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Cu, Zn and Cd content in different tissues of the Antarctic scallop *Adamussium colbecki*: role of metallothionein in heavy metal homeostasis and detoxication

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ABSTRACT: Cu and Zn concentrations in the gills of the Antarctic scallop *Adamussium colbecki* (Smith, 1902) were found to be significantly lower than in the gills of mussel *Mytilus galloprovincialis* (Lam.) and scallop *Pecten jacobaeus* (L.); however, the Cd concentration was significantly higher. In digestive gland, copper concentrations were not significantly different for all 3 species, but *A. Colbecki* had a lower Zn concentration and an extremely high Cd concentration (ca 27 µg g^{-1} wet wt). In *A. colbecki* digestive gland, about 70 % of the total Cd was associated with the particulate fraction, and the rest was mostly bound to a metallothionein-type protein. In *P. jacobaeus* a major fraction (about 60 %) of the Cd present in the digestive gland was bound to cytosolic metallothioneins. Aging was found not to affect the concentration of Cd bound to metallothioneins in *A. colbecki* digestive gland, although the Cd concentration is slightly reduced in scallops >10 yr old compared to younger (3 to 7 yr old) scallops.

INTRODUCTION

The mechanisms of heavy metal cation homeostasis in cells of marine lamellibranch molluscs have been widely investigated (Coombs 1980, Viarengo 1989). These filter feeding organisms are known to accumulate high concentrations of heavy metals (such as Cu, Cd, Zn and Hg) in their tissues. This is due to the fact that in the cells of these organisms heavy metal cations can be rapidly detoxified by different mechanisms, i.e. compartmentalization into lysosomes, accumulation into granules and membrane-bound vesicles or binding to specific soluble ligands such as metallothioneins (Viarengo 1989, George & Viarengo 1985, Viarengo & Nott 1993). Metallothioneins are a class of metal-rich, low-molecular-weight, cysteine-rich proteins, with high affinity and binding capacity for heavy metal cations. These biochemical characteristics make them unique in playing a primary role in regulating the concentration of free heavy metal ions in the cells (Kagi & Kojima 1987). Little is known, however, about the mechanisms of metal homeostasis and about the role of metallothioneins in Antarctic marine organisms which have adapted evolutionally to peculiar environmental conditions (including low temperature, high levels of dissolved oxygen and characteristic photoperiod).

In this work both the heavy metal content and the level of metallothioneins in the tissues of the Antarctic scallop *Adamussium colbecki* (Smith, 1902) were evaluated. The results were compared to data obtained from the Mediterranean molluscs *Pecten jacobaeus* (L.) and *Mytilus galloprovincialis* (Lam.). In addition, the effect of aging on metal and metallothionein content in the digestive gland cells of the Antarctic scallop was studied.

MATERIALS AND METHODS

Molluscs. Specimens of *Mytilus galloprovincialis* (6 cm shell length) and *Pecten jacobaeus* (6 cm shell length) were obtained from La Spezia and Chioggia (Mediterranean Sea), respectively, in October 1990.

Specimens of *Adamussium colbecki* of the same size, corresponding to ca 7 yr old individuals, were obtained from Terranova Bay (74° S, 163° E; Antarctic Ross Sea). Tissues from pools of 8 to 10 individuals were dissected and immediately frozen at -70 °C.

In the analysis concerning the effects of aging on Cd and metallothionein content, specimens of *Adamussium colbecki* of 3 different sizes corresponding to different-aged animals (Stockton 1984) (3-4, 6-7, ≥ 10 yr) were utilized. All Antarctic scallops were sampled during austral summer (January) 1990.

An experiment involving exposure to Cd was also performed in order to obtain a Cd-thionein enriched fraction from the tissues of *Mytilus galloprovincialis*. Mussels 6 cm long were kept for 3 d in an aquarium in static tanks containing aerated, EDTA-free artificial sea water (1 l per mussel) at 15 °C (Viarengo et al. 1985). Mussels were then exposed to Cd (200 µg ind.$^{-1}$ l^{-1}) for 9 d. During the experiments the artificial sea water and the metal, added in the form of $CdCl_2$ stock solutions, were changed daily.

Preparation of subcellular fractions. Tissues were homogenized in 3 volumes of 0.5 M sucrose, 150 mM NaCl, 20 mM Tris-HCl pH 8.6, 0.006 mM leupeptine, 0.5 mM PMSF (phenylmethylsulphonyl fluoride) and 1 mM DTT (dithiothreitol). The homogenate was then centrifuged at 100 000 × *g* for 90 min at 0 to 4 °C to obtain the cytosolic fraction. The pellet, containing the particulate fraction, was washed once with homogenization buffer, resuspended in the same buffer, and utilized for the determination of Cd content.

Metallothioneins were partially purified by heating the cytosol at 60 °C for 10 min in the presence of 1 mM DTT. Samples were then centrifuged at 20 000 × *g* for 15 min at 0 to 4 °C; the supernatant contained metallothionein. Before HPLC (high performance liquid chromatography) analysis, the samples were treated with ethanol as described by Kimura et al. (1979) to remove low-molecular-weight soluble thiols.

The pellet obtained by this procedure was washed once with 5 mM K-phosphate buffer, pH 7.4, resuspended with the same buffer and used to measure the metal bound to heat-denaturated cytosolic proteins.

Determination of Cd, Zn and Cu by inductively coupled plasma - atomic emission spectroscopy (ICP-AES). Determination of the metal content in whole homogenates and in the different subcellular fractions was performed by ICP-AES utilizing a Jobin Yvon (JY24) apparatus at a wavelength of 324.754 nm for Cu, 213.856 nm for Zn, and 214.438 nm for Cd. The samples (aliquots of 1 ml homogenate, cytosolic fraction, or particulate fraction) were hydrolyzed with 2 ml of 65 % HNO_3 at 70 °C for 2 h as described previously (Viarengo et al. 1988). The emission intensity of the samples was evaluated at a wavelength typical of each metal. The metal concentration was calculated with the standard addition method (SMA), utilizing another set of samples to which known amounts of Cu, Zn, Cd had been added (final concentrations ranging from 0.05 to 2 µg ml^{-1}) to give a linear response under ICP-AES. A standard reference material, oyster tissue (NBS 1566), was included in the analyses as a quality control sample. This standard was treated in the same manner as other samples. The NBS oyster standard, with certified concentrations (µg g^{-1} dry wt) of Cd 3.5, Cu 63, and Zn 852, was found to contain Cd 3.7 ± 0.15, Cu 65 ± 1.2, and Zn 860 ± 5.1 (mean of quadruplicate determinations ± SD).

Evaluation of metallothionein content. Metallothioneins were separated by HPLC on an Ultropac TSK G3000 SW gel filtration column (LKB Pharmacia), using as an eluent 5 mM K-phosphate buffer pH 7.4, at a flow rate of 0.7 ml min^{-1}. The elution of the protein was monitored at 280 or 254 nm with a Varian Star 9065 Polychrom Ultraviolet Diode Array detector and the metal content evaluated by directly connecting the chromatographic column to the torch of the ICP system (Mazzucotelli et al. 1991). Metallothioneins are characterized by a low absorbance at 280 nm, with a broad shoulder at 254 nm (Cd-thioneins; see Fig. 1C) or 272 nm (Cu-thioneins) (Stillman et al. 1987).

Alternatively, the column was connected to an electrochemical detector (Waters 460) set at +0.4 V to evaluate the sulphydrylic (SH) group content in the eluate. Metallothioneins, which bind large amounts of heavy metal cations by the formation of tetrathiolate clusters, can be identified by their extremely high thiol content (20 to 30 % cysteine) compared to other proteins.

The system was calibrated utilizing standard rabbit liver Cd,Zn-thionein, bovine erythrocyte Cu,Zn superoxide dismutase (SOD) and soluble low-molecular-weight thiols (cysteine and reduced glutathione).

Statistical analysis. Data, representing the means ± SD of 6 experiments, each consisting of triplicate samples, were compared by either Mann-Whitney *U*-test or Student's *t*-test (Siegel 1956).

Materials. All reagents were of analytical or HPLC grade. Rabbit liver Cd,Zn-thionein (M-7641), bovine erythrocyte superoxide dismutase (EC 1.15.1.1). (S-2515), reduced glutathione (GSH) (G-4251) and L-cysteine (C-7755) were from Sigma Chimica (Milano, Italy).

RESULTS

The results presented in Table 1 show that in the gills of *Adamussium colbecki* the concentration of Cu was lower than in those of *Pecten jacobaeus* and *Mytilus galloprovincialis* ($p \leq 0.05$). The Zn content was lower in

Table 1. *Adamussium colbecki*, *Pecten jacobaeus* and *Mytilus galloprovincialis*. Heavy metal content (Cu, Zn, Cd) in the gills and digestive gland (μg g^{-1} wet wt). Heavy metal content was evaluated by ICP-AES as described in the 'Materials and methods' Data are means ± SD of at least 6 experiments. Identical letters in each row indicate that values are not significantly different at the $p \leq 0.05$ level (Mann-Whitney *U*- and Student's *t*-tests)

	A. colbecki	*P. jacobaeus*	*M. galloprovincialis*
Gills			
Cu	1.39 ± 0.31^{A}	2.42 ± 0.57^{B}	2.28 ± 0.14^{B}
Zn	11.66 ± 3.66^{A}	13.43 ± 2.45^{A}	31.83 ± 14.71^{B}
Cd	2.80 ± 0.38^{A}	0.25 ± 0.18^{B}	0.29 ± 0.02^{B}
Digestive gland			
Cu	3.52 ± 0.49^{A}	3.13 ± 0.55^{A}	3.17 ± 0.26^{A}
Zn	17.42 ± 2.91^{A}	28.63 ± 3.29^{B}	34.15 ± 4.61^{C}
Cd	26.51 ± 6.09^{A}	7.19 ± 1.23^{B}	3.69 ± 1.65^{C}

both the scallops than in mussels ($p \leq 0.002$ and $p \leq 0.001$, respectively). On the other hand, in *A. colbecki* Cd tissue content was significantly higher than in *M. galloprovincialis* ($p \leq 0.01$) and *P. jacobaeus* ($p \leq 0.001$).

Comparable values of Cu were observed in the digestive gland of the 3 different molluscs. As in the gills, the Zn concentration was lower in the 2 Pectinidae than in mussels. In the digestive gland of *Adamussium colbecki*, an extremely high Cd concentration was found (about 4 times higher than in *Pecten jacobaeus* and 7 times higher than in *Mytilus galloprovincialis*) ($p \leq 0.001$). Such a large amount of Cd has been previously reported in the digestive gland of mussels (*M. galloprovincialis*) exposed for 3 d to Cd (200 μg l^{-1} $ind.^{-1}$) (Viarengo et al. 1985).

Data reported in Table 2 demonstrate that in the digestive gland of *Adamussium colbecki* about 70 % of the total Cd was associated with the particulate fraction, whereas the remaining 30 % was present in the cytosol and mainly bound to a specific soluble, heat-stable protein fraction. In *Pecten jacobaeus* about 60 % of the Cd present in the digestive gland was bound to a soluble metalloprotein.

Table 2. *Adamussium colbecki* and *Pecten jacobaeus*. Cd concentration in the cytosol, metallothionein and particulate fraction obtained from the digestive gland (μg g^{-1} wet wt). Cd content was evaluated by HPLC/ICP-AES as described in the 'Materials and methods' Data are means ± SD of at least 6 experiments

	Cytosolic Cd content	Metallothionein-associated Cd	Particulate fraction Cd content
A. colbecki	4.88 ± 0.92	4.10 ± 0.96	18.83 ± 2.96
P. jacobaeus	4.91 ± 0.98	4.15 ± 0.92	1.60 ± 0.21

To investigate the nature of the Cd-binding protein present in the cytosol of the digestive gland cells of the Pectinidae, this Cd-rich protein was partially purified and biochemically characterized. These data were compared with those obtained from the digestive gland of Cd-exposed mussels in which, as previously demonstrated, cytosolic Cd is mostly bound to a metallothionein (Frankenne et al. 1980, Viarengo et al. 1985, Viarengo 1989).

Soluble extracts obtained from scallop and mussel digestive gland were analyzed by HPLC on a gel filtration column. The system was opportunely calibrated with a mixture of standard rabbit liver Cd,Zn-thionein, superoxide dismutase (SOD), reduced glutathione (GSH) and cysteine. Fig. 1 shows the chromatograms obtained by detecting the UV absorbance (Panel A) and the Cd and Cu content (Panel B) in the eluate. In Panel C the typical UV absorbance spectrum of the standard Cd,Zn-thionein peak is shown. Fig. 1A also indicates that the GSH peak elutes close to the metallothionein peak. Therefore, samples were treated with organic solvents prior to injection into the column, to remove possible interference of low-molecular-weight thiols (Kimura et al. 1979).

In Fig. 2 the chromatograms for the HPLC separation of metallothionein obtained from the digestive gland of Cd-exposed *Mytilus galloprovincialis* and non-exposed *Adamussium colbecki* and *Pecten jacobaeus* are presented. When cytosolic extracts of gills and digestive gland of Cd-exposed mussels are analyzed by gel filtration chromatography, a large Cd-thionein peak is usually resolved, corresponding to 10 000 daltons molecular weight (Frankenne et al. 1980, Viarengo et al. 1985). The eluates were monitored by a diode array detector to evaluate the absorbance at 280 nm and by ICP-AES to detect Cu and Cd content (Fig. 2, Panels 1A, 2A & 3A). The absorption spectra of the 3 Cd-proteins are also shown (Fig. 2, Panels 1B, 2B & 3B). In addition, the distribution of the SH residues of the peptides was evaluated by an electrochemical detector (Fig. 2, Panels 1C, 2C & 3C).

As shown in Fig. 2, the Cd-thionein peak in the soluble extracts of Cd-exposed mussels had the same retention time as the Cd peak present in the extracts from the digestive gland of non-exposed *Adamussium colbecki* and *Pecten jacobaeus*. Moreover, the low UV absorption spectra were similar and typical of Cd-thioneins, due to the virtual lack of aromatic amino acids, with a maximum at 254 nm due to the presence of Cd-tetrathiolate clusters. Finally, the data obtained by electrochemical detection show that the Cd-protein

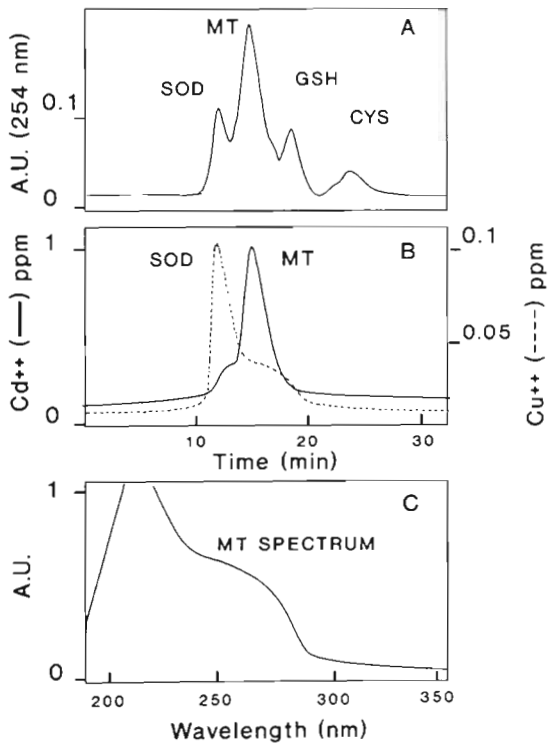

Fig. 1. Elution profiles from HPLC gel filtration chromatography of a mixture containing bovine erythrocyte superoxide dismutase (SOD), standard rabbit liver Cd, Zn-thionein (MT), reduced glutathione (GSH) and cysteine (CYS). (A) Absorbance at 254 nm. (B) Cd and Cu content. (C) Absorbance spectrum, obtained by diode array detection, of the standard Cd,Zn-thionein peak shown in (A)

from *A. colbecki* and *P. jacobaeus*, similarly to the Cd-thionein from mussel digestive gland, has an extremely high sulphydryl content, characteristic of metallothioneins.

In Table 3 data are presented on total and thionein-bound Cd in the digestive gland of *Adamussium colbecki* of 3 different ages (3–4, 6–7, ≥10 yr). The results indicate that the Cd concentration is higher in smaller and younger, growing individuals than in larger, older ones, although the difference is not statistically significant. However, the percentage of thionein-bound Cd is significantly higher in >10 yr old individuals (36%) than in younger ones (about 24%) ($p \leq 0.01$ Mann-Whitney *U*-test).

DISCUSSION

The data demonstrate that the gills and digestive gland of the 3 molluscs, *Adamussium colbecki*, *Pecten jacobaeus*, and *Mytilus galloprovincialis*) contain different but comparable concentrations of essential elements such as Cu and Zn. The observed differences in metal content could reflect requirements of essential heavy metals specific for the 3 molluscs, but they could be also interpreted as seasonal fluctuations due to the different physiological status of the animals (reproductive cycle, food intake). Indeed, in both scallops and mussels the heavy metal tissue concentration can vary by about 2 to 3 times among the different seasons (Bryan 1973, Martino 1987).

The concentration of Cd, a metal usually considered an environmental contaminant, is extremely high in the tissues of the Antarctic mollusc *Adamussium colbecki* relative to the 2 Mediterranean species. High Cd levels in the digestive gland of scallops from United Kingdom waters have previously been reported (Bryan 1973, Stone et al. 1986). In the digestive gland of the 2 scallops, higher concentrations of Cd are associated with lower Zn levels. The blue mussel *Mytilus edulis* is often utilized in monitoring programs due to its ability to concentrate pollutants and, among these, heavy metals such as Cd, Hg and Pb (Viarengo & Canesi 1991). However, this organism shows gill and digestive gland Cd levels which are significantly lower than those observed not only in the Mediterranean but also in the Antarctic scallop.

The fact that the digestive glands of Pectinidae from both temperate and Antarctic seas contain such a large amount of Cd, as also indicated by the total Cd body burden (Berkman & Nigro 1992), appears likely to be related to their high feeding rates (Palmer & Rand 1977). Indeed, the data concerning the Cd tissue distribution seem to indicate that in these molluscs most of the metal is taken up with the food, since the metal concentration is low in the gills and extremely high in the digestive gland. In Cu-exposed mussels copper, when present as a solute in the sea water, is mainly accumulated in the gills. In contrast, the metal concentration increases more rapidly in the digestive gland when copper is present in the sea water associated with particulate matter (Viarengo unpubl.).

Moreover, data on metal content of the plankton and particulate fraction from the Mediterranean (Baffi et al. 1983) and the Antarctic Ross Sea (Frache et al. 1990) show that the ratio between Cd and some essential metals such as Fe, Cu and Zn is higher in the Antarctic samples.

These data seem to confirm the possibility that the high Cd concentration found in the digestive gland of *Adamussium colbecki* could reflect a 'physiological' accumulation of Cd with its food.

Regarding the subcellular distribution of Cd, in the digestive gland cells of the 2 Pectinidae this metal is partly associated with the particulate fraction and partly present in the cytosol. However, it is important to note that cytosolic Cd is mostly bound in a non-toxic form to a protein which is classed with the

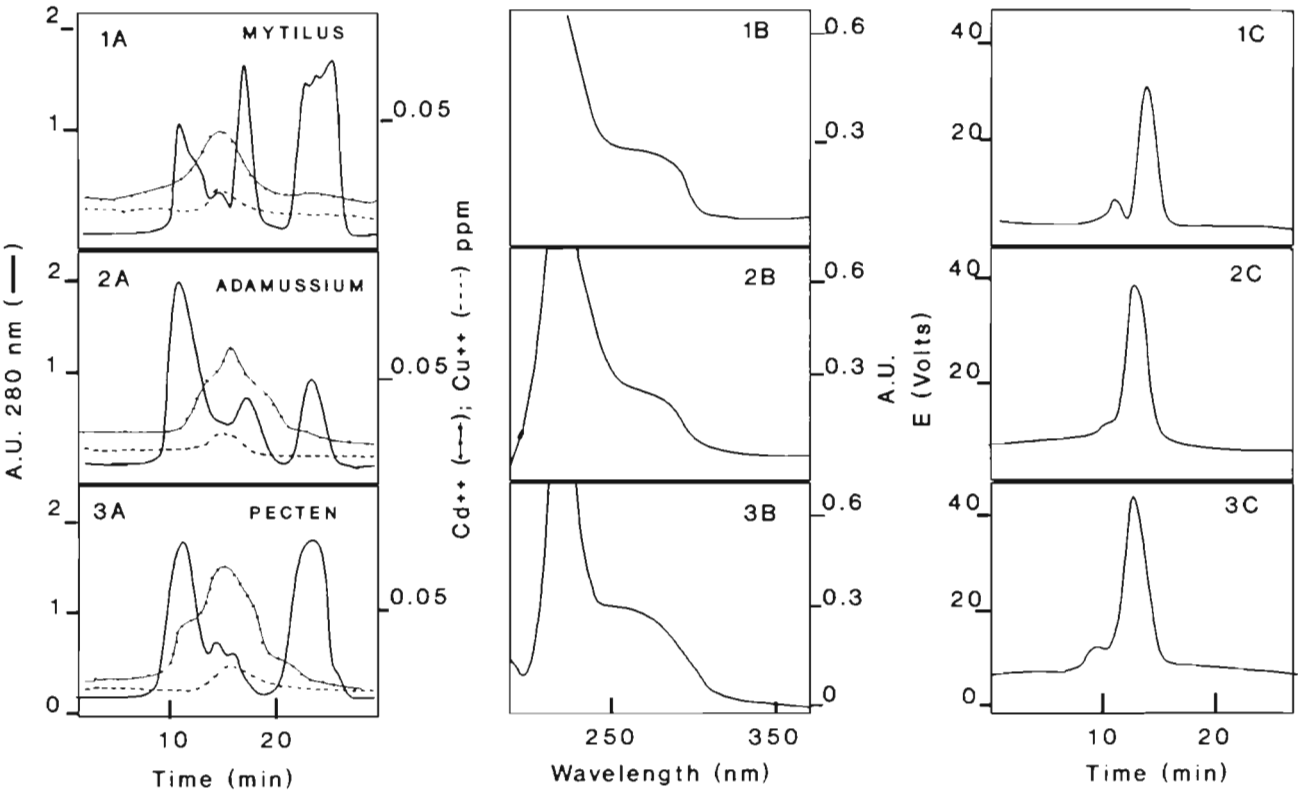

Fig. 2. Elution profiles from HPLC gel filtration chromatography of soluble extracts obtained from the digestive gland of Cd-exposed *Mytilus galloprovincialis* (1A, 1B, 1C), and non-exposed *Adamussium colbecki* (2A, 2B, 2C) and *Pecten jacobaeus* (3A, 3B, 3C). Panels 1A to 3A: Absorbance at 280 nm and Cd and Cu content. Panels 1B to 3B: Absorption spectra of the metalloprotein peak. Panels 1C to 3C: Electrochemical detection of SH groups

metallothioneins. In fact, this Cd-binding protein has some typical characteristics of metallothioneins, i.e. it is a soluble, low-molecular-weight, heat-stable protein with high metal content. Moreover, the absorption spectra of the Cd-protein obtained by diode array detection during HPLC gel filtration are typical of metallothionein, as they have a maximum at 254 nm and are almost identical to the absorption spectrum of the Cd-thionein obtained from Cd-exposed mussels (George et al. 1979, Frankenne et al. 1980). In addition, the Cd protein present in the 2 Pectinidae, similarly to the mussel Cd,Zn-thionein, shows an extremely high SH content, as judged by electrochemical detection.

Table 3. *Adamussium colbecki*. Total Cd and thionein-associated Cd in the digestive gland of scallops of 3 different ages. Heavy metal content was evaluated by ICP-AES as described in the 'Materials and methods' Data, expressed as $\mu g\ g^{-1}$ tissue wet wt, are means ± SD of at least 6 experiments

Age of scallops (yr)	Cd concentration	Metallothionein-associated Cd
3–4	22.87 ± 7.48	5.43 ± 0.54
6–7	22.96 ± 7.80	5.41 ± 1.64
≥10	15.78 ± 5.77	5.61 ± 1.14

The data on the subcellular distribution of Cd in the digestive gland seem to demonstrate that in the 2 Pectinidae the compartmentalization of Cd in the cell particulate fraction could represent a second mechanism of Cd ion homeostasis (in *Adamussium colbecki* it accounts for about 70% of total Cd). Recent data, obtained by electron microscopic analysis of *A. colbecki* digestive gland did not show Cd-containing granules (Mauri et al. 1990). Therefore, the possibility that Cd is associated with membrane components also cannot be ruled out at present. Different authors have recently proposed that Cd could play some physiological role in molluscs (Evtushenko et al. 1990, Wlostowski 1992). Considering that in the digestive gland of both Pectinidae the high Cd concentration is associated with low Zn levels, it could be argued that, in these organisms, Cd plays some physiological role by substituting for Zn in digestive gland cell structures and/or enzymes.

Finally, in *Adamussium colbecki* the total Cd content of the whole digestive gland is higher in larger (≥ 10 yr)

individuals than in smaller (3 to 4 yr) ones, being proportional to the digestive gland weight (data not shown). On the other hand, Cd concentration (expressed as $\mu g\ g^{-1}$ wet wt) is lower in older *A. colbecki*, which is probably related to lower feeding rates.

Usually Cd, due to its long biological half-life, (5 to 6 mo in mussels; Viarengo et. al 1985), is accumulated in scallop tissues with increasing age (Evtushenko et al. 1990). However, as mentioned before, seasonal factors can influence metal metabolism in marine organisms. In particular, plankton and particulate matter that constitute the diet of these animals are particularly rich in Cd in the Antarctic Ross Sea. The animals utilized in this study were sampled during the austral summer, when food uptake is presumably very high. Therefore, it seems that the influence of seasonal factors could be stronger than that of aging in determining the accumulation of Cd in the tissues of Antarctic scallops.

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LITERATURE CITED

Baffi, F., Dadone, A., Frache, R. (1983). Heavy metals in inshore waters near Genoa. Mar. Pollut. Bull. 14: 469–471

Berkman, P. A., Nigro, M. (1992). Trace metal concentrations in scallops round Antarctica: extending the Mussel Watch Programme to the Southern Ocean. Mar. Pollut. Bull. 24: 322–323

Bryan, G. W. (1973). The occurence and seasonal variation of trace metals in the scallops *Pecten maximus* (L.) and *Chlamys opercularis* (L.). J. mar. biol. Ass. U.K. 53: 145–166

Coombs, T. L. (1980). Heavy metal pollutants in the aquatic environment. In: Gilles, R. (ed.) Animal and environmental fitness, Vol 1. Pergamon Press, Oxford, p. 283–302

Evtushenko, Z. S., Lukyanova, O. N., Belcheva, N. N. (1990). Cadmium bioaccumulation in organs of the scallop *Mizuhopecten yessoensis*. Mar. Biol. 104: 247–250

Frache, R., Baffi, F., Cosma, B., Mazzucotelli, A., Bottinelli, C., Sena, F., Soggia, F. (1990). Trace metal distribution in water, particulate matter and sediments in the Ross Sea and Inland lakes (Antarctica). Proceedings from 'Environmental impact in Antarctica', Roma, June 1990, p. 34–47

Frankenne, F., Noel-Lambot, F., Disteche, A. (1980). Isolation and characterization of metallothioneins from cadmium-loaded mussel *Mytilus edulis*. Comp. Biochem. Physiol. 66C: 179–186

George, S. G., Carpené, E., Coombs, T. L., Overnell, J., Joungson, A. (1979). Characterization of cadmium-binding proteins from the common mussel *Mytilus edulis*. Biochim. biophys. Acta B 580: 225–233

George, S. G., Viarengo, A. (1985). An integration of current knowledge of the uptake, metabolism and intracellular control of heavy metals in mussels. In: Vernberg, F. J., Thurberg, F. P., Calabrese, A., Vernberg, W. B. (eds.) Marine pollution and physiology: recent advances, University of South Carolina Press, Columbia, p. 125–144

Kagi, J. H. R., Kojima, Y (eds.) (1987). Metallothionein. Experientia 52 (Suppl.). Birkhauser, Basel

Kimura, M., Otaki, N., Imano, M. (1979). Rabbit liver metallothionein tentative amino acid sequence of metallothionein B. In: Kagi, J. H. R., Nordberg, M. (eds.) Metallothionein. Experientia, Suppl. 34. Birkhauser, Basel, p. 163–168

Martino, G. (1987). Studio degli effetti di alcuni parametri ambientali quali temperatura, salinitá, ossigeno e rame sull'omeostasi e sulla tossicitá dei metalli pesanti nel mitilo *Mitylus galloprovincialis* Lam. Possibili applicazioni nell'ambito dell'ecotossicologia. Ph.D. thesis, Univ. of Genoa

Mauri, M., Orlando, E., Nigro, M., Regoli, F. (1990). Heavy metals in the Antarctic scallop *Adamussium colbecki*. Mar. Ecol. Prog. Ser. 67: 27–33

Mazzucotelli, A., Viarengo, A., Canesi, L., De Paz, F., Ponzano, E., Rivaro, P. (1991). Investigation of organometallics in the marine ecosystem by high performance liquid chromatography with inductively coupled plasma atomic emission spectrometric and electrochemical detection. Analyt. Proc. 28: 79–80

Palmer, J. B., Rand, G. M. (1977). Trace metal concentration in two shellfish species of commercial importance. Bull. environ. Contam. Toxicol. 18: 512–519

Siegel, S. (1956). Non-parametric statistics for the behavioral sciences. McGraw-Hill, New York

Stillman, M. J., Law, A. Y. C., Cai, W., Zelazowski, A. J. (1987). Information on metal binding properties of metallothioneins from optical spectroscopy. In: Kagi, J. H. R., Nordberg, M. (eds.) Metallothionein. Experientia, Suppl. 34. Birkhauser, Basel, p. 203–211

Stockton, W. L. (1984). The biology and ecology of the epifaunal scallop *Adamussium colbecki* on the west side of McMurdo Sound, Antarctica. Mar. Biol. 78: 171–178

Stone, H. C., Wilson, S. B., Overnell, J. (1986). Cadmium binding components of scallop (*Pecten maximus*) digestive gland. Partial purification and characterization. Comp. Biochem. Physiol. 85C: 259–268

Viarengo, A. (1989). Heavy metals in marine invertebrates: mechanisms of regulation and toxicity at the cellular level. CRC crit. Rev. aquat. Sci. 1: 295–317

Viarengo, A., Canesi, L. (1991). Mussels as biological indicators of pollution. Aquaculture 94: 225–243

Viarengo, A., Mancinelli, G., Martino, G., Pertica, M., Canesi, L., Mazzucotelli, A. (1988). Integrated cellular stress indices in trace metal contamination: critical evaluation in a field study. Mar. Ecol. Prog. Ser. 46: 65–70

Viarengo, A., Nott, J. A. (1993). Mechanisms of heavy metal cation homeostasis in marine invertebrates. Comp. Biochem. Physiol. (in press)

Viarengo, A., Palmero, S., Zanicchi, G., Orunesu, M. (1985). Role of metallothioneins in Cu and Cd accumulation and elimination in the gill and digestive gland cells of *Mytilus galloprovincialis* Lam. Mar. environ. Res. 16: 23–26

Wlostowski, T. (1992). Seasonal changes in subcellular distribution of zinc, copper, cadmium and metallothionein in the liver of bank vole (*Clethrionomys glareolus*): a possible essential role of cadmium and metallothionein in the hepatic metabolism of copper. Comp. Biochem. Physiol. 101C: 155–162

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