



Correlations between metal uptake in the soft tissue of *Perna perna* and gill filament pathology after exposure to mercury

M.A. Gregory^{a,*}, D.J. Marshall^b, R.C. George^b, A. Anandraj^b, T.P. McClurg^c

^a Electron Microscope Unit, University of Durban-Westville, Private Bag X54001, Durban, Natal 4000, South Africa

^b Department of Zoology, University of Durban-Westville, Durban 4000, South Africa

^c Council For Scientific & Industrial Research (CSIR), Division of Water, Environment and Forestry Technology, King George V Avenue, Durban 4000, South Africa

Abstract

The accumulation of metal in soft tissues, filtration rate and gill filament morphology are correlated in the southern African rock mussel, *Perna perna*, during exposure to mercury (24 days) and recovery (24 days). The amount of Hg in soft tissues increased from 0.13 to 87.5 µg/g dry weight after 24 days exposure, and declined to 13 µg/g during recovery. Mean filtration rate fell from 3979 to 1818 ml/h/g dry weight by day 2, but recovered slightly through days 4 and 8 (3037 ml/h/g), with a higher average rate (5030 ml/h/g) being maintained over the 24–48 days recovery period. The initial decline in filtration coincided with epithelial cell deterioration presented as interstitial oedema, neural and epithelial cell degeneration and reduced ciliation. Between days 8 and 24, cilia regenerated and there was a general improvement in cell morphology. Gill filament morphology returned to near normal during the metal-free recovery period. The usefulness of *P. perna* as an indicator of pollution is discussed.

© 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Mussels; Bioindicators; Pollution; Electron microscopy

1. Introduction

Heavy metals are introduced into the marine environment in a number of ways. They may be deposited in the sea “naturally” as a consequence of erosion from ore bearing rock, wind blown dust, volcanic activity, and forest fires. Increasingly, however, they are introduced via contaminated rivers, marine outfalls and through the deliberate dumping of wastes in coastal waters. While the atmosphere provides a route for some metals, it has been estimated that anthropogenic releases of Hg, Pb, Zn, Cd, and Cu are between one and three orders of magnitude greater than natural input (Schindler, 1991). Considerable effort has been made by the international community to monitor the distribution of these metals in the sea and determine their effect on marine ecosystems. While analyses of water samples may give accurate, local, transient information, this methodology

cannot determine the long-term effect of these pollutants on benthic communities.

Mussels are long lived and easily sampled and their sedentary nature means that their geographical relationship to a pollution source can be easily ascertained. Mussels, therefore, exhibit desirable characteristics to serve as sentinel organisms. Many studies have demonstrated that over extended periods of time, mussels sequester and may accumulate heavy metals in their shells (Prakash and Rao, 1993) and soft tissues (Cossa, 1988; Broman et al., 1991; Andersen et al., 1996; Szefer et al., 1999). Observations that mussels are bio-accumulators of heavy metals, has led them to be used in marine pollution impact assessments (Goldeberg et al., 1987; Ritz et al., 1982; Watling and Watling, 1982; Grace and Gainey, 1987; Ward, 1990; Stronkhorst, 1992; Kraak et al., 1992; Odzak et al., 1994; Depledge et al., 1995). The importance of bivalve molluscs in pollution impact studies is shown by the magnitude and longevity of the International Mussel Watch (Goldberg, 1975; Calow, 1994; Beliaeff et al., 1997). This programme originated in the late 1960s and continues to maintain momentum

* Corresponding author.

E-mail address: mgregory@pixie.udw.ac.za (M.A. Gregory).

today. Most of the studies have been concentrated in Europe and North America and have principally centered about their local indigenous species, *Mytilus californianus*, *Mytilus edulis* and *Mytilus galloprovincialis*. However, interest has been increasing in areas such as the Asia-Pacific region, Australia and South America (Tavares et al., 1988; Higashiyama et al., 1991; Martin and Richardson, 1991; Murray et al., 1991; Richardson et al., 1994; Tanabe, 1994) on other mussel genera, including *Perna* (Caihuan et al., 2000; Sze and Lee, 2000). South Africa has never formally adopted a mussel watch programme, but has subscribed to its principles in that bivalve molluscs have been used in its national marine pollution surveys (Hennig, 1985). The present study critically examines the potential use of our local brown mussel *P. perna* as a bio-indicator species. This is the only rock mussel species occurring in the sub-tropical regions of southern Africa but extends its distribution to the warm and cool temperate regions where it cohabits with endemic, introduced mussel species.

Pollution monitoring techniques using mussels include measurement of physiological rates (Watling and Watling, 1982; Marshall and McQuaid, 1993), evidence of bioaccumulation of heavy metals (Regolli and Orlando, 1994) and estimation of the biotoxicity of metals from pathological changes in soft-tissue morphology (Sunila, 1988a,b; Gregory et al., 1999). Metals are known to reduce the physiological performance of bivalve molluscs, including *P. perna* (Watling and Watling, 1982; Marshall and McQuaid, 1993). Bivalve molluscs exposed to elevated concentrations of heavy metals produce fewer byssus threads (Martin et al., 1975; Szefer et al., 1997) and have reduced heart rates (Grace and Gainey, 1987). Measurement of physiological parameters such as the filtration and oxygen uptake rate have been used to demonstrate pathological stress in bivalves exposed to high, but sub-lethal levels of heavy metals (Abel, 1976; Watling and Watling, 1982; Axiak and George, 1987; Kraak et al., 1994; Eisler, 1977; Niamo et al., 1992; Depledge et al., 1995). In addition to heavy metal mediated changes in mussel physiology, there are studies that describe the patho-morphological response of mussels to heavy metal pollution at the histological (Sunila, 1986, 1987, 1988a,b) and ultrastructural level (Moore, 1985; Ballan-Difrancais et al., 1990; Regolli and Orlando, 1994). Target organs include the digestive gland (Ward, 1990) and especially the gills, organs that appear able to take-up dissolved metals directly from sea-water (Burgess and McKinney, 1999) and where heavy metals and other pollutants are known to accumulate (Axiak et al., 1988).

While there is a relatively good understanding of the response of the northern hemisphere *Mytilus* species to pollution, relatively little work has been published on equivalent species in the southern hemisphere. *P. perna* is dominant on the east coast of South Africa

and is an appropriate sentinel organism. This study was undertaken in order to increase our understanding of its response to pollution. Using a sub-lethal concentration of mercury as a test pollutant, experiments were performed to obtain some clarity on the following three issues:

1. The effect of sub-lethal concentrations of mercury on filtration rate.
2. The rate at which mercury is accumulated in soft tissues and subsequently eliminated.
3. The morphological response of gill filaments when chronically exposed to mercury.

2. Materials and methods

2.1. Collection and maintenance of mussels

Specimens of *P. perna* (43–60 mm; $n = 400$) were collected at spring, low tide from an unpolluted rocky shore near Isipingo Beach, south of Durban (29° 05' S 30° 56' E). The animals were kept in well oxygenated, flow through, plastic sea water aquaria (75.5 × 40.5 × 21 cm³) and a glass aquarium (75 × 45 × 13 cm³). Using a sharp blade, the surfaces of shells were scraped clean of all epiphytes before being placed in the aquaria. In order that the alimentary canal was cleared of all faecal matter and that the animals became acclimatised to their new conditions, they were kept for five days prior to the commencement of experiments. While in the aquaria, the animals were supplied with a suspension (7000–27 000 cells/ml) of unicellular alga, *Chlorella* (12 µm). Each of the two aquaria containing 100 animals was cleaned every two days, by siphoning the faecal matter. Ambient room and sea water temperatures were recorded daily and found to remain at 23 ± 1 °C, even without temperature control mechanisms. An algae concentration of ±4800 cells/ml (500 ml concentrated algae suspension into 100 l sea water) was maintained in both tanks for the duration of the experiment.

2.2. Tank system

A system was designed that allowed a constant supply of high quality seawater (drawn from the intake of the Durban aquarium—“Seaworld”) and transported to the laboratory in a stainless steel tanker to each of two aquaria. Seawater was fed by gravity from two reservoir tanks (>1000 l) to the holding tanks (100 l each; two per aquaria). The holding tanks were manually filled with fresh seawater on a daily basis. This supplied each aquarium with seawater at a flow rate of 12 l/h (288 l/day). Two separate delivery systems were created to run simultaneously: Tank 1 delivered fresh seawater (control), Tank 2 delivered Hg. Mercuric chloride

(HgCl_2 —0.007 g), was dissolved in 2 ml of distilled water and added to tank 2 each day to provide an estimated final concentration of 50 $\mu\text{g/l}$. Mercury was administered to tank 2 for 24 days, thereafter, fresh seawater was supplied to surviving animals for a further 24 days. The control was run for 48 days. Using atomic absorption (AA) spectrometry, the concentration of Hg in the water of tank 2 was found to be 44 $\mu\text{g/l}$ on day 2, 46 $\mu\text{g/l}$ on day 8 and 45 $\mu\text{g/l}$ on day 24.

2.3. Preparation of tissue for mercury analysis

After removal of the specimens for transmission electron microscopy, the remaining fresh, soft tissues of these and an additional five bivalves from each group were pooled, ground and 2.5 g (wet weight) placed in 100 ml stoppered flasks. 10 ml of 5% $\text{K}_2\text{S}_2\text{O}_8$ and 10 ml H_2SO_4 was added to each flask which were then left to stand for 2 h. This was followed by heating for 4–6 h (60 °C) in a water bath and allowing to stand overnight at RTP. Thereafter, 15 ml 50 g/l KMnO_4 was added and after standing overnight at RTP, 1 ml conc. HNO_3 was added and the solution allowed to stand for a further 12 h. The subsequent solutions were analysed by AA. Three blanks containing no tissue were prepared simultaneously to serve as negative controls. A single reading was made of each pooled sample using a Varian Spectra 30 model AA Spectrometer. All readings were converted from $\mu\text{g/l}$ to $\mu\text{g/g}$ dry tissue weight.

2.4. Determination of filtration rates

Estimation of the filtration rates was made by determining the rate of removal of algal particles from a known volume of water. Filtration rate was calculated according to the following equation (Coughlan, 1969):

$$m = M \ln[C_0] / nt [C_t]$$

where m = filtering rate (ml/min); M = volume of test solution (ml); n = number of bivalves; C_0 = particles in initial algal suspension; C_t = particles in final algal suspension; t = time between C_0 and C_t (min).

The filtration apparatus consisted of a beaker containing an aerated, algal/seawater medium, immersed in a temperature controlled water bath (23 ± 1 °C). An individual mussel was placed in a cone-shaped nylon mesh suspended in the glass beaker. Measurements of particles of algal suspension were made before (C_0) and after (C_t) 30 min of immersion for each animal so as to determine an average of three Coulter Counter readings from 100 ml water samples. Filtration rates were calculated for 10 animals from each tank for each time interval considered. Data was obtained for animals immersed in tank 1 (control) after 2, 8, 24 and 48 days and for tank 2 animals (metal exposure) after 2, 4, 8, 16, 24, 32, 40 and 48 days.

2.5. Preparation of tissue for microscopy

Five mussels were removed from each tank at the same times that filtration rates and O_2 uptake rates were determined. The shells were forced open and a solution of 2% glutaraldehyde in filtered fresh seawater adjusted to pH 7.2 at 22 °C was poured over the gills. Undamaged areas of approximately 2 mm were dissected through the full thickness (ascending and descending lamellae) of the central regions of each gill and placed in fresh fixative for 1 h. The tissue was washed in fresh seawater, post-fixed in 1% osmium tetroxide in seawater (pH 7.2), dehydrated through graded ethanols, cleared in propylene oxide and embedded in Spurr (1969) epoxy resin.

Sections, 1 μm in thickness, were cut of the resin embedded tissue and stained with aqueous, alkaline toluidine blue. Areas showing cross-sectioned gill filaments were photographed and selected for electron microscopy. Each block was trimmed and aligned in such a manner as to ensure that at least 10 cross-sectioned filaments were available for fine-structural evaluation. Sections of 50–80 nm were cut of cross-sectioned filaments with diamond knives, picked up on uncoated copper grids and double stained with uranyl acetate and lead citrate (Reynolds, 1962). Each section was examined using either a Jeol 1010 transmission electron microscope at 80 kv.

3. Results

Within 24 h of immersion in the aquaria, two animals had died, possibly as a consequence of stress (one in tank 1 and one in tank 2). During the course of the experiment, five animals died in aquarium 2 (2 and 3 on days 2 and 16 respectively). The remaining 93 animals remained apparently healthy throughout the experiment.

3.1. Time related concentration of Hg in soft tissues

There was a gradual increase in the amount of Hg in soft tissues over the 24 days experimental period (Fig. 1). From day 2–8, Hg in tissues increased at the rate of 0.05 $\mu\text{g/g/h}$ (3–13 $\mu\text{g/g}$), from day 8–16 at 0.15 $\mu\text{g/g/h}$ (44 $\mu\text{g/g}$) and from day 16–24 at 0.2 $\mu\text{g/g/h}$ (87 $\mu\text{g/g}$ dry weight). These data show that the concentration of mercury in soft tissues increased nearly 30 fold from day 2–24. Although there was a massive decline from days 24–32 (87–17 $\mu\text{g/g}$), the rate at which mercury declined during the remaining post exposure period was much slower. From days 32–40, Hg in the tissue was reduced by a rate of 0.0007 $\mu\text{g/g/h}$ (17–16 $\mu\text{g/g}$) and from days 40–48 at 0.02 $\mu\text{g/g/h}$ (13 $\mu\text{g/g}$ dry weight). However,

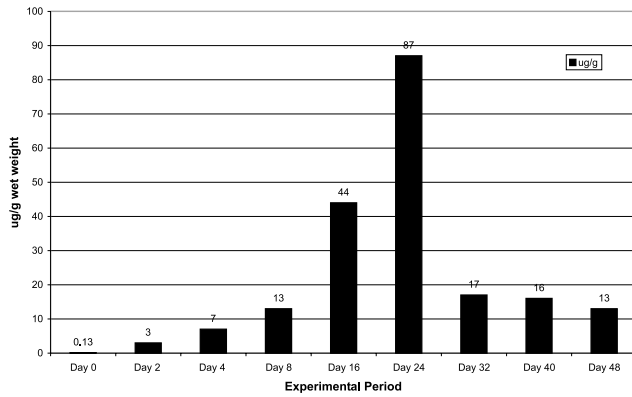


Fig. 1. Bar graph showing the amount of Hg ($\mu\text{g/g}$ —dry weight) in the soft tissues of *P. perna* during the experimental period.

although the amount of Hg in soft tissues was drastically reduced, Hg levels did not return to normal within the 24 days recovery period.

3.2. Filtration rates

Mean filtration rates of experimental animals were less than those of control bivalves for all recording intervals over the 24 days that the animals were exposed to Hg (Table 1). Even though the differences in filtration rates were significant ($p < 0.05$) on only two occasions (days 2 and 16), the significantly lesser mean filtration rates for all exposure periods of experimental animals ($2707 \text{ ml h}^{-1} \text{ g}^{-1}$ dry weight) compared to that of control animals ($3978 \text{ ml h}^{-1} \text{ g}^{-1}$ dry weight) indicates a real effect. Our data show no clear incremental decline in filtration rates of experimental animals in response to longer exposure, with similar filtration rates for animals after initial exposure as for those exposed for 24 days. During the recovery period (day 24–48), however, filtration rates of the experimental animals increased markedly soon after the exposure to Hg had ceased. These significantly ($p < 0.05$) exceeded the levels observed for the control mussels during this period (5030 versus $3979 \text{ ml h}^{-1} \text{ g}^{-1}$ dry weight for the experimental and controls respectively). The filtration rates of the control group remained similar during the course of the experiment ($3978 \text{ ml h}^{-1} \text{ g}^{-1}$ dry weight).

Table 1

Mean filtration rates (FR $\text{ml h}^{-1} \text{ g}^{-1}$ dry weight) and standard deviation (SD; $n = 10$) for control and experimental mussels

	Time (days)										
	2*	4	8	16*	24	32	40	48	2–24	24–48	2–48
Control (\pm SD)	3422 1125	– –	5154 1858	– –	4770 2485	– –	– –	3882 319	– –	– –	3979 1559
Experimental (\pm SD)	1818 1097	3603 1287	3037 2073	1149 562	3727 1608	5475 1697	5171 2109	4252 4063	2707 1675	5030 1939	– –

Asterisk (*) denotes significant difference between experimental and control rates (Newman Keul's multiple range test; $P < 0.05$).

3.3. Transmission electron microscopy

3.3.1. Tissue from controls

Irrespective of when the samples were taken, the architecture and fine structure of gill filaments from animals in Tank 1 (controls) was similar in every respect to the detailed description of gill filament morphology provided by this group in an earlier study (Gregory et al., 1996).

3.3.2. Tissue from experimental animals

It is interesting to note that unlike *Mytilus edulis*, (Sunila, 1986, 1987, 1988a,b), the shape and general architecture of gill filaments did not change during or after exposure to mercury.

3.3.3. Two days Hg

Irrespective of position on the gill filament, many epithelial cells contained myelin figures, multivesicular bodies and lysosomes. Focal areas of cytoplasm were often devoid of any organelles giving the matrix an electron-lucent appearance. Microvilli (m) projecting from many cells were elongated and appeared normal. However, those projecting from squamous lateral cells (S) were often swollen and had the appearance of vesicles separating from the plasmalemma. In some instances, there were short stretches of plasmalemma where m were absent. There was considerable intercellular oedema, especially between S (Fig. 2), ciliated lateral cells (lc) and ciliated lateral frontal cells (lfc) (Fig. 3). In many instances, appeared anchored to the basement membrane by small foot processes (Figs. 2 and 3). It is interesting to note that while intercellular oedema was a consistent feature, there was rarely evidence of intracellular oedema in any cell type. A regular abnormality was the “de-ciliation” of lc and lfc. In normal tissue, there are four, well ciliated lc and one ciliated lfc. In these specimens only one or two lc were ciliated, and these and lfc had fewer cilia than those projecting from the same cell types in control specimens. In some instances, cells in the lc and lfc positions showed little or no evidence of ciliation (Fig. 3). In addition to fewer cilia projecting from lc and lfc, there was often an apparent reduction or absence of cilia projecting from abfrontal cells (ac). Also, the amount of mucus in ad-

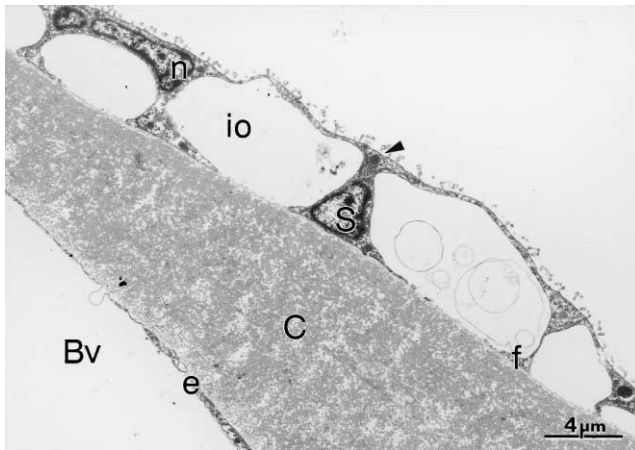


Fig. 2. Two days Hg: Lateral aspect of gill filament showing squamous lateral cells (S) and severe intercellular oedema (io). Note that some microvilli are swollen while others appear “vesicular” (arrowed). Bv = branchial vein; C = chitin; e = endothelial cell; f = foot process; n = nucleus.

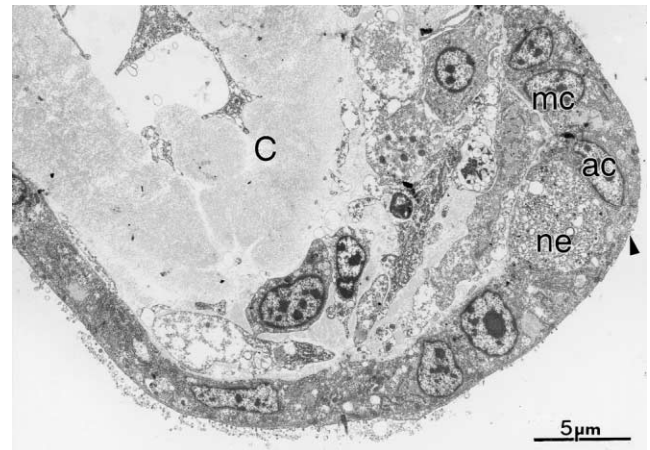


Fig. 4. Two days Hg: Abfrontal aspect of gill filament showing abfrontal cells (ac) devoid of cilia and microvilli (arrowed). Note some axons in the abfrontal nerve (ne) are dilated and there are very few mucus droplets in mucous cells (mc).

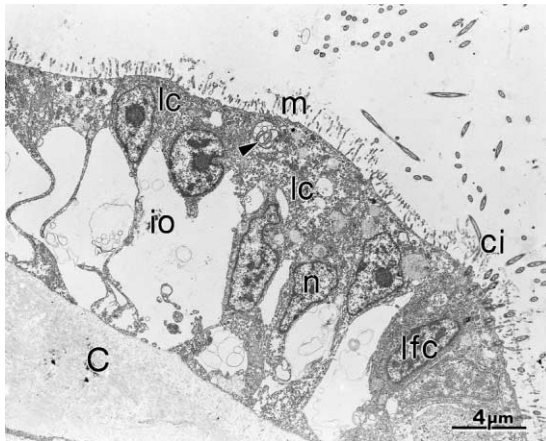


Fig. 3. Two days Hg: Lateral frontal aspect of gill filament showing non-ciliated cells in the position normally occupied by ciliated lateral cells (lc). Note gross intercellular oedema and random cilia (ci) rather than organised cirri projecting from the lateral frontal cell (lfc). m = microvilli; myelin figure (arrowed).

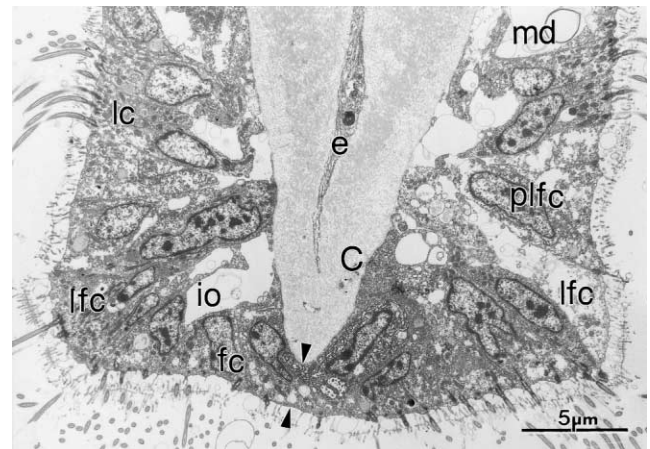


Fig. 5. Four days Hg: Lateral-frontal and frontal aspect of gill filament showing membranous debris (md) in oedematous intercellular spaces and necrotic lfc and post-lateral frontal cell (plfc). Note thinning of frontal cell (fc) cytoplasm at the chitin “tip” (arrowed).

joining mucus secreting cells (mc) was considerably reduced (Fig. 4). Frontal cells (fc) appeared morphologically normal. Numerous axons, especially those in lateral and abfrontal nerves (ne) had swollen axons (Fig. 4). The endothelium, while normal over most of the branchial vessel wall, was swollen in others (Fig. 2). There were numerous granulocytes in the branchial vein, with many infiltrating the epithelium, especially in the lateral and lateral-frontal regions.

3.3.4. Four days Hg

While there was little or no evidence of intercellular oedema between cells populating the abfrontal “shoulder”, large intercellular spaces between S, lc and lfc, were often filled with membranous debris (Fig. 5). In

some filaments, fc retained their normal cuboidal appearance while in others they were elongated over the chitin (Fig. 5). In some instances the chitin appeared to have “pushed” through the frontal epithelium. Many cells in the lateral and lateral frontal regions appeared necrotic with large electron-lucent spaces in the cytoplasmic matrix (Fig. 5). Microvilli were similar to those described in 2 days Hg above. There were still fewer cilia projecting from lc and lfc than in control specimens, but ciliary numbers of ac had returned to normal (Fig. 6). The axons of lateral and abfrontal nerves were swollen, their neurolemmas convoluting to form extensive myelin figures (Fig. 6). Although the endothelium lining the branchial vein was generally intact, there were regions where endothelial cells (e) were swollen, fragmented or



Fig. 6. Four days Hg: Abfrontal nerve showing vacuolation and apparent necrosis of axons (arrowed). Note normal complement of cilia and “vesiculated” microvilli. Gr = granulocyte.

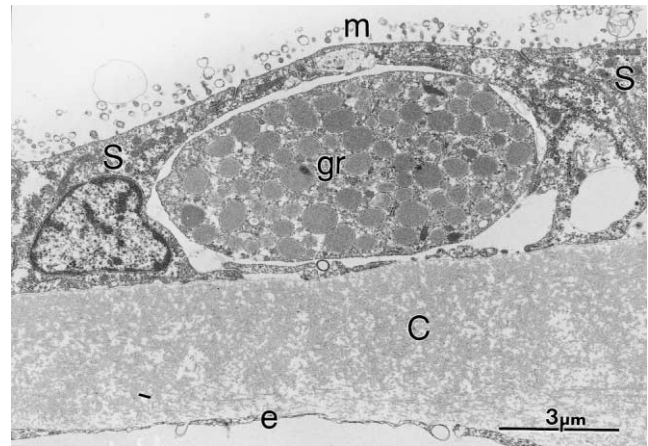


Fig. 8. Eight days Hg: Granulocyte in the intercellular space between lateral squamous cells. Note vesicular m and swollen e cytoplasm.

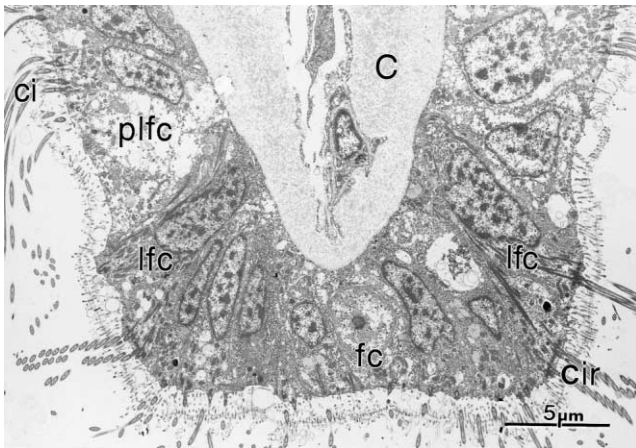


Fig. 7. Eight days Hg: Frontal region showing necrotic plfc and large vacuoles in some fc (arrowed). Note apparent normal complement of lc cilia and lfc cirri (cir).

absent. There were still large number of granulocytes in the interstitial spaces between S, lc and lfc.

3.3.5. Eight days Hg

Interstitial oedema was considerably reduced and all normally ciliated cells had an apparent normal complement of cilia and cirri (Fig. 7). Some S, lc and post lateral frontal cells (plfc) appeared necrotic and were in various stages of autolysis (Fig. 7). Microvilli projecting from S were reduced in numbers and were generally “vesicular” in appearance (Fig. 8). The thickness of fc cytoplasm covering the frontal aspect of chitin, while still thin in some specimens but had returned to normal in others (Fig. 7). Endothelial cells lining the branchial vein although generally continuous, were often swollen (Fig. 8). There were, however, areas where the endo-

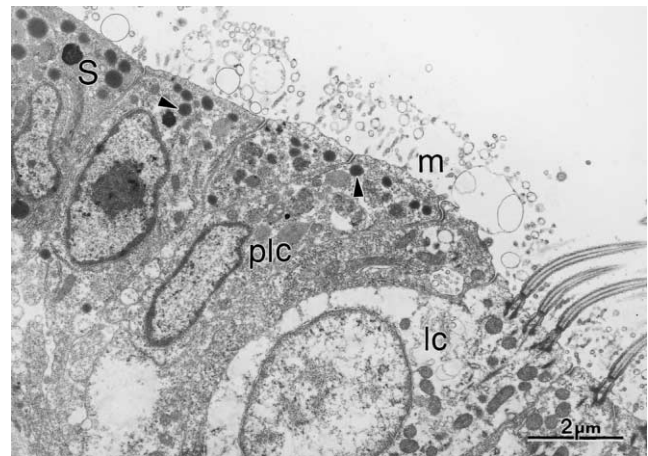


Fig. 9. Sixteen days Hg: Numerous granules of varying electron density (arrowed) in S and post-lateral cells (plc). Note electron lucent cytoplasm of lc and complete disruption of m. The lc cilia (ci) appear normal.

thelium appeared to have fragmented. Granulocytes (gr) were still present in higher than normal numbers in both the branchial vein and intercellular spaces (Fig. 8). Although nerves still contained myelin figures, the tissue appeared less pathological than after two and four days exposure to Hg.

3.3.6. Sixteen days Hg

Interstitial oedema, while still present in some areas of some specimens, was considerably less than from specimens exposed to Hg for two, four and eight days. Indeed, no oedema was observed in many filaments from each specimen. The cytoplasm of many ciliated lc was particularly electron lucent, giving the cells a “necrotic” appearance (Fig. 9). The numbers of cilia projecting from lc and lfc had returned to normal. Frontal cells had

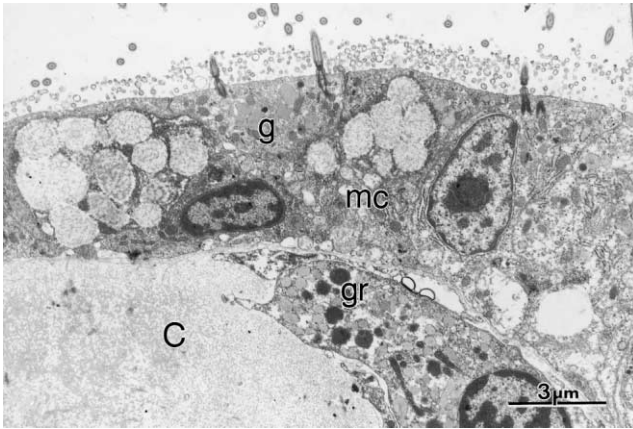


Fig. 10. Sixteen days Hg: Increased number of mucus cells in abfrontal region. Note occasional cilia projecting from normally non-ciliated cells and electron-pale granules (g) in epithelial cells.

returned to their cuboidal shape and adequately covered the frontal aspects of branchial vein chitin. Abfrontal mucus cells (mc) were present in greater than normal numbers (Fig. 10) with mc interspersed between S and post-lateral cells (plc). In addition, many otherwise non-secretory cells on the abfrontal shoulder contained droplets (g) that were probably secretory granule precursors (Fig. 10). Most cells, especially S, lc, and plc contained electron dense and less electron dense granules (Fig. 9). Microvilli projecting from most cells of all cell types had become “vesicular” (Figs. 9 and 10). While ac morphology and complement of cilia appeared normal, cilia emanated from normally non-ciliated cells on, and beyond the abfrontal “shoulder” (Fig. 10). The nerves still contained myelin figures and there were still increased numbers of granulocytes in intercellular spaces. There were occasional epithelial and endothelial cells undergoing autolysis, however, the overall morphology of filaments after exposure to 16 days of Hg appeared less pathological than those exposed for shorter periods.

3.3.7. Twenty four days Hg

There were considerable variations in the morphological appearance of epithelia in different specimens. In some, the endothelium lining the branchial vein was continuous and appeared morphologically normal (Fig. 11) while in others endothelial cells were swollen and/or very necrotic (Fig. 12). The m projecting from some epithelial cells were swollen, “vesiculated” or absent (Fig. 12), while in others they appeared normal (Figs. 11 and 13). All normally ciliated cells had an apparently normal complement of cilia (Fig. 13). There were also cilia projecting from S, especially near the region of the abfrontal shoulder. Most S, plc, lc, plfc and lfc contained numerous, round or oval, membrane-bound osmiophilic inclusions (Fig. 11). Some S, plfc and fc contained larger, less osmiophilic, possibly secretory

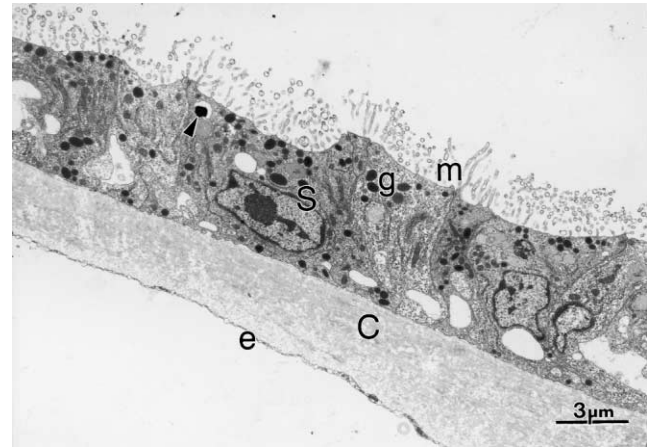


Fig. 11. Twenty four days Hg: Cuboidal cells in areas normally occupied by elongated lateral squamous cells (S). Note elongated m, numerous granules (g) and occasional irregular shaped, osmiophilic inclusions (arrowed).

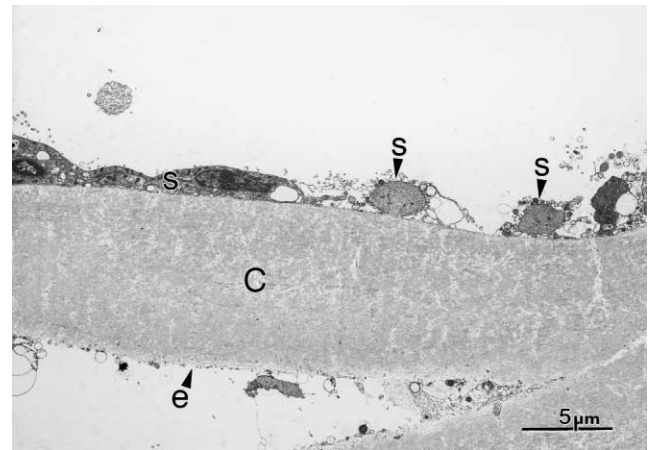


Fig. 12. Twenty four days Hg: Elongated S and necrotic, lateral squamous (s-arrowed) and endothelial cells (e-arrowed).

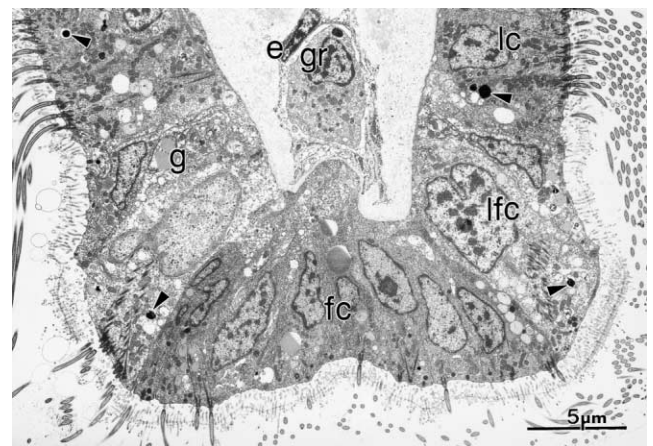


Fig. 13. Twenty four days Hg: Morphologically normal cells populating lateral frontal and frontal regions. Note osmiophilic inclusions in lc (arrowed) and lfc and large electron-pale granules (g) in some fc and plfc.

granules (Fig. 13). Most cells, irrespective of position contained distinctive, irregularly shaped, electron dense membrane-bound inclusions, that appeared to be surrounded by an electron-lucent halo (Figs. 11 and 13). Lateral squamous cells were often more cuboidal than elongated (Fig. 11) and in some filaments there were discrete areas where necrotic S were exfoliating from the epithelium (Fig. 12). In such areas, endothelial cells were also necrotic. There were numerous myelin figures in the abfrontal and lateral nerves of some filaments while in others the nerves appeared essentially normal. Granulocytes were present in all filaments from all specimens.

3.3.8. Thirty two days after Hg—eight days during recovery

Most cells populating abfrontal regions appeared normal. No abnormal cilia projected from normally non-ciliated mc, S or plc. Mucus cell numbers had returned to normal and no intercellular oedema was observed. While osmiophylic inclusions and paler granules were still present in some cells, their numbers were dramatically reduced. The epithelium contained numerous electron lucent, apparently necrotic cells that were immediately adjacent to morphologically normal cells. This was especially the case in the lateral frontal (Fig. 14) and frontal regions (Fig. 15). Irrespective of their apparent patho-morphological status, lc and fc retained some apparently functional cilia. Squamous lateral cells (S) had returned to their elongated shape, however, many m projecting from these cells were swollen or “vesicularised”. Far fewer granulocytes were infiltrating the epithelium and endothelial cells, while not always forming a continuous layer over the inner aspect of the branchial vein, appeared morphologically normal (Figs. 14 and 15). Many axons were swollen and

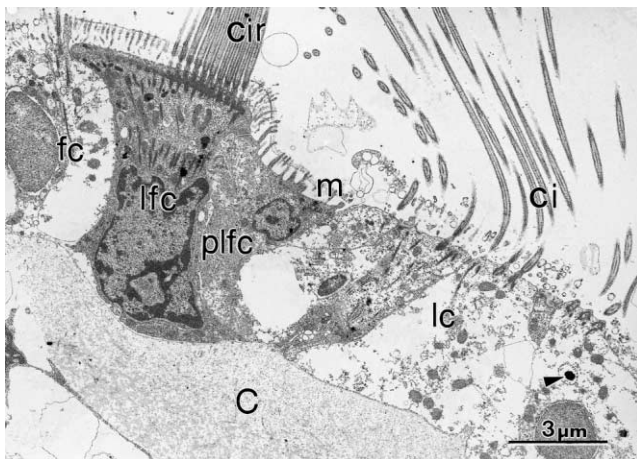


Fig. 14. Eight days recovery: Electron-pale cytoplasm in apparently necrotic ciliated lc and fc. Note lfc and plfc appear morphologically normal with elongated m projecting from their surfaces. Microvilli projecting from necrotic cells are “vesiculated”.

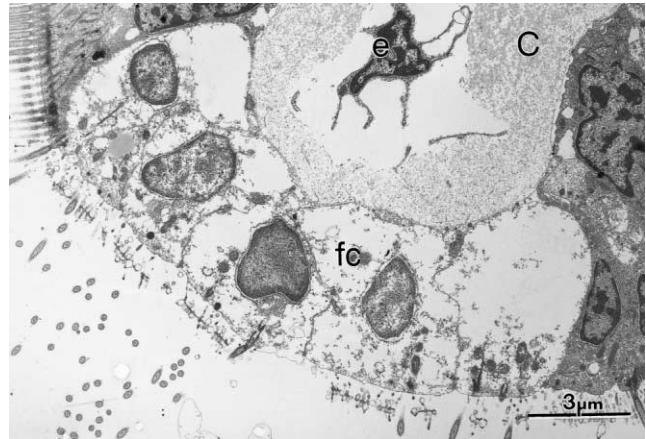


Fig. 15. Eight days recovery: Many fc have an electron-lucent cytoplasm and appear necrotic while adjoining cells appear healthy (lfc).

myelin figures were still present, especially in the large abfrontal nerve.

3.3.9. Forty eight days after Hg—twenty four days during recovery

The morphological appearance of the epithelium over most filaments was essentially normal. Occasional electron lucent, “necrotic” cells were present in lateral-frontal and frontal regions. These cells may well have been pre-exfoliative and part of the normal process of epithelial turnover. Occasional osmiophylic inclusions were still present in some S, lc, lfc and fc but no electron-pale “secretory” droplets were in evidence. While normal, elongated microvilli projected from S and between the cilia of cells in the lateral frontal and frontal regions, m were still swollen and/or “vesicular” from ac (Fig. 16). Very occasional granulocytes were present in the epithelium and the endothelium lining the branchial vein was intact over its entire surface. There were, however,

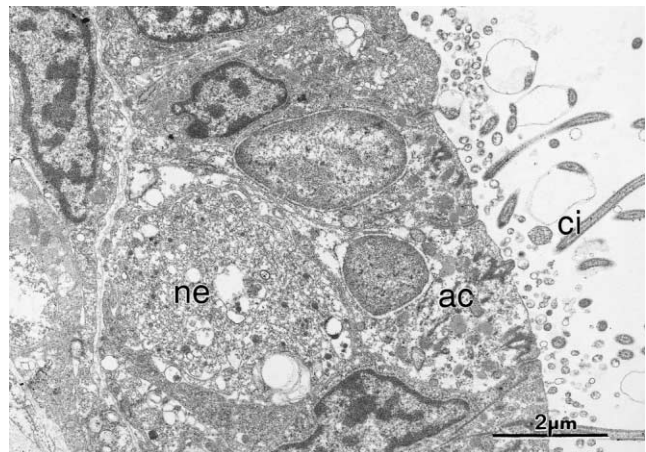


Fig. 16. Sixteen days recovery: While most epithelial cells appear morphologically normal, some axons remain swollen (arrowed) in the abfrontal nerve (ne). Microvilli projecting from ac remain vesiculated.

some ec with swollen processes. Nerves still contained occasional swollen axons and myelin figures, but were less obvious than in specimens from preceding times (Fig. 16).

4. Discussion

Bivalves are reported to sequester Hg from their environment (Clark, 1996). The metal gradually accumulates in the tissues (Roesijadi and Fellingham, 1987; Lakshmanan and Nambisan, 1989; Micallef and Tyler, 1990), especially the viscera, with the highest concentrations in *P. viridis* occurring in the gills (Roesijadi and Fellingham, 1987). While there are numerous reports that describe the accumulation of metals in *P. viridis* and *M. edulis* (Lakshmanan and Nambisan, 1989; Manly et al., 1996; Caihuan et al., 2000; Sze and Lee, 2000), to our knowledge, this is the first study that quantifies Hg uptake in *P. perna* and correlates such uptake with physiological and morphological criteria.

There was a progressive increase of Hg in the soft tissues of *P. perna* from 0.13 $\mu\text{g/g}$ in control animals to 87 $\mu\text{g/g}$ after 24 days in a Hg environment. There was not, however, a concomitant deterioration in physiological function nor gill filament pathomorphology over this period. Filtration rates were generally reduced while the animals were exposed to Hg, with a maximum reduction of more than 50% on day 16. Severe pathomorphological changes were observed in the gills two days after exposure to Hg and remained, albeit with differing morphological characteristics and at apparently reduced levels of pathological severity, during and up to 24 days after exposure to the metal.

Filtration of particles from water is primarily a function of the lateral frontal cirri. Irrespective of species, cirri in bivalves are structures whose tips are reported to serve as nets to capture water-borne food particles and whose motion is thought to deliver particles to the frontal cilia for “further processing” (Silverman et al., 1996). Studies by this group have shown that *P. perna* have 18–23 pairs of cilia per latero-frontal cirrus with each pair averaging 18 μm in length (Gregory et al., 1996; Gregory and George, 2000). While the present study did not focus on filament or cell morphometrics, there was an obvious, substantial depletion of cirri emanating from lfc, especially two days after exposure to Hg.

While de-ciliation may explain the reduced filtration rate during the early stages of the experiment, it does not explain the greater reduction when the animals had been exposed to Hg for 16 days. In bivalves, filtration is controlled by the neurochemicals, serotonin (5-HT) and dopamine (DOP), which are synthesised in the nervous system (Salanki and Hiripi, 1990). High 5-HT is associated with increased filtration, while high DOP levels

correlates to rest (Hiripi and Salanki, 1973; Catapane, 1979). Shortly after exposure to Hg, Salanki and Hiripi (1990) showed that 5-HT levels were depressed by up to 20% while longer exposure caused DOP levels to rise. Our morphological data showed neural pathology throughout the study. Perhaps nerve damage, together with other pathomorphological criteria “peaked” 16 days after exposure resulting in significantly reduced filtration at this time.

Other studies also report a rapid decrease in the filtration rate of bivalves, when exposed to Hg. Dorn (1976) reported a substantial reduction in the filtration rate of mussels exposed to Hg (400–2800 $\mu\text{g Hg l}^{-1}$) for 48 h. Abel (1976) and Watling and Watling (1982) report an EC_{50} of 40 $\mu\text{g Hg l}^{-1}$ and 25 $\mu\text{g Hg l}^{-1}$ for the filtration rate of *M. edulis* and *P. Perna* respectively. While these studies did not explore a morpho-mechanical aetiology for impaired filtration, reduced filtration in the present study was probably a consequence of a combination of factors including a reduction in the number of lateral frontal cirri and damage to nerves, lfc and fc.

Exposure to Hg unquestionably caused considerable changes in gill filament morphology. Further, pathomorphology varied in nature and apparent degree of severity with time of exposure. Surprisingly, the most obvious changes occurred shortly after exposure and presented as a substantial loss and/or absence of lc, lfc, and by four days, ac cilia. In addition there was a “thinning” of the frontal aspects of the epithelium, generalised intercellular oedema, axon oedema and degeneration, progressive endothelial cell deterioration and swelling and loss of Mv, especially from squamous lateral cells. While there was ciliary recovery by eight days after exposure and far less intercellular oedema, there was a progressive increase in the number of osmiophylic, cytoplasmic inclusions and number of electron-pale, apparently necrotic epithelial cells. From days 16–24, while there was little evidence of intercellular oedema, mucus cells had increased in numbers and secretory droplets and osmiophylic inclusions were present in most cells. In addition, many filaments contained numerous, apparently necrotic ciliated fc, lc and lfc and groups of now “cuboidal” S had either exfoliated or were exhibiting necrotic features. At this time, there was also an increase of cilia projecting from otherwise non-ciliated cells lining the abfrontal “shoulder”. This phenomenon had been reported by this group in an earlier study (Gregory et al., 1999). There was an increase in the number of granulocytes in both the lumen of the branchial vessel and the epithelium throughout the entire period that the animals were exposed to Hg.

Many of these features have been described in other bivalves exposed to heavy metals and other toxins. Changes in the shape and general architecture of gill filaments have been reported together with progressive

necrosis of epithelial cells, increased exfoliation and elevated mucus secretion following prolonged exposure to heavy metals (Sunila, 1987, 1988a,b, Hieteman et al., 1988; Axiak et al., 1988). Exposure of bivalves to low concentrations of metal induces the synthesis of metallothionins which form a complex with the metal. The metal is stored as membrane-bound granules, which effectively isolates the toxic material from the cell (Calow, 1994). Cunningham (1979) considers that metal is also bound to secretory products, which may be removed from the surface of filaments by motile granulocytes. This could explain both the progressive increase in membrane-bound osmiophylic inclusions and granulocytes in the epithelium. The increase in numbers of cilia projecting from lateral cells and cells in abfrontal regions, the change in the shape of squamous cells together with an increase in mucus cells and mucus droplets in otherwise non-secretory cells, suggests that Hg destabilises the epithelium. NB: While a non-polluted environment promotes normal differentiation, Hg encourages a metaplastic response, with non-ciliated cells becoming ciliated and otherwise non-secretory cells taking up a secretory function. In addition, while there is an apparent increase in necrotic cells, there was no evidence to suggest a concomitant increase in exfoliation. This suggests that Hg also influences epithelial cell turnover.

While most studies focus on the effect that heavy metals have on the metabolism of bivalves during exposure to a pollutant, this study also examined their physiology and morphology during a 24 days recovery period. After placement in clean water, there was a dramatic reduction of Hg in the soft tissues. Within eight days, Hg levels had reduced from 87–17 $\mu\text{g/g}$ and to 13 $\mu\text{g/g}$ 16 days later. It is important to note that even after 24 days in unpolluted water, Hg levels still remained 100 times higher than in control animals. A reduction of Hg during the post exposure period has also been reported in the bivalve *Crassostrea virginica*, which when exposed to 100 $\mu\text{g/l}$ for 45 days showed a subsequent reduction of Hg after transfer to uncontaminated seawater (Clark, 1996) a phenomenon also reported by Calow (1994). Loss of Hg from *P. perna* tissues during the recovery period correlated with the recovery of the filtration rate. Within eight days, filtration rates had returned to normal and remained within normal values until sacrifice. Over the 24 days recovery period, the endothelium lining the branchial vein became continuous and endothelial cells appeared morphologically normal. Granulocytes were far less evident in the epithelium and epithelial cell morphology gradually returned to near normal. There remained, however, occasional necrotic cells and myelin figures in nerves, Mv were sometimes swollen and there were some re-

sidual, osmiophylic inclusion in lc, lfc and fc after 24 days in non-contaminated water.

This study shows the remarkable resilience of *P. perna* to high concentrations of a particularly toxic, heavy metal. Few animals died during the experiments and the animals returned to physiological normality (as reflected in filtration rates) within eight days of exposure to Hg. In addition, while their gill filaments exhibited severe pathomorphology during exposure to Hg, gill filament morphology returned to near normal within 24 days of being returned to clean seawater. While our findings agree with those of other authors that bivalves efficiently accumulate metal in their soft tissues (Ólafsson, 1986; De Gregori et al., 1996; Ahn et al., 1996; O' Connor and Baliaeff, 1994; O' Connor, 1996), our results show that *P. perna* is also able to rapidly depurate its soft tissues.

In conclusion, sub-lethal concentrations of mercury were rapidly accumulated in the soft tissues of *P. perna*, had a negative effect on filtration rates and induced marked changes in gill filament morphology. These changes were readily reversed on return of the mussels to clean seawater, with near normal conditions being re-attained within 24 days. This resilience suggests that caution should be applied in using *P. perna*, and probably other mussel species as biomonitors over long time frames since widely spaced, intermittent releases may not be detected.

Acknowledgements

The authors would like to thank the National Research Foundation (NRF) for funding this project and the Council for Scientific and Industrial Research (CSIR) for the use of their facilities during the experimental stages of the study.

References

- Abel, P.D., 1976. Effect of some pollutants on the filtration rate of *Mytilus*. Mar. Pollut. Bull. 7, 228–231.
- Ahn, I.Y., Lee, S.H., Kim, K.T., Shim, J.H., Kim, D.Y., 1996. Baseline heavy metal concentrations in the Antarctic clam, *Laternula elliptica* in Maxwell Bay, King George Island, Antarctica. Mar. Pollut. Bull. 32, 592–598.
- Andersen, V., Maage, A., Johannessen, P.J., 1996. Heavy metals in blue mussels (*Mytilus edulis*) in the Bergen Harbor area, western Norway. Bull. of Environ. Contam. Toxicol. 57, 589–596.
- Axiak, V., George, J.J., 1987. Effects of exposure to petroleum hydrocarbons on the gill function and ciliary activities of a marine bivalve. Mar. Biol. 94, 157–162.
- Axiak, C., George, J.J., Moore, M.N., 1988. Petroleum hydrocarbons in the marine bivalve *Venus verrucosa*: accumulation and cellular responses. Mar. Biol. 97, 225–230.

- Ballan-Difrancais, C., Jeantet, A.Y., Coulon, J., 1990. Cytological features of mussels (*Mytilus edulis*) *in situ* exposed to an effluent of the titanium dioxide industry. *Ann. Inst. Oceanogr.* 66 (12), 118.
- Belialieff, B., O'Connor, T.P., Daskalakis, D.K., Smith, P.J., 1997. US mussel watch data from 1986–1994. Temporal trend detection at large spatial scales. *Am. Chem. Soc.* 31 (5), 1411–1415.
- Broman, D., Lindqvist, L., Lundbergh, L., 1991. Cadmium and Zinc in *Mytilus edulis* L. from the Bothnian Sea and northern Baltic proper. *Environ. Pollut.* 74, 227–244.
- Burgess, R.M., McKinney, M., 1999. Importance of interstitial, overlying water and whole sediment exposures to bioaccumulation by marine bivalves. *Environ. Pollut.* 104, 373–382.
- Caihuan, K., Yu, K.N., Lam, P.K.S., 2000. Uptake and depuration of cesium in the green mussel *Perna viridis*. *Mar. Biol.* 137, 567–575.
- Calow, P., 1994. Heavy metals. In: *Handbook of Ecotoxicology*, vol. 2. Oxford Blackwell Scientific Publications, London, pp. 79–97.
- Catapano, E.J., 1979. Neurophysiological correlates of the dopaminergic cilio-inhibitory mechanism of *Mytilus edulis*. *J. Exp. Biol.* 83, 315–323.
- Clark, R.B., 1996. Metals. In: *Marine Pollution*. Oxford University Press, Oxford, New York, pp. 64–82 (Chapter 5).
- Cossa, D., 1988. Cadmium in *Mytilus* spp: worldwide survey and relationship between seawater and mussel content. *Marine Environ. Res.* 26, 265–284.
- Coughlan, J., 1969. The estimation of filtering rate from the clearance of suspensions. *Mar. Biol.* 2, 356–358.
- Cunningham, P.A., 1979. The use of bivalve molluscs in heavy metal pollution research. *Mar. Poll. Functional Responses*, 183–214.
- De Gregori, I., Pinochet, H., Gras, N., Munoz, L., 1996. Variability of cadmium, copper and zinc levels in Molluscs and associated sediments from Chile. *Environ. Pollut.* 92, 359–368.
- Depledge, M.H., Aagaana, A., Gyorkos, P., 1995. Assessment of trace metal toxicity using molecular, physiological and behavioural biomarkers. *Mar. Pollut. Bull.* 31, 19–27.
- Dorn, P., 1976. The feeding behaviour of *Mytilus edulis* in the presence of methyl mercury acetate. *Bull. Environ. Contam. Toxicol.* 15, 714–719.
- Eisler, R., 1977. Acute toxicity of selected heavy metals to the softshell clam. *Bull. Environ. Contam. Toxicol.* 17, 137–145.
- Goldberg, E.D., 1975. The mussel watch—A first step in global marine monitoring. *Mar. Pollut. Bull.* 6, 111.
- Goldeberg, E.D., Bowen, V.T., Farrington, J.W., Harvey, G., Martin, J.H., Parker, P.L., Risebrough, R.W., Robertson, W., Schneider, E., Gamble, E., 1987. The mussel watch. *Environ. Conserv.* 5 (2), 101–125.
- Grace, A.L., Ganey Jr., L.F., 1987. The effects of copper on the heart rate and filtration rate of *Mytilus edulis*. *Mar. Pollut. Bull.* 18, 87–91.
- Gregory, M.A., George, R.C., McClurg, T.P., 1996. The architecture and fine structure of gill filaments in the brown mussel, *Perna perna*. *S. Afr. J. Zool.* 31, 193–206.
- Gregory, M.A., George, R.C., Marshall, D.J., Anandraj, A., McClurg, T.P., 1999. The effects of mercury exposure on the surface morphology of gill filaments in *Perna perna* (Mollusca: Bivalvia). *Mar. Pollut. Bull.* 39, 116–121.
- Gregory, M.A., George, R.C., 2000. The structure and surface morphology of gill filaments in the brown mussel *P. perna*. *Afr. Zool.* 35 (1), 121–129.
- Hennig, H.F. 1985. Review of metal concentrations in southern African coastal waters, sediments and organisms. South African National Scientific Programmes Report no.108, FRD, CSIR, Pretoria. p. 40.
- Hieteman, B., Sunila, I., Kristofferson, R., 1988. Toxic effects of zinc on the common mussel *Mytilus edulis* L. (Bivalvia). *Ann. Zool. Fennici* 25, 341–347.
- Higashiyama, T., Shiraiishi, H., Otsuki, A., Hashimoto, S., 1991. Concentrations of organotin compounds in blue mussels from the wharves of Tokyo Bay. *Mar. Pollut. Bull.* 22 (12), 585–587.
- Hiripi, L., Salalanki, J., 1973. 5-HT-DOPA decarboxylase in the nervous system and other tissues of *Anodonta cygnea* L. (Pelecypoda). In: Salánki, J. (Ed.), *Neurobiology of invertebrates. Mechanisms of Rhythm Regulation*. Akademiai Kiadó, Budapest, pp. 291–401.
- Kraak, M.H.S., Lavy, D., Peters, W.H.M., Davids, C., 1992. Chronic exotoxicity of copper and cadmium to the Zebra Mussel *Dreissena polymorpha*. *Arch. Environ. Contam. Toxicol.* 23, 363–369.
- Kraak, M.H.S., Toussaint, M., Lavy, D., Davids, C., 1994. Short term effects of copper and cadmium on the Zebra Mussel *Dreissena polymorpha*. *Environ. Pollut.* 84, 139–143.
- Lakshmanan, P.T., Nambisan, P.N.K., 1989. Bioaccumulation and depuration of some trace metals in the mussel, *Perna viridis* (Linnaeus). *Bull. Environ. Contam. Toxicol.* 43, 131–138.
- Manly, R., Blundell, S.P., Fifield, F.W., McCabe, P.J., 1996. Trace metal concentrations in *Mytilus edulis* L. From the Laguna San Rafael, Southern Chile. *Mar. Pollut. Bull.* 32, 444–448.
- Marshall, D.J., McQuaid, C.D., 1993. Differential physiological and behavioural responses of the intertidal mussels, *Coromytilus meridionalis* (kr.) and *Perna perna* L., to exposure to hypoxia and air: a basis for spatial separation. *J. Exp. Mar. Biol. Ecol.* 171, 225–237.
- Martin, J.M., Piltz, F.M., Reish, D.J., 1975. Studies on *Mytilus edulis* community in Alamitos Bay, California, V. The effect of heavy metals on byssal thread production. *Veliger* 18, 183–188.
- Martin, M., Richardson, B.J., 1991. Long term contaminant biomonitoring: views from southern and northern hemisphere perspectives. *Mar. Pollut. Bull.* 22 (11), 533–537.
- Micallef, S., Tyler, P.A., 1990. Effect of mercury and selenium on the gill function of *Mytilus edulis*. *Mar. Pollut. Bull.* 21, 288–292.
- Moore, M.N., 1985. Cellular responses to pollutants. *Mar. Pollut. Bull.* 16, 134–139.
- Murray, A.P., Richardson, B.J., Gibbs, C.F., 1991. Bioconcentration factors for petroleum hydrocarbons, PAHs, LABs and biogenic hydrocarbons in the blue mussel. *Mar. Pollut. Bull.* 22 (12), 595–603.
- Niamo, T.J., Atchison, G.J., Hollan-Bartels, L.E., 1992. Sublethal effects of cadmium on the physiological responses in the pocket-book mussel, *Lampsilis ventricosa*. *Environ. Toxicol. Chem.* 11, 1013–1021.
- O'Connor, T.P., Baliaeff, B., 1994. The National Oceanic and Atmospheric Administration (NOAA) National Status and Trends Mussel Watch Program: National monitoring of chemical contamination in the coastal United States. In: Cothern, C.R., Ross, N.P. (Eds.), *Environmental Statistics Assessment and Forecasting*. Lewis Publishers, Boca Raton, pp. 331–339.
- O'Connor, T.P., 1996. Trends in chemical concentrations in mussels and oysters collected along the US Coast from 1986 to 1993. *Mar. Environ. Res.* 41, 183–200.
- Odzak, N., Martinic, D., Zvonaric, T., Branica, M., 1994. Bioaccumulation rate of Cd and Pb in *Mytilus galloprovincialis* foot and gills. *Mar. Chem.* 46, 119–131.
- Ólafsson, J., 1986. Trace metals in mussels (*Mytilus edulis*) from southwest Iceland. *Mar. Biol.* 90, 223–229.
- Prakash, T., Rao, K.S.J., 1993. Relationship between metal content in bivalve shell and its' physical parameters. *Fresenius Environ. Bull.* 2, 509–513.
- Regolli, F., Orlando, E., 1994. Accumulation and subcellular distribution of metals (Cu, Fe, Mn, Pb and Zn) in the Mediterranean mussel *Mytilus galloprovincialis* during a field transplant experiment. *Mar. Pollut. Bull.* 28, 592–600.

- Reynolds, E.S., 1962. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* 17, 208–212.
- Richardson, B.J., Garnham, J.S., Fabris, J.G., 1994. Trace metal concentrations in mussels (*Mytilus edulis planulatus* L.) transplanted into southern Australian waters. *Mar. Pollut. Bull.* 28 (6), 392–396.
- Ritz, D.A., Swain, R., Elliot, N.G., 1982. Use of the mussel *Mytilus edulis planulatus* (Lamarck) in monitoring heavy metal levels in seawater. *Aust. J. Mar. Freshwat. Res.* 33, 491–506.
- Roesijadi, G., Fellingham, G.W., 1987. Influence of Cu, Cd, and Zn pre-exposure on Hg toxicity in the mussel *Mytilus edulis*. *Can. J. Fish Aquat. Sci.* 44, 680–684.
- Salanki, J., Hiripi, L., 1990. Effects of Heavy metals on the serotonin and dopamine systems in the central nervous system of the freshwater mussel. *Comp. Biochem. Physiol.* 95, 301–305.
- Schindler, P.W., 1991. The regulation of heavy metals in natural aquatic systems. In: Vernet, J.P. (Ed.), *Heavy metals in the environment*. Elsevier, Amsterdam, pp. 95–123.
- Silverman, H., Lynn, J.W., Dietz, T.H., 1996. Particle capture by the gills of *Dreissena polymorpha*: structure and function of latero-frontal cirri. *Biol. Bull.* 191, 42–54.
- Spurr, A.R., 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* 26, 31–34.
- Sunila, I., 1986. Chronic histopathological effects of short-term copper and cadmium exposure on the gill of the mussel, *Mytilus edulis*. *Invert. Path.* 47, 125–142.
- Sunila, I., 1987. Histopathology of mussels (*Mytilus edulis* L.) from Tvärminne area, the Gulf of Finland (Baltic Sea). *Ann. Zool. Fennici.* 24, 55–69.
- Sunila, I., 1988a. Acute histological responses of the gill of the mussel, *Mytilus edulis*, to exposure by environmental pollutants. *J. Invert. Path.* 52, 137–141.
- Sunila, I., 1988b. Pollution related histopathological changes in the mussel *Mytilus edulis* L. in the Baltic Sea. *Mar. Environ. Res.* 24, 277–280.
- Sze, P.W.C., Lee, S.Y., 2000. Effects of chronic copper exposure on the green mussel *Perna viridis*. *Mar. Biol.* 137, 379–392.
- Szefer, P., Ikuta, K., Kushiyama, S., Szefer, K., Frelek, K., Geldon, J., 1997. Distribution and association of trace metals in soft tissue and byssus of *Mytilus edulis* from the east coast of Kyushu Island, Japan. *Arch. Environ. Contam. Toxicol.* 32, 184–190.
- Szefer, P., Ali, A.A., Ba-Haroon, A.A., Rajeh, A.A., Geldon, J., Nabrzyski, M., 1999. Distribution and relationship of selected trace metals in mollusks and associated sediments from the Gulf of Aden, Yemen. *Environ. Pollut.* 106, 299–314.
- Tanabe, S., 1994. International mussel watch in Asia-Pacific phase. *Mar. Pollut. Bull.* 28 (9), 518.
- Tavares, T.M., Rocha, V.C., Porte, C., Barcelo, D., Albaiges, J., 1988. Application of the mussel watch concept in studies of hydrocarbons, PCBs and DDT in the Brazilian Bay of Todos os Santos (Bahia). *Mar. Pollut. Bull.* 19 (11), 575–578.
- Ward, R.E., 1990. Metal concentrations and digestive gland lysosomal stability in mussels from Halifax Inlet, Canada. *Mar. Pollut. Bull.* 21, 237–240.
- Watling, H.R., Watling, R.J., 1982. Comparative effects of metals on the filtering rate of the brown mussel (*Perna-perna*). *Bull. Environ. Contam. Toxicol.* 29, 651–657.