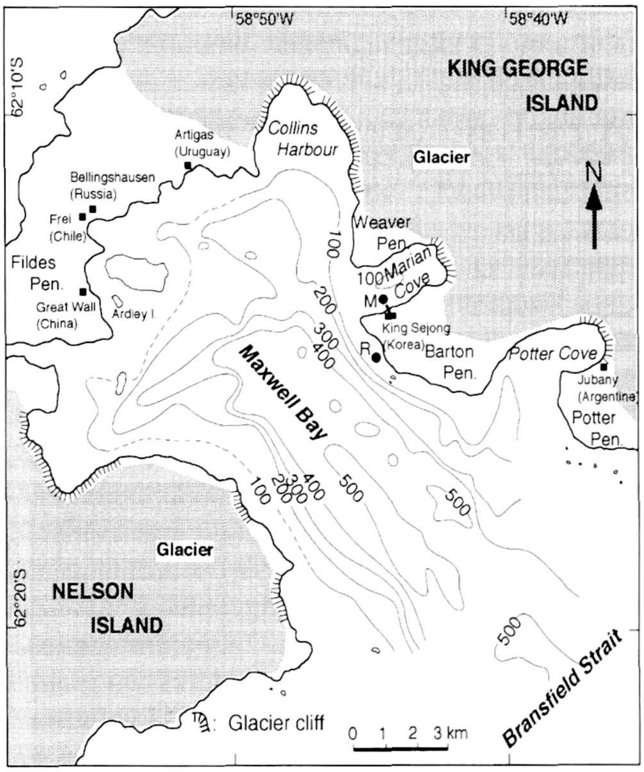

Fig. 1. Sampling sites in King George Island, Antarctica. M = Monitoring site; R = Reference Site.

After freeze-dried for about 3 days, tissue dry weight of each limpet was determined to the nearest 1 mg.

Analytical procedures

Heavy metal concentrations in the tissues of *Nacella concinna* were determined using the methods described by Ahn *et al.* (1996) and Zdanowiez *et al.* (1993). Metal concentrations were determined by flame (Mn, Cu, Zn, Fe) and flameless (Cd) spectrophotometer (Spectra AA-20, Varian). The accuracy of the analytical method used for the limpet tissues was tested using the standard reference materials of oyster (SRM 1566a, NIST) and mussel (CRM 278, Commision of the European Communities). Data were analyzed using statistical program MINITAB.

Results

Morphological characteristics of Nacella concinna *from the two sites*

Shell dimension and tissue dry weight of the

Table 1. Shell dimensions and tissue dry weights of *Nacella concinna* used for metal analysis. Means and standard deviations are represented (n = 35). SWt = Shell Weight, SL = Shell Length, SH = Shell Height, SW = Shell Width.

	SWt (g)	SL (mm)	SH (mm)	SW (mm)	SH/SL ×10	SH/SW ×10	SW/SL ×10	TDW (mg)
Monitoring Site	0.95 ± 0.31	29.9 ± 3.1	9.6 ± 1.3	21.1 ± 2.3	3.2 ± 0.2	4.6 ± 0.4	7.1 ± 0.3	764 ± 250
Reference Site	1.34 ± 0.47	29.6 ± 3.2	10.9 ± 1.9	20.8 ± 2.3	3.7 ± 0.4	5.2 ± 0.6	7.0 ± 0.3	501 ± 178

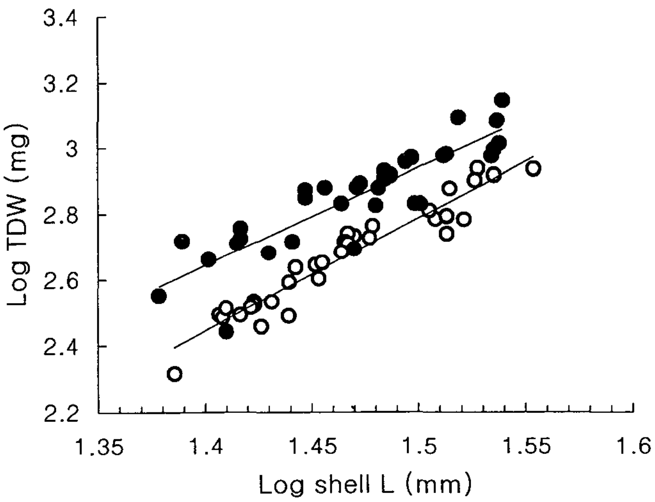

Fig. 2. The relationship between shell length (SL, mm) and tissue dry weight (TDW, mg) of *Nacella concinna* from the monitoring Site (●); regression line, TDW = $10^{-1.53}$ $SL^{2.98}$ (n = 35, SE_b = 0.282, r^2 = 0.771, F = 111.26, $p < 0.001$) and the reference site (○); regression line, TDW = $10^{-2.36}$ $SL^{3.43}$ (n = 35, SE_b = 0.174, r^2 = 0.922, F = 387.8, $p < 0.001$).

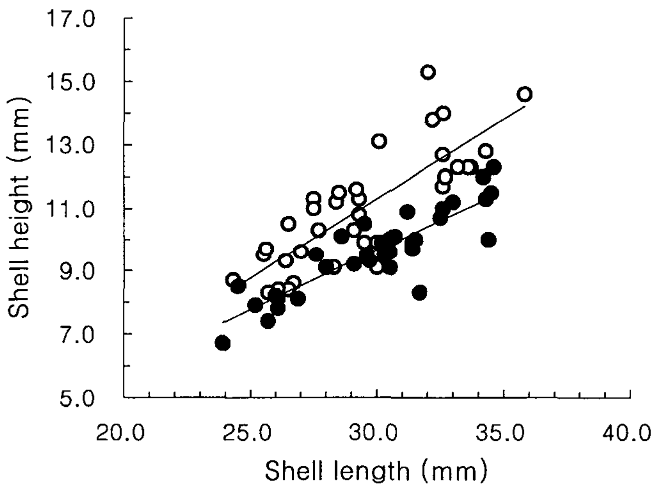

Fig. 3. The relationship between shell length (SL, mm) and shell height (SH, mm) of *Nacella concinna* from the monitoring Site (●); regression line, SH = -1.71 + 0.379 SL (n = 35, SE_b = 0.037, r^2 = 0.763, F = 106.13, $p < 0.001$) and the reference site (○); regression line, SH = -3.85 + 0.505SL (n = 35, SE_b = 0.063, r^2 = 0.661, F = 64.31, $p < 0.001$).

limpets used for the metal analysis were presented in Table 1. Average shell length of the limpets from the monitoring site (29.9 mm) was very similar to the average value of those from the reference site (29.6 mm). However, for a same shell length, the average tissue weight of the monitoring site was greater than that of the reference site (Differences among adjusted means: F=108.27 with degrees of freedom, 1 and 67, p<0.001, Fig. 2). On the other hand, the shell weight of the reference site was heavier than that of the monitoring site by 40%, and the shell height to shell length ratio was significantly higher in the limpets of the reference site (Differences among adjusted means: F=52.28 with degrees of freedom, 1 and 67, p<0.001, Fig. 3).

Comparison of metal concentrations of* N. concinna *between two sites

Analytical results for the reference materials were shown in Table 2, and metal concentration in the tissues of *N. concinna* in Table 3. Cadmium concentrations were significantly higher in the limpets of the reference site (degrees of freedom, *d.f.* = 55, t = 8.41, $p < 0.001$), while Fe and Mn were higher in the limpets of the monitoring site (Fe: *d.f.* = 66, t = -2.83, $p < 0.01$; Mn: *d.f.* = 61, t = -1.87, $p < 0.05$). No significant differences were detected in Cu and Zn concentrations between the limpets of two sites. Regression analysis (Figs. 4-8) also showed that for a limpet of a same tissue dry weight, Cd concentration per unit tissue weight was higher at the reference site (Differences among adjusted means: F=37.60 with degrees of freedom, 1 and 65, p<0.001), while Fe and Mn concentrations were higher at the monitoring site (Fe: Differences among adjusted means: F=20.83 with degrees of freedom, 1 and 64, p<0.001, Mn:

Table 2. Comparison of the certified and measured concentrations ($\mu g\ g^{-1}$ tissue dry weight) of the standard reference materials of oyster and mussel tissues. Means and standard deviations are represented (n = 6).

Element	Oyster (SRM 1566a)			Mussel (CRM 278)		
	Certified	Measured	% Recovery	Certified	Measured	% Recovery
Cd	4.15 ± 0.38	4.01 ± 0.17	97	0.34 ± 0.02	0.36 ± 0.02	106
Cu	66.3 ± 4.3	68.7 ± 0.6	104	9.6 ± 0.1	10.0 ± 0.0	104
Mn	12.3 ± 1.5	11.7 ± 0.2	95	7.3 ± 0.2	7.5 ± 0.2	103
Zn	830 ± 57	883 ± 21	106	76 ± 2	75.4 ± 1.1	99
Fe	539 ± 15	521 ± 33	97	133 ± 4	131 ± 12	98

Table 3. Comparison of metal concentrations ($\mu g\ g^{-1}$ tissue dry weight) in the tissues of *Nacella concinna* from the monitoring site and the reference site, King George Island. Means and standard deviations are represented (n = 35 except [a]: n = 33 and [b]: n = 34).

	Cd	Cu	Fe	Mn	Zn
Monitoring Site	4.9 ± 2.2	20.8 ± 3.8	2265 ± 1005	39.9 ± 10.4[a]	69.4 ± 5.2
Reference Site	10.7 ± 3.3[a]	20.8 ± 4.7	1627 ± 863[b]	34.6 ± 12.6[a]	71.4 ± 6.5

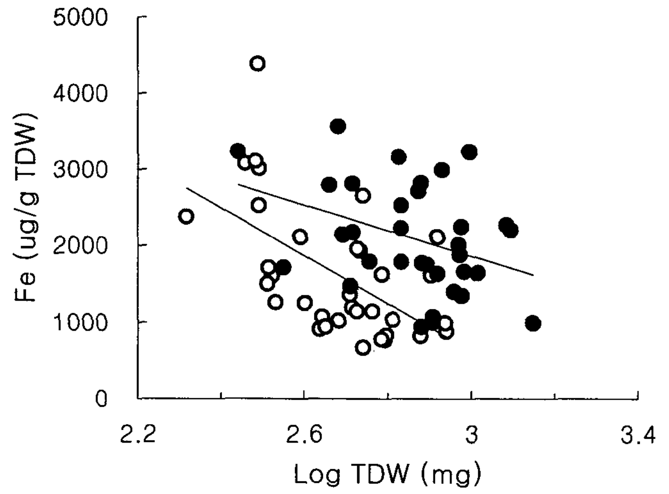

Fig. 4. The relationship between Fe concentration ($\mu g\ g^{-1}$ tissue dry wt) and tissue dry weight (TDW, mg) of *Nacella concinna* from the monitoring Site (●); regression line, Fe Conc. = 6843 - 1661 Log TDW (n = 33, SE_b = 0.770, r^2 = 0.130, F = 4.65, $p < 0.05$) and the reference site (○); regression line, Fe Conc. = 9925 - 3100 Log TDW (n = 34, SE_b = 0.7996, r^2 = 0.32, F = 15.03, $p < 0.001$).

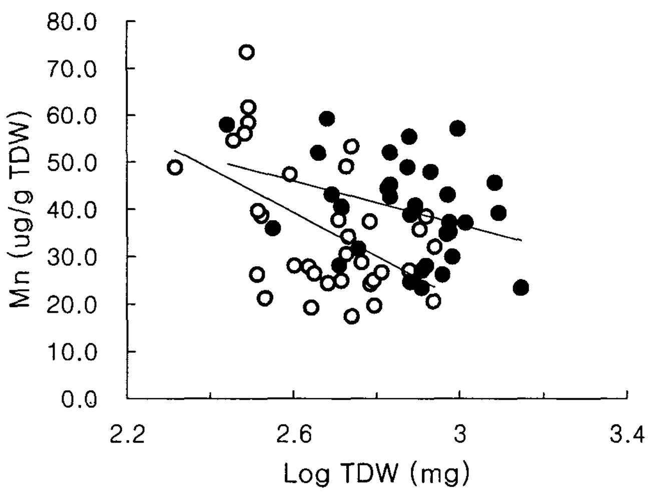

Fig. 5. The relationship between Mn concentration ($\mu g\ g^{-1}$ tissue dry wt) and tissue dry weight (TDW, mg) of *Nacella concinna* from the monitoring site (●); regression line, Mn Conc. = 106 - 23.0 Log TDW (n = 33, SE_b = 11.28, r^2 = 0.119, F = 4.17, $p < 0.05$) and the reference site (○); regression line, Mn Conc. = 160 - 46.4 Log TDW (n = 34, SE_b = 13.58, r^2 = 0.268, F = 11.69, $p < 0.005$).

Differences among adjusted means: F=11.15 with degrees of freedom, 1 and 64, $p<0.01$). Since shell morphology was significantly different between the limpets from the two sites, the regression curves of metal concentrations were plotted against tissue dry weight.

Effects of body size of **N. concinna** *on the metal accumulation*

Fe and Mn concentrations per unit body weight

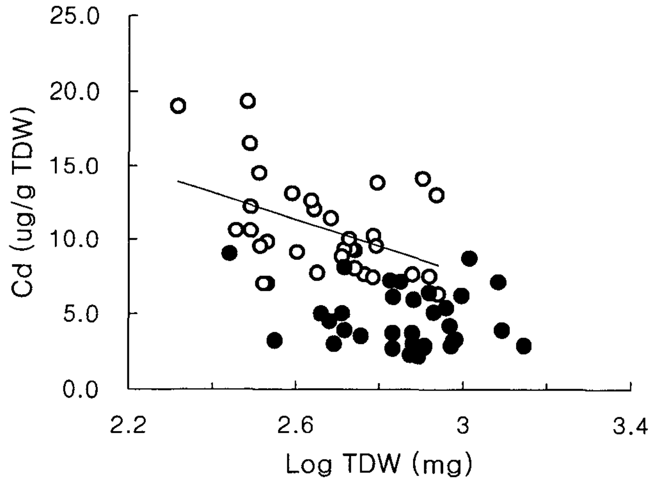

Fig. 6. The relationship between Cd concentration ($\mu g\ g^{-1}$ tissue dry wt) and tissue dry weight (TDW, mg) of *Nacella concinna* from the monitoring site (●) and the reference site (○); regression line, Cd Conc. = 35.0 - 9.12 Log TDW (n = 33, SE_b = 3.350, r^2 = 0.193, F = 7.41, $p < 0.01$). No significant relationship was found at the monitoring site.

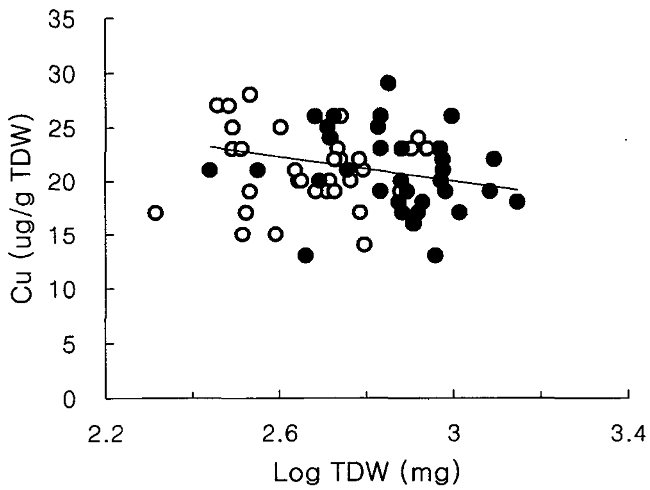

Fig. 8. The relationship between Cu concentration ($\mu g\ g^{-1}$ tissue dry wt) and tissue dry weight (TDW, mg) of *Nacella concinna* from the monitoring Site (●); regression line, Cu Conc. = 43.8 - 8.05 Log TDW (n = 33, SE_b = 3.725, r^2 = 0.131, F = 4.67, $p < 0.05$). No significant relationship was found at the reference site (○).

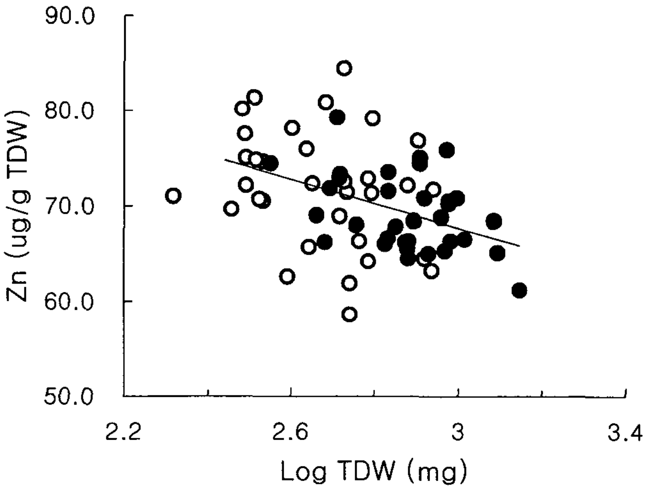

Fig. 7. The relationship between Zn concentration ($\mu g\ g^{-1}$ tissue dry wt) and tissue dry weight (TDW, mg) of *Nacella concinna* from the monitoring site (●); regression line, Zn Conc. = 105 - 12.6 Log TDW (n = 33, SE_b = 4.808, r^2 = 0.181, F = 6.87, $p < 0.05$). No significant relationship was found at the reference site (○).

decreased significantly with increase in body weight within the size range analyzed at both sites (Figs. 4 and 5). Cd concentrations significantly varied with body size only at the reference site; Cd concentration of a 300-mg *N. concinna* (12.4 $\mu g\ g^{-1}$ TDW) was one and half times higher than that of a 800-mg individual (8.5 μg Cd g^{-1} TDW) (Fig. 6). On the other hand, Zn and Cu varied signicantly with body size only at the monitoring site (Figs. 7 and 8).

Discussion

Morphological characteristics of Nacella concinna *from the two sites*

The limpets from the two sampling sites significantly differed in shell shape (Table 1, Fig. 3). For a same shell length, the limpets from the reference site were shown to have steeper and heavier shell. The morphological difference in shell shape between the two sites was also confirmed by the data from the regression analysis. The mean slope of regression of shell height on shell length is 0.505 for the limpets from the reference site and 0.379 for those from the monitoring site. These figures are very close to those for the littoral form (0.52) and the sub-littoral form (0.38), respectively given by Walker (1972). Equivalent figures for the two forms are 0.49 and 0.32 by Beaumont and Wei (1991) and 0.416 and 0.299 by Nolan (1991). According to these previous studies on the shell morphometry, the limpets from the reference site fall into the littoral group, but those from the monitoring site seem to lie between the littoral and the sublittoral forms. It appears that the limpets were collected nearer the sublittoral zone at the monitoring site than at the reference site, and those from the monitoring site may be mixed with some sublittoral forms.

Comparisons of metal concentrations in **N. concinna** *with those of other limpets in temperate coastal waters*

The metal concentrations in *N. concinna* were relatively high in comparison with those of the representative limpet species from other geographical regions and comparable to those found in *Patella* spp. in some polluted coastal waters (Butterworth *et al.*, 1972; Nickless *et al.*, 1972; Preston *et al.*, 1972; Peden *et al.*, 1973; Stenner and Nickless, 1974). In particular, Cd and Cu concentrations were highly elevated in comparison with those found in *Patella vulgata* from a clean site (Miramand and Bentley, 1992).

Elevation of Cd level was also reported in the Antarctic bivalves, *Adamussium colbecki* (26-49 $\mu g\ g^{-1}$ tissue dry weight) (Mauri *et al.*, 1990) and *Laternula elliptica* (7.5 $\mu g\ g^{-1}$ tissue dry weight) (Ahn *et al.*, 1996). Similar values were also reported for *N. concinna* collected from a different site on King George Island (Moreno *et al.*, 1997). The elevation of Cd seems to be a unique feature of the Antarctic herbivorous organisms living in the naturally Cd-enriched waters. Cadmium concentrations are several times higher in the Southern Ocean than in any other oceans, and this may be accounted for the upwelling of enriched deep water to the surface (Orren and Monteiro, 1985; Honda *et al.*, 1987). Cd levels comparable to those in contaminated area were also reported in Antarctic macroalgae and other invertebrates in the Antarctic Peninsula region (Moreno *et al.*, 1997). Cd having a high affinity to phosphate in general shows a positive correlation with phosphate concentration in sea water (Orren and Monteiro, 1985). Cadmium then seems to be taken up by primary producers along with phosphate, and as a result, appears to be accumulated in herbivores grazing on primary producers, and to a considerable degree in the Antarctic herbivorous organisms. In addition, *N. concinna* is known to grow very slowly compared to other limpets in temperate waters. For a same-sized individual, *N. concinna* is older than the limpets in temperate waters, and probably accumulate metal for a longer period of time. This may contribute in part to the elevation of Cd in *N. concinna*.

The elevation of Cu, on the other hand, seems to be associated with a local geochemical process during summer, *metal-laden glacial-melt water runoff*. Ahn *et al.* (1996) reported that Cu concentrations in the nearshore coastal sediment near the study area were highly elevated (68-88 $\mu g\ g^{-1}$ dry weight) and contended that the elevated Cu has originated from erosion of terrestrial volcanic rocks by ice-melt water during summer. Terrestrial rocks on King George Island are composed mainly of volcanic rocks containing high amounts of Cu, Mn and Fe, and especially Cu is highly enriched (>100-186 $\mu g\ g^{-1}$ dry weight) in the rocks near the study area (Jwa and Lee, 1992). Berkman *et al.* (1992) also reported that trace metal concentrations were higher in the shells of the Antarctic scallop *Adamussium colbecki* in shallow water adjacent to glacial meltwater runoff, suggesting that the significant decreases in the concentrations of Fe, Mn, Cu and Zn with depth be caused by the decreasing influence of the glacial runoff towards the offshore.

Variations of Fe, Mn and Cd in **N. concinna** *between the two sites*

There were significant differences in Fe, Mn and Cd concentrations between the two groups of limpets from the monitoring and from the reference sites, and this also seems to be related to the metal-laden ice-melt water input. The input of ice-melt water was reported to vary regionally within the Maxwell Bay (Ahn *et al.*, 1996). Higher Fe and Mn concentrations in the limpets from the monitoring site indicate larger amount of input of ice-melt water laden with the heavy metals. On the other hand, Cd concentration appears to be higher in the sea water than in ice-melt water, and the higher Cd concentrations in the limpets of the reference site indicate less influence of ice-melt water. Apart from the geochemical process, Cd concentration in the tissues of *N. concinna* may be differentiated also by difference in food availability between the two sites. Density of microphytobenthos which is a significant food source for

N. concinna (Brêthes *et al.*, 1994) could be highly variable over short vertical and/or horizontal distance, and the amount of Cd accumulated in *N. concinna* would be expected to be variable site to site depending on food amount consumed.

Effects of body size of **N. concinna** *on the metal accumulation*

Body size can be important in making data comparable especially when there are considerable differences in metal concentrations among individuals of various body size, and therefore should be taken into consideration in sampling and data analysis. The regression analysis (Figs. 4-8) showed that in general concentrations of the metals decreased with increase in body size. Similar relationships between body size and tissue metal concentrations were frequently reported in other gastropods and bivalves in temperate waters (Peden *et al.*, 1973; Boyden, 1977; Ramelow, 1985). Boyden (1977) observed that Cu, Fe, Pb and Zn varied inversely with size for *Patella* sp., with the only exception of Cd showing a positive correlation. Ramelow (1985), on the other hand, reported that Cd, Cr, Cu, Ni, Pb, Zn decreased with increasing shell length of *Patella* sp., while As, Sr, Fe increased with the size. Thus, the pattern of size-dependent variation seems to differ depending on species, physiological condition and possibley geographic regions.

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