

TRACE METAL RESIDUES IN TISSUES OF TWO CRUSTACEAN SPECIES FROM THE BAHIA BLANCA ESTUARY, ARGENTINA

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Abstract. Trace metals (total mercury, cadmium and zinc) were studied in several tissues of the edible shrimps *Pleoticus muelleri* and *Artemesia longinaris* from the Bahía Blanca estuary, Argentina. The digestive gland was shown to be the main organ in which metals accumulate, followed by the stomach–alimentary canal and abdominal muscle.

The metal contents found in the present study largely exceeded those of the same species caught from the Argentine Sea. Mercury concentrations increased proportionally to the total length of the shrimps studied. Only little variation in metal concentrations among individuals seemed to exist.

Finally, the usefulness of these species as bioindicators of trace metal pollution in the Bahía Blanca estuary is discussed.

1. Introduction

Marine organisms usually have levels of heavy metals higher than the surrounding seawater, and the corresponding concentration factors may reach many thousand-fold. Those organisms living in contact with the sediments where metals accumulate are known to concentrate trace metals in their body tissues (Anderson *et al.*, 1978). Thus, through biological amplification some aquatic organisms may build up concentrations of metals present at low amounts in the environment to levels which are harmful to the organisms, and which exceed public health standards (Phillips, 1976; Ahsanullah *et al.*, 1981). This ability of various aquatic organisms to concentrate trace metals has led to their use as indicators of metal levels in coastal and estuarine environments (Popham *et al.*, 1980; Romeo and Gnassia-Barelli, 1988; Fischer, 1989), including several large-scale coastal monitoring programmes (e.g., Goldberg *et al.*, 1978).

Although a large volume of data on trace metal accumulation in crustaceans from different environments has been published (Bryan *et al.*, 1985; Bagatto and Alikhan, 1987(a); 1987(b); White and Rainbow, 1987), information on South Western Atlantic Ocean species is scarce, and only a few papers are available (Pérez *et al.*, 1986; Marcovecchio *et al.*, 1988(a)).

The main purpose of this paper is to supply basic data on the heavy metal levels – total mercury, cadmium and zinc – of two penaeid species (*Pleoticus muelleri* and *Artemesia longinaris*) which inhabit the seawater of Bahía Blanca, Argentina.

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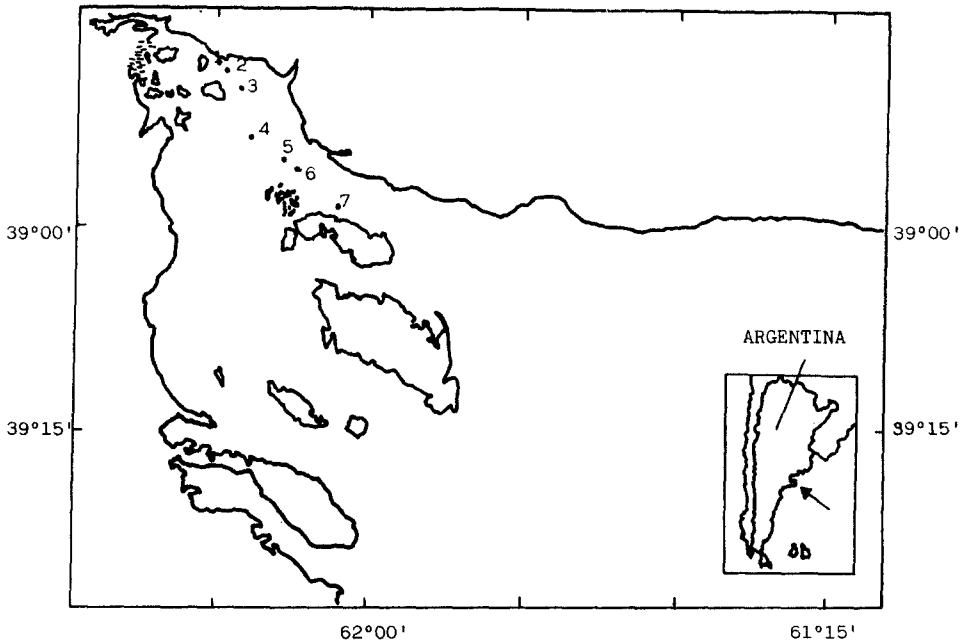


Fig. 1. Location of sampling stations in the Bahía Blanca estuary.

Simultaneously, (1) the variation of metal concentrations among individual and species, and (2) the relationship between metal content and size of the shrimp were considered. Furthermore, we intended to evaluate the extent to which these shrimp species are representative indicators of their environments.

2. Materials and Methods

2.1. STUDY AREA

The Bahía Blanca estuary is located between $38^{\circ}45'$ and $39^{\circ}40'S.$, and $61^{\circ}45'$ and $62^{\circ}30'W.$, in the south-eastern Buenos Aires Province, Argentina (Figure 1). Water interchange within the bay is influenced by a semi-diurnal tidal regime. The bay encompasses an area of 400 km^2 , and at high tide the total area is nearly 1300 km^2 (Villa and Pucci, 1987). The hydrography of the estuary is strongly influenced by climatological conditions (Freije *et al.*, 1981). Several streams and canals flow into the bay, most of them affected by anthropogenic activities. The freshwater contribution is approximately $4000 \text{ m}^3 \text{ day}^{-1}$. The average tides of 3 m, together with an associated tidal excursion of about 80 km, ensure thorough vertical mixing of the water column (Villa and Pucci, 1987).

The northern boundaries of the bay are the sites of various ports, towns and industries. Nearly $10 \text{ m}^3 \text{ day}^{-1}$ of raw sewage is discharged into the study area. Furthermore, the bay is extensively used by shallow draft vessels, fishing boats,

oil tankers, and cargo vessels throughout the whole year, and requires regular maintenance dredging. As an example, during the period 1980–1981, nearly 3×10^3 m³ of dredged materials were dumped into the bay.

All the abovementioned features mean that one can assume that the estuary receives a substantial amount of contaminant wastes from different sources, which could endanger its natural balance. These circumstances, together with the estuary-type dynamics, make the Bahía Blanca estuary an appropriate environment for developing pollution studies.

2.2. SAMPLING AND ANALYSIS

A programme for sampling marine organisms was carried out monthly at Bahía Blanca esuary during 1986–1987. Samples of two shrimp species – *Pleoticus muelleri* and *Artemesia longinaris* (Crustacea; Decapoda; Penaeidae) – were taken by fishing nets from seven sampling stations in the estuary (Figure 1). The sample included specimens of all the sizes caught by the net, which were mainly adults; in the same way, animals of both sexes were included for the present study. After biometric measurements were taken, the specimens were dissected, and the abdominal muscle (in the case of *Artemesia longinaris*), and digestive gland, stomach–alimentary canal and abdominal muscle (in the case of *Pleoticus muelleri*) were removed. All samples were stored in plastic bags and frozen at -20°C until analysis.

Total mercury was determined by flameless atomic absorption spectrophotometry (AAS), after wet digestion according to a previously reported method (Uthe *et al.*, 1970). The concentrations of cadmium and zinc were determined by AAS – with an air/acetylene flame – and with deuterium background correction, as previously reported by Marcovecchio *et al.* (1988(b)). A Shimadzu AA–640–13 instrument was used for the analyses. Analytical grade reagents were used for the blanks and calibration curves. Accuracy and precision were checked against the standard reference material No. 6 of the National Institute of Environmental Studies (NIES), Tsukuba (Japan), and were always within the range of certified values.

Comparisons among metal concentrations from species were performed by one-way analysis of variance (ANOVA).

3. Results and Discussion

The distribution of total mercury, cadmium and zinc in tissues of the shrimps *Pleoticus muelleri* and *Artemesia longinaris* show that the digestive gland is the most important organ for the accumulation of the three metals, followed by the stomach–alimentary canal and muscle (Table I). Analysis of variance showed that significant differences ($p < 0.01$) existed between the metal concentrations of the three tissues analyzed. This sequence of metal tissue concentrations found in Bahía Blanca shrimps fully coincide with that reported by White and Rainbow (1986(a))

TABLE I

Metal concentrations ($\mu\text{g/g}$, wet wt.) in tissues of the crustacean species analyzed from the Bahía Blanca estuary. S.D. = standard deviation; CV% = coefficient of variation; Min–Max = minimum and maximum values; n = number of samples analyzed.

		<i>Pleoticus muelleri</i> ($n = 85$)			<i>Artemesia longinaris</i> ($n = 165$)
		Muscle	Digestive gland	Stomach and gut	Muscle
Mercury	Mean	0.04	5.04	0.74	0.04
	S.D.	0.04	1.03	0.12	0.03
	CV%	103	20.4	16.2	70.0
	Min–Max.	0.0–0.16	0.0–6.18	0.61–0.96	0.0–0.09
Cadmium	Mean	0.16	0.25	0.21	0.13
	S.D.	0.06	0.05	0.06	0.04
	CV%	34.8	20.2	28.6	30.1
	Min–Max.	0.09–0.34	0.15–0.34	0.14–0.32	0.06–0.23
Zinc	Mean	9.35	67.6	22.2	0.71
	S.D.	1.97	15.2	5.21	0.13
	CV%	21.1	22.5	23.4	7.85
	Min–Max.	4.36–14.5	0.0–88.0	14.26–30.05	7.56–10.7

for *Palaemon elegans* from the United Kingdom coasts. The high accumulation of metals in the digestive gland of *Pleoticus muelleri* might be related to the occurrence of low molecular weight binding (metallothionein-like) proteins, which have been extensively reported for different crustacean species (Olafson *et al.*, 1979; Overnell, 1982; White and Rainbow, 1986(b)), but additional research is necessary to confirm this.

On the other hand, White and Rainbow (1986(a)) have opportunely reported that the hepatopancreas (or digestive gland) of crustaceans can accumulate metals at a rate of approximately 100-fold that of muscle. Furthermore, this is also sustained by the fact that the digestive gland – according to Brown (1982) – is the main regulatory organ in crustacean species, and as such would be the major site for metal storage and detoxification in these animals.

The metal concentrations found in stomach–alimentary canal of *Pleoticus muelleri* are likely related to their contents. These shrimps are basically detritivorous feeders (Angelescu and Boschi, 1959; Boschi, 1969), and large amounts of sediments and particulate matter are usually included in the food. These species have partially benthic habits, and they are strongly related to the sediments, which are a major reservoir of metals in the aquatic environment (Salomons and Förstner, 1984). Many vertical migrating benthic organisms – which alternately inhabit the

TABLE II

Correlation matrix of length vs. metal contents, and metal vs. metal in tissues of *Pleoticus muelleri*. ** = significance level 99% ($p < 0.01$); * = significance level 95% ($p < 0.05$).

	length	Hg musc.	Cd musc.	Zn musc.	Hg d.gl	Cd d.gl	Zn d.gl
length	–						
Hg in muscle	0.849**	–					
Cd in muscle	0.437	0.420	–				
Zn in muscle	0.662	0.679	0.590	–			
Hg in dig.gland	0.738*	0.667	0.357	0.622	–		
Cd in dig.gland	0.535	0.393	0.389	0.492	0.587	–	
Zn in dig.gland	0.560	0.545	0.410	0.670	0.501	0.866**	–

relatively uncontaminated pelagic zone and the contaminated sediments – may accumulate metals from the sediments and return them to the pelagic zone during their vertical migrations (Van Duyn-Anderson and Lasenby, 1986). Consequently, fish that feed on these organisms may be exposed to a significant source of metals. Concentrations of mercury in benthic invertebrates living in uncontaminated sediments are usually lower than 0.01 ppm (Huckabee *et al.*, 1979). The concentrations observed in the present study are therefore higher than what should have been expected in uncontaminated ecosystems. In addition, sediments of Bahía Blanca estuary have been recognized to have high levels of heavy metals (Marcovecchio *et al.*, 1986(a); Pucci, 1988).

The abdominal muscle of the shrimps studied turned out to have the lowest metal contents, even though these values largely exceeded the heavy metal levels reported by Pérez *et al.* (1986) for the same species from the Argentine Sea continental shelf. When the heavy metal concentrations in muscle of both *Artemesia longinaris* and *Pleoticus muelleri* were compared by analysis of variance, no significant differences ($p < 0.01$) were found. This is an important fact, since marine organisms do not bioaccumulate metals to the same extent or at the same rate. Differences in metal bioaccumulation in marine organisms are usually linked with differences in their ecology, in their life cycles, or in their trophic status (Allard and Stokes, 1989).

Vermeer (1972), and Stinson and Eaton (1983) noted that mercury concentrations in the abdominal muscle increased in larger individuals in the crustacean species they examined. To assess whether a similar relationship exists in *Pleoticus muelleri*, metal concentrations were regressed with the corresponding total length (Figure 2). The dependence of muscular mercury on shrimp size correlated significantly ($p < 0.01$), while mercury contents in the digestive gland did so at a low significance level ($p < 0.05$) (Table II). This kind of relationship was not observed

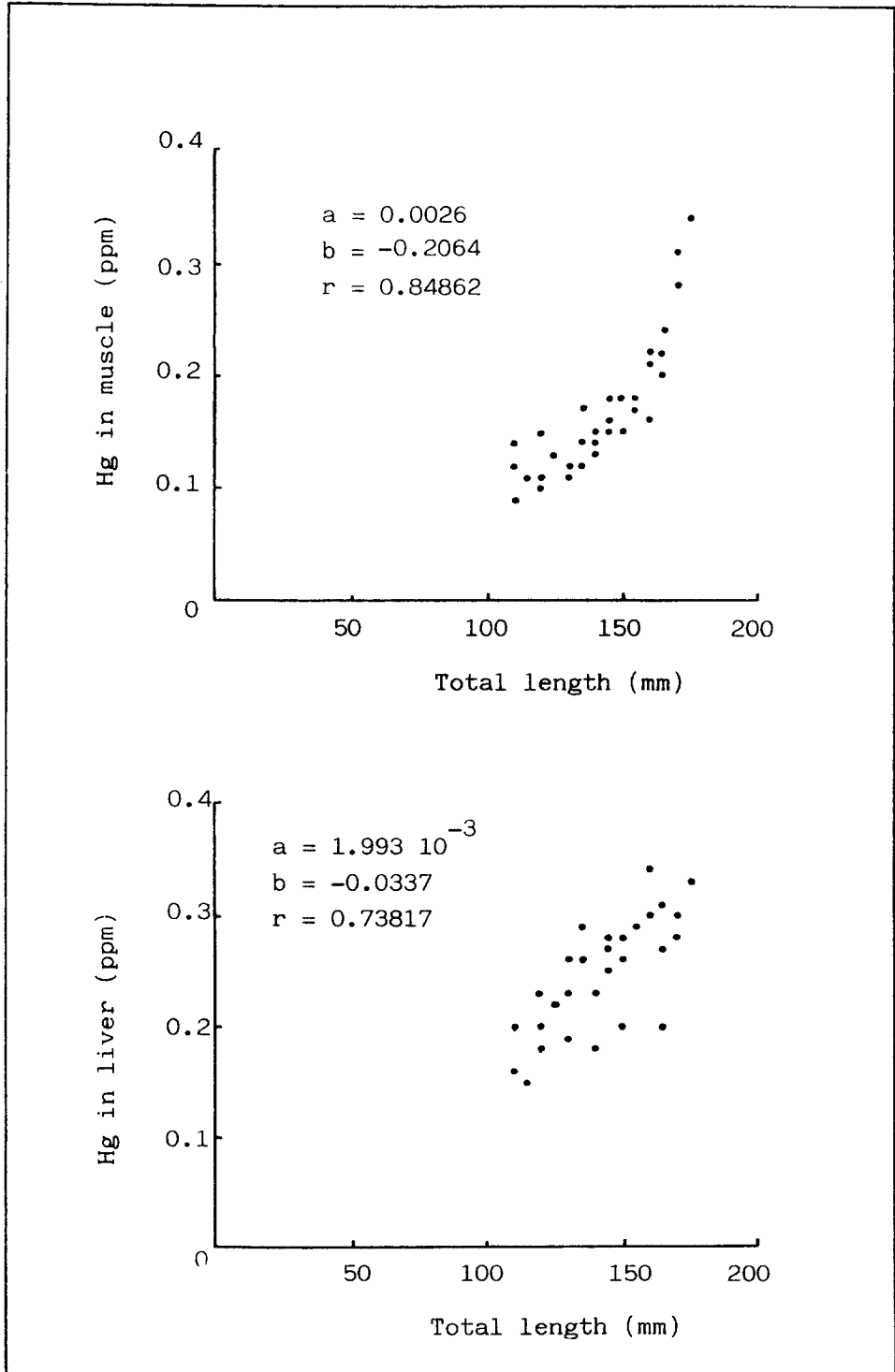


Fig. 2. Total mercury vs. total length relationship in *Pleoticus muelleri*.
(a) Muscle; (b) Digestive gland.

for either cadmium or zinc, neither in muscle nor in digestive gland of the species studied. Cadmium and zinc could probably be regulated by these organisms – up to certain concentrations – and thus these metals would be in equilibrium between the shrimp and the environment. This fact was previously suggested by Bryan *et al.* (1986) for the lobster *Homarus gammarus*. On the other hand, mercury would not be in equilibrium, presumably because excretion rates could not keep pace with uptake rates of this element, and so net uptake would be positive throughout the lifetime of the shrimp, as been suggested by Phillips (1980).

The coefficient of variation (CV%) was calculated for each metal concentration in the two shrimp species analyzed, in order to assess the variation among individuals (Table I). The CV% were in the range 16.2–20.4% for cadmium, 7.85–23.4% for zinc, and 20.2–34.8% for mercury. In the particular case of cadmium in muscle of *Pleoticus muelleri* and *Artemesia longinaris*, high CV% values were obtained (103% and 107%, respectively), and this is explained on the basis of the extremely low cadmium levels in these tissues, which implies that mean values and standard deviations lie very close to each other.

Finally, the possibility of using these shrimp species as indicators of trace metal pollution in the Bahía Blanca estuary was considered. Crustaceans could be a potential source of metals, since they serve as food for fish and wildlife. For instance, the peneid species studied in this paper are one of the most important items in the diet of sharks (Menni, 1986), and other fish species inhabiting the area, which have been shown to concentrate high amounts of trace metals (Marcovecchio *et al.*, 1986(b); 1988(b)). Furthermore, crustacean species can transfer heavy metals to other organisms, not only through the trophic route, but also via other routes. Thus, the importance of moulted crustacean exoskeletons (Martin, 1970) and of fecal material (Boothe and Knauer, 1972) for the transport and biological amplification of trace metals in the marine environment has been underlined.

If we strictly consider those characteristics required to define an organism as a 'bioindicator species' – e.g., that it is abundant and easily collectable, non-migratory, that it has an homogeneous distribution throughout the year in the studied environment, etc. (Phillips, 1980) – the shrimp species assessed in the present study would be appropriate. In addition, if their condition as a potential contributor of trace metals to other steps of the trophic web is considered, they deserve to be used as bioindicator species, and they would be a very useful tool for future monitoring programmes on heavy metal levels in the Bahía Blanca estuary.

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References

- Ahsanullah, M., Negilski, D.S., and Mobley, M.C.: 1981, 'Toxicity of Zinc, Cadmium and Copper to the Shrimp *Callinassa australiensis*. III Accumulation of Metals', *Mar. Biol.* **64**, 311–316.
- Allard, M. and Stokes, P.M.: 1989, 'Mercury in Crayfish Species from Thirteen Ontario Lakes in Relation to Water Chemistry and Smallmouth Bass (*Micropterus dolomieu*) Mercury', *Can. J. Fish. Aqu. Sci.* **46**, 1040–1046.
- Anderson, R.V., Vinikour, W.S., and Brower, J.E.: 1978, 'The Distribution of Cd, Cu, Pb and Zn in the Biota of Two Freshwater Sites with Different Trace Metal Inputs', *Holarctic Ecol.* **1**, 377–384.
- Angelescu, V. and Boschi, E.E.: 1959, 'Estudio biológico-pesquero del langostino de Mar del Plata, en conexión con la operación Nivel Medio', *Serv. Hidrogr. Nav.*, Bs.As. H-1017, pp. 1–135.
- Bagatto, G. and Alikhan, M.A.: 1987(a), 'Copper, Cadmium and Nickel Accumulation in Crayfish Populations near Copper–Nickel Smelters at Sudbury, Ontario, Canada', *Bull. Environ. Contam. Toxicol.* **38**, 540–545.
- Bagatto, G. and Alikhan, M.A.: 1987(b), 'Zinc, Iron, Manganese and Magnesium Accumulation in Crayfish Populations near Copper–Nickel Smelters at Sudbury, Ontario, Canada', *Bull. Environ. Contam. Toxicol.* **38**, 1076–1081.
- Boothe, P.N. and Knauer, G.A.: 1972, 'The Possible Importance of Fecal Material in the Biological Amplification of Trace and Heavy Metals', *Limnol. Oceanogr.* **17** (2), 270–274.
- Boschi, E.E.: 1969, 'Estudio biológico-pesquero del camarón *Artemesia longinaris* Bate de Mar del Plata', *Bol. Inst. Biol. Mar.* **18**, 47 pp.
- Brown, B.E.: 1982, 'The Form and Function of Metal-Containing "Granules" in Invertebrates Tissues', *Biol. Rev.* **57**, 621–667.
- Bryan, G.W., Langston, W.J., Hummerstone, L.G., and Burt, G.R.: 1985, 'A Guide to the Assessment of Heavy Metal Contamination in Estuaries using Biological Indicators', *Mar. Biol. Ass. U.K.*, Occ. Publ., **4**, 92 pp.
- Bryan, G.W., Hummerstone, L.G. and Ward, E.: 1986, 'Zinc Regulation in the Lobster *Homarus Gammarus*: Importance of Different Pathways of Absorption and Ecretion', *J. Mar. Biol. Ass. U.K.* **66**, 175–199.
- Fischer, H.: 1989, 'Cadmium in Seawater Recorded by Mussels: Regional Decline Established', *Mar. Ecol. Progr. Ser.* **55**, 159–169.
- Freije, R.J., Asteasuain, R.O., Schmidt, A.S. and Zavatti, J.R.: 1981, 'Relaciones de la salinidad y temperatura del agua de mar con las condiciones hidrometeorológicas de la porción interna del estuario de Bahía Blanca', *Inst. Arg. Oceanogr.*, Contrib. Científ. No. 57, 20 pp.
- Goldberg, E.D., Bowen, V.T., Farrington, J.W., Harvey, G., Martin, J.H., Parker, P.L., Risebrough, R.W., Robertson, W., Schneider, E., and Gamble, E.: 1978, 'The Mussel Watch', *Environ. Conserv.* **5**, 101–125.
- Huckabee, J.W., Elwood, J.W., and Hildebrand, S.G.: 1979, 'Accumulation of Mercury in Freshwater Biota', in: Nriagu, J.O. (Ed.), *The Biogeochemistry of Mercury in the Environment*, Elsevier/North Holland Biomedical Press, pp. 277–302.
- Marcovecchio, J.E., Moreno, V.J., and Pérez, A.: 1986(a), 'Biomagnification of Total Mercury in Bahía Blanca Estuary Shark', *Mar. Pollut. Bull.* **17** (6), 276–278.
- Marcovecchio, J.E., Lara, R.J., and Gómez, E.A.: 1986(b), 'Total Mercury in Marine Sediments near a Sewage Outfall. Relation with Organic Matter', *Environ. Tech. Lett.* **7**, 501–507.
- Marcovecchio, J.E., Moreno, V.J., and Pérez, A.: 1988(a), 'Total Mercury Contents in Marine Organisms of the Bahía Blanca Estuary Trophic Web', in: Seelinger U., Lacerda L.D., and Patchineelam, S.R. (Eds.), *Metals in Coastal Environments of Latin America*, Springer-Verlag, Heidelberg, pp. 122–129.
- Marcovecchio, J.E., Moreno, V.J., and Pérez, A.: 1988(b), 'Determination of Heavy Metal Concentrations in the Biota of Bahía Blanca, Argentina', *Sci. Tot. Environ.* **75**, 181–190.

- Martin, J.H.: 1970, 'The Possible Transport of Heavy Metals via Moulded Copepod Exoskeletons', *Limnol. Oceanogr.* **15** (5), 756–761.
- Menni, R.C.: 1986, 'Shark Biology in Argentina: A Review', in: Uyeno, T., Arai, R., Taniuchi, T., and Matsumura, K. (Eds.), *Indo-Pacific Fish Biology: Proc. 2nd. Int. Conf. Indo Pacific Fishes*, Ichthyolog. Soc. Jap. Tokyo, pp. 425–436.
- Olafson, R.W., Kearns, A., and Sim, R.G.: 1979, 'Heavy Metal Induction of Metallothionein Synthesis in the Hepatopancreas of the Crab *Scylla Serrata*', *Comp. Biochem. Physiol.* **62B**, 417–424.
- Overnell, J.: 1982, 'A Method for the Isolation of Metallothionein from the Hepatopancreas of the Crab *Cancer pagurus* that Minimizes the Effect of the Tissues Proteases', *Comp. Biochem. Physiol.* **73B**, 547–553.
- Pérez, A., Moreno, V.J., Moreno, J.E., and Malaspina, A.: 1986, 'Distribución de mercurio total en pescados y mariscos del Mar Argentino', *Rev. Invest. Desarr. Pesq.* **6**, 103–115.
- Phillips, D.J.H.: 1976, 'The Common Mussel *Mytilus Edulis* as an Indicator of Pollution by Zinc, Cadmium, Lead and Copper. I. Effects of Environmental Variables on Uptake of Metals', *Mar. Biol.* **38**, 59–69.
- Phillips, D.J.H.: 1980, *Quantitative Aquatic Biological Indicators*, Applied Sci. Publ. Ltd., London, 455 pp.
- Popham, J.D., Johnson, D.C., and D'Auria, J.M.: 1980, 'Mussels (*Mytilus edulis*) as 'Point Source' Indicators of Trace Metal Pollution', *Mar. Pollut. Bull.* **11**, 261–263.
- Romeo, M. and Gnassia-Barelli, M.: 1988, '*Donax trunculus* and *Venus verrucosa* as Bioindicators of Trace Metal Concentrations in Mauritanian Coastal Waters', *Mar. Biol.* **99**, 223–227.
- Salomons, W. and Förstner, U.: 1984, *Metals in the Hydrocycle*, Springer-Verlag, Heidelberg, 349 pp.
- Stinson, M.D. and Eaton, D.L.: 1983, 'Concentrations of Lead, Cadmium, Mercury and Copper in the Crayfish (*Pacifasticus leniusculus*) Obtained from a Lake Receiving Urban Runoff', *Arch. Environ. Contam. Toxicol.* **12**, 693–700.
- Uthe, J.F., Armstrong, F.A.J., and Stainton, M.P.: 1970, 'Mercury Determination in Fish Samples by Wet Digestion and Flameless Atomic Absorption Spectrophotometry', *J. Fish. Res. Bd. Can.* **27** (4), 308–312.
- Van Duyn-Henderson, J.A. and Lasenby, D.C.: 1986, 'Zinc and Cadmium Transport by the Vertical Migrating Opossum Shrimp', *Mysis relicta*', *Can. J. Fish. Aqu. Sci.* **43**, 1726–1732.
- Vermeer, K.: 1972, 'The Crayfish, *Orconectes virilis*, as an Indicator of Mercury Contamination', *Can.Fld. Nat.* **86**, 123–125.
- Villa, N. and Pucci, A.E.: 1987, 'Seasonal and Spatial Distributions of Copper, Cadmium and Zinc in the Seawater of Blanca Bay', *Est. Coast. Shelf Sci.* **25**, 67–80.
- White, S.L. and Rainbow, P.S.: 1986(a), 'Accumulation of Cadmium by *Palaemon Elegans* (Crustacea: Decapoda)', *Mar. Ecol. Progr. Ser.* **32**, 17–25.
- White, S.L. and Rainbow, P.S.: 1986(b) 'A Preliminary Study of Cu-, Cd-, and Zn-Binding Components in the Hepatopancreas of *Palaemon elegans* (Crustacea: Decapoda)', *Comp. Biochem. Physiol.* **83C**, 111–116.
- White, S.L. and Rainbow, P.S.: 1987, 'Heavy Metal Concentrations and Size Effects in the Mesopelagic Decapod crustacean *Styellapis debilis*', *Mar. Ecol. Progr. Ser.* **37**, 147–151.