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MINI REVIEW

A review of the effects of heavy metals on freshwater mussels

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The widespread recent decline in the species diversity and population density of freshwater mussels in North America may be partly related to chronic, low-level exposure to toxic metals. As benthic filter-feeding organisms, freshwater mussels are exposed to metals that are dissolved in water, associated with suspended particles and deposited in bottom sediments. Thus, freshwater mussels can bioaccumulate certain metals to concentrations that greatly exceed those dissolved in water. In adult mussels, the most common site of metal uptake is the gill, followed by the mantle and the kidney. The toxic effects of metals on freshwater mussels have been examined in a few acute toxicity tests, but the sublethal effects of long-term exposure to low environmental concentrations are little understood. Sublethal exposure to metals can alter growth, filtration efficiency, enzyme activity and behaviour. Sublethal effects are frequently observed at concentrations that are only half the lethal concentrations. However, few toxicity tests have used environmentally realistic exposure concentrations. Total concentrations of Cd, Cu, Hg and Zn in many oxic surface waters are in the $ng\,l^{-1}$ range, yet many toxicity studies have exposed mussels to concentrations in the $\mu g\,l^{-1}$ or even the $mg\,l^{-1}$ range. An understanding of the processes by which metals affect freshwater mussels would provide insights on the ecotoxicological significance of metal contamination to natural mussel populations and aid in the development of water-quality criteria that adequately protect mussels.

Keywords: freshwater mussels; metals; effects; bioaccumulation; toxicity; review.

Introduction

Freshwater mussels are ecologically and economically important in aquatic ecosystems. Mussels can comprise a significant proportion of the total standing crop in freshwater benthic communities (Mann 1964, Negus 1966, Cameron *et al.* 1979), they can be important in the cycling of calcium in lakes (Green 1980) and they can mix surficial sediments through bioturbation (McCall *et al.* 1979). Mussels also serve as food for aquatic mammals, including raccoons, muskrats and otters (Van der Schalie and Van der Schalie 1950).

The density and species diversity of freshwater mussels in North America have declined substantively during the past 30 years, but the causal factors are seldom known. Such declines have been documented for several rivers, including the Illinois (Starrett 1971), the Tennessee system (Isom 1969, Gordon and Layzer 1989), Ohio (Williams and Schuster 1989) and upper Mississippi (Coon *et al.* 1977). These declines

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have been attributed to an array of factors, including sedimentation (Ellis 1936, Stansbery 1970), changes in fish-host distribution (Isom and Yokley 1968), impoundment of rivers (Bates 1982) or creation of wing dams (Fuller 1974). Although not sufficiently documented, exposure to toxic contaminants may also be contributing to these declines. Chemical spills and other point sources of contaminants can cause localized mortality; however, the widespread decreases in density and diversity may result in part from the subtle, pervasive effects of chronic, low-level contamination.

Mussels are exposed to an array of anthropogenic contaminants. This review focuses primarily on four metals (cadmium (Cd), copper (Cu), mercury (Hg) and zinc (Zn)), which are widespread, persistent and potentially toxic. Many freshwater ecosystems are contaminated with these metals, as a result of human activities.

The chemical form, bioavailability and toxicity of most metals are greatly influenced by water and sediment chemistry. The aquatic chemistry of metals has been widely studied (Forstner and Wittmann 1981, Moore and Ramamoorthy 1984, Salomons and Forstner 1984, Leland and Kuwabara 1985). However, little is known about the effects of long-term sublethal exposures to metals on freshwater mussels.

I critically review the literature on bioaccumulation, tissue distribution, uptake, elimination, detoxification and ecotoxicological effects of certain metals on freshwater mussels. The review focuses on freshwater mussels of the family Unionidae; however, information on other freshwater molluscs (such as *Corbicula* and *Dreissena*) and on marine invertebrates was occasionally used if no data on unionid mussels were available.

Metal pollution and environmental exposure

Concentrations of many metals in natural waters are much lower than previously believed (before about 1985) because of recent advances in the use of trace metal-free protocols to reduce sample contamination during handling and analyses (Nriagu *et al.* 1993). For example, recently reported dissolved trace metal concentrations for the Mississippi River are 10- to 100-fold lower than those previously reported (Windom *et al.* 1991). However, nearly all bodies of water in the Northern Hemisphere are contaminated with metals such as mercury, cadmium and lead due to long-range atmospheric transport and deposition from anthropogenic sources (Norton *et al.* 1990, Spry and Wiener 1991, Rognerud and Fjeld 1993).

Recent studies using clean techniques have documented that total concentrations in oxic surface waters are in the ranges 0.6–100 ng $Hg\,l^{-1}$, 7–350 ng $Cd\,l^{-1}$, 100–2000 ng $Cu\,l^{-1}$ and 30–560 ng $Zn\,l^{-1}$ (Table 1). In pristine and lightly contaminated systems, the ranges of total concentrations are 0.6–4.0 ng $Hg\,l^{-1}$, 10–70 ng $Cd\,l^{-1}$, 100–500 ng $Cu\,l^{-1}$ and 30–200 ng $Zn\,l^{-1}$ (Table 1).

Many toxic metals that enter aquatic systems are absorbed onto suspended particles and subsequently accumulate in surficial sediments (Salomons *et al.* 1987, Tessier and Campbell 1987). Toxic concentrations of dissolved metals are uncommon in oxic surface waters. In the Mississippi River, for example, more than 90% of the trace metal load is associated with particles (Trefry *et al.* 1986). Thus, these metals can be accumulated by and directly affect filter-feeding benthic organisms such as freshwater mussels (Giesy and Hoke 1989).

Biology of unionid mussels

Certain natural history characteristics make freshwater mussels useful bioindicators of water and sediment pollution. They are macroscopic, long-lived benthic invertebrates that obtain food principally by filter feeding and are consequently exposed to contaminants that are dissolved in water, associated with suspended particles and deposited in bottom sediments. The association of freshwater mussels with fine-grained sediments increases their exposure to sediment-associated contaminants.

Many of the ecologically significant aspects of mussel biology are revealed by an examination of their life cycle (see Pennak 1978, McMahon 1991). Eggs are produced in the ovaries and released into the female's suprabranchial chamber where they are fertilized by sperm, concurrently released by males. Embryos are held in marsupial gills, where they develop into parasitic glochidia (Fig. 1). Species characterized as short-term brooders usually spawn in spring and release glochidia soon after they become fully developed. Long-term brooders spawn in late spring and early summer and the glochidia overwinter in the gills of females to be released in the following spring. Once released, glochidia are obligate parasites on the gills or fins of fish hosts, where they metamorphose and, after dropping off their fish host, become free-living juveniles. The specificity of glochidia to fish hosts is species-dependent; some mussel species can successfully metamorphose on only a single fish species, while others can use a wide variety of fishes as hosts. Thus, the species diversity and population density of freshwater molluscs may be attributed, at least in part, to changes in the availability of suitable fish hosts.

A unique physiological feature of bivalve molluscs is their ability to filter large volumes of water. Water filtration rates (ml per individual per h) are in the ranges 10–100 in *Dreissena polymorpha*, 0.6–8.3 in Sphaeridae, 60–490 in Unionidae and 60–800 in *Corbicula* spp. (Stanczykowska *et al.* 1976, Mackie 1991). Given a conservative filtration rate of 300 ml per individual per h, a life span of 25 years, 100% assimilation efficiency and the dissolved and particulate Cd concentrations present in the Mississippi River (Trefry *et al.* 1986), a unionid mussel could accumulate (assuming no elimination) more than 0.85 mg of dissolved Cd and more than 4.3 mg of particulate Cd in its lifetime.

Bioaccumulation

A contaminant is bioaccumulated when its rate of uptake exceeds its rate of elimination. Freshwater mussels meet many of the requirements of a good biological sentinel organism (Phillips 1977) – they are somewhat sedentary, regionally abundant, long-lived and have adequate tissue mass for analysis. They readily accumulate many metals and their body burden seems to reflect mean exposure levels over time. Consequently, they have been used as sentinel organisms in many freshwater contaminant-monitoring programmes (Adams *et al.* 1981, Schmitt *et al.* 1987). In 1978, a large-scale biomonitoring programme, termed 'International Mussel Watch', was initiated to monitor pollution levels in the world's coastal waters. This programme uses bivalve molluscs to assess environmental pollution in the coastal marine environment (Goldberg *et al.* 1978).

The availability of metals for uptake by organisms is influenced by an array of

Table 1. Range of concentrations of total and dissolved Cd, Cu, Hg and Zn in oxic fresh waters, from studies in which trace metal-free protocols were used during handling and analyses of samples

Location	Number and type of surface waters sampled	Concentration ($ng\,l^{-1}$)		Reference
		Total	Dissolved	
Mercury				
California, USA	Silver Lake (pristine)	0.6	0.4	Gill and Bruland (1990)
North American Great Lakes	Lake Ontario	0.9	0.7	Gill and Bruland (1990)
Nevada, USA	Pyramid Lake (desert lake)	1.9	0.9	Gill and Bruland (1990)
Sweden	Eight drainage lakes	1.4–15	–	Lee and Iverfeldt (1991)
Northern Manitoba, Canada	Burntwood River	2.1	–	Ramsey (1990)
New York State, USA	Onondaga Lake (Hg polluted)	7–19	2–10	Bloom and Effler (1990)
California, USA	Clear Lake (Hg polluted tailings)	3.6–104	1.1–1.5	Gill and Bruland (1990)
	Davis Creek Reservoir (Hg polluted tailings)	2.7–13	1.9–4.0	Gill and Bruland (1992)
Brazil	Madeira River and tributaries (Hg polluted)	20–33	10–17	Nriagu *et al.* (1992)
Cadmium				
Antarctica	Lake Vanda (pristine)	10–70	–	Green *et al.* (1986)
Sweden	Fifty-nine forest lakes	7–36	–	Borg (1987)
Southern USA	Mississippi River (at mouth)	–	8–16	Trefry *et al.* (1986)
Louisiana, USA	Mississippi River (at St. Francisville)	–	101	Taylor *et al.* (1990)
North American Great Lakes	Lake Erie	–	7–11	Coale and Flegal (1989)
North American Great Lakes	Lake Ontario	–	0.7–9	Coale and Flegal (1989)
France	Rhone River (drains industrial area)	20–117	17–80	Huynh-Ngoc *et al.* (1988a)
Sweden	Lake Langsjon	100–350	–	Andersson and Borg (1988)

Copper				
Sweden	Fifty-nine forest lakes	100–2000	–	Borg (1987)
Antarctica	Lake Vanda (pristine)	400–600	200–400	Green *et al.* (1986)
Southern USA	Mississippi River (at mouth)	–	1810–1960	Trefry *et al.* (1986)
Louisiana, USA	Mississippi River (at St. Francisville)	–	1569	Taylor *et al.* (1990)
North American Great Lakes	Lake Erie	–	546–820	Coale and Flegal (1989)
North American Great Lakes	Lake Ontario	–	724–1010	Coale and Flegal (1989)
France	Rhone River (drains industrial area)	405–1340	119–1240	Huynh-Ngoc *et al.* (1988b)
Zinc				
Sweden	Fifty-nine forest lakes	< 26–556	–	Borg (1987)
China	Yangtze River (at mouth)	–	39–78	Shiller and Boyle (1985)
South America	Amazon River	–	20–248	Shiller and Boyle (1985)
North American Great Lakes	Lake Erie	–	26–55	Coale and Flegal (1989)
North American Great Lakes	Lake Ontario	–	3–115	Coale and Flegal (1989)
Louisiana, USA	Mississippi River (at St. Francisville)	–	131	Taylor *et al.* (1990)
Ohio, USA	Ohio River (drains industrial area)	–	288–3203	Shiller and Boyle (1985)
	Sciotto River (drains metropolitan area)	–	1307–1438	Shiller and Boyle (1985)
New Jersey, USA	Delaware River (at West Trenton) (drains metropolitan area)	–	3922–3988	Shiller and Boyle (1985)

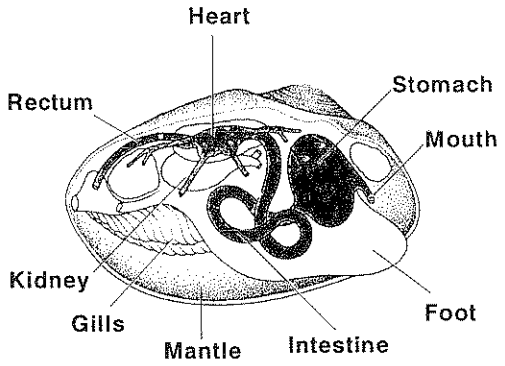

Fig. 1. The internal anatomy of a freshwater bivalve mollusc showing the major internal organs. (From *Zoology* by L. Mitchell, J. Mutchmor and W. Dolphin, copyright 1988 by the Benjamin/Cummings Publishing Company, reprinted by permission.)

factors, including speciation, water quality (particularly pH and hardness) and the concentration and composition of particulate material (Luoma and Bryan 1979). The form of metal available for uptake is often metal and pH specific. For example, in the Mississippi River, a neutral to basic pH system with a mean total suspended matter concentration greater than 100 $mg\,l^{-1}$, 70–90% of the Cd and Cu is associated with particulates (Trefry *et al.* 1986). Conversely, in systems with neutral to acidic waters, Cd exists largely as the free Cd^{2+} ion, with minor contributions from various inorganic complexes; compared to other divalent metals it shows relatively little tendency to associate with organic colloids or organic chelators (Campbell and Tessier 1987). The mean dissolved concentrations of Cd and Cu in the Mississippi River, for example, are in the ranges 8–16 $ng\,l^{-1}$ and 1810–1960 $ng\,l^{-1}$, respectively; the mean particulate concentrations (0.4 μm pore size, reported as μg metal/g suspended matter) are in the ranges 0.6–0.8 $\mu g\,g^{-1}$ and 27–34 $\mu g\,g^{-1}$, respectively (Trefry *et al.* 1986).

Several investigators have measured metal concentrations in overlying surface water, pore water and surficial oxic sediments to determine which of these variables was most likely to predict metal levels in indigenous benthic organisms. This approach seems theoretically simple, but is fraught with misconceptions. One of the primary misconceptions is that the *total* metal concentrations are the appropriate standard measure of metal concentrations (Lee 1991, Tessier *et al.* 1993). The appropriate measure varies between the aqueous and particulate phases and perhaps among metals.

For example, Tessier *et al.* (1993) measured Cd concentrations in oxic sediments, overlying water and in soft tissues of *Anodonta grandis* along a Cd contamination gradient in 38 lakes in Canada. They found that variations in Cd levels in *A. grandis* were related to dissolved Cd^{2+} concentrations at the sediment–water interface; dissolved Cd^{2+} was calculated based on lake water pH and sediment–water sorption equilibria. In contrast, they found no relation between the Cd concentrations in *A. grandis* and the total Cd concentrations in sediment. Furthermore, they found no relation between the Cd in *A. grandis* and any sediment extract (an operationally defined measure of bioavailability), even when extractable Cd was normalized to Fe oxyhydroxides or organic carbon. In other studies, these geochemical normalizations

have been used successfully in predicting Cu, lead (Pb) and Zn bioaccumulation in marine (Luoma and Bryan 1978) and freshwater bivalves (Tessier *et al.* 1984).

The uptake of metals in mussels may partly depend on the principle contaminant sources of metals (food, water and sediment). Laboratory and field experiments show that marine bivalves accumulate heavy metals associated with ingested sediment (Luoma and Jenne 1977, Bryan and Uysal 1978). In a 14 day laboratory experiment, *Macoma balthica* obtained 75–89% of radiolabelled silver from ingested sediment (Luoma and Jenne 1977). The significance of ingested sediment to metal uptake in freshwater mussels has not been sufficiently assessed, perhaps because fewer freshwater species are deposit feeders than marine bivalves. Furthermore, the importance of phytoplankton as a dietary source of metals for freshwater mussels may have been under-emphasized.

The most contaminated sediments in many temperate lakes and rivers are often in the top 30 cm (Rada *et al.* 1989, 1990). For example, Rada *et al.* (1989) found the most Hg-contaminated sediments in 11 lakes in northern Wisconsin were in the top 15 cm. Furthermore, the highest sediment concentrations of Cd, Cu, Chromium (Cr) and Zn from Lake Pepin, a natural lake on the upper Mississippi River, were in the top 30 cm (Rada *et al.* 1990). Adult freshwater mussels typically burrow from 1 to 25 cm in sediment and feed by filtering phytoplankton and detritus out of the water column (Pennak 1978, McMahon 1991). Conversely, juvenile mussels typically burrow less than 8 cm (Neves and Widlak 1987) and feed on bacteria (2–5 μm), detritus and colloidal particles in the pore water (Yeager and Cherry 1994). Recently, Yeager and Cherry (1994) demonstrated that although juvenile *Villosa iris* burrowed less than 1 cm into the sediment, they were not exposed to the overlying water. Thus, although freshwater mussels, in general, can be exposed to metals that are dissolved in water, associated with suspended particles and deposited in bottom sediments, juvenile mussels are most likely exposed to elevated metal concentrations found in association with sediment or pore water.

Comparative data on modes of metal uptake in freshwater mussels are needed to design contaminant-monitoring programmes and to develop water-quality criteria that protect mussels adequately. Such programmes should focus on the environmental matrices (food, water and/or sediment) that most strongly control exposure. The critical criterion for the development of such programmes in the marine environment has focused on distinguishing metal uptake between food (particles) and water (solution) (Luoma and Jenne 1976).

Tissue distribution

Organ distribution. Metal concentrations in freshwater mussels are generally greatest in the gills and the mantle (Manly and George 1977, Tessier *et al.* 1984, Hemelraad and Herwig 1988). However, accumulation in other organs appears to be metal specific. Cadmium concentrations, for instance, are highest in the digestive gland, gills and kidney and lowest in the shell and muscle (Adams *et al.* 1981, Hemelraad *et al.* 1986b, Herwig *et al.* 1989). In marine bivalves, metal concentrations are similarly highest in the gills and digestive system (Cunningham 1979, Janssen and Scholz 1979, Robinson and Ryan 1986). The accumulation of metals within a certain tissue may be partly due to the presence of specific binding sites and perhaps due to the detoxification

mechanisms within that tissue. Cadmium, for example, may preferentially bind to the many sites associated with calcium concretions in the gills of freshwater mussels (Pynnonen *et al.* 1987).

An extensive series of laboratory experiments on Cd accumulation and distribution in the freshwater mussel *Anodonta* spp. was conducted by Hemelraad and co-workers (Hemelraad *et al.* 1986a,b, Hemelraad and Herwig 1988, Holwerda *et al.* 1988, 1989). In one series of experiments, Hemelraad *et al.* (1986a) exposed *Anodonta anatina* and *Anodonta cygnea* to 29 $\mu g\,Cd\,l^{-1}$ for 16 weeks. Rates of accumulation did not differ between the two species during the first 11 weeks; thereafter, only *A. anatina* continued to accumulate Cd. The total dry weight of soft tissues was similar for the two species; however, the dry weights of the gills, kidney and digestive gland in individual *A. cygnea* were twice those in *A. anatina*. Hence, interspecific differences in Cd accumulation were small when expressed on a whole organism basis and large when expressed on an organ basis. Furthermore, the pattern and rate of metal accumulation can vary considerably between species (Hemelraad *et al.* 1986a) and the size of the mussel (Naimo *et al.* 1992b).

In natural waters, freshwater mussels are exposed to a mixture of metals. Most laboratory studies have focused on a single metal to investigate metal-specific uptake and tissue distribution. Such studies are critical to understanding contaminant dynamics, but cannot be used to assess effects of multiple-metal exposures. Hemelraad *et al.* (1987), for example, demonstrated that Zn (exposure concentration 25 $mg\,l^{-1}$) in freshwater mussels was antagonistic to Cd (exposure concentration 25 $\mu g\,l^{-1}$), inhibiting Cd uptake in gills and accelerating the redistribution of Cd from the gills to internal organs. They hypothesized that Zn competes with Cd for binding sites in the gills.

Marine and freshwater mussels can accumulate certain metals to high concentrations without adverse effects (Ravera 1984; Ray 1984). Cadmium, for example, can concentrate in the kidney for detoxification (such as by chelation) without adversely affecting that organ (Ravera 1984). In addition, the rate of metal accumulation within an organism likely varies as a function of the exposure concentration; the importance of exposure concentration on test results cannot be overemphasized. Few ecotoxicologists are conducting toxicity tests on freshwater mussels at concentrations found in natural waters (Table 1).

Mussels are often not fed during tests examining the localization and toxicity of metals. The lack of food may eliminate confounding factors in studies which examine mechanistic processes, but it can physiologically affect the test animal. For instance, *M. balthica* accumulated Hg, Zn and Cd more rapidly from solution when unfed than when fed (Luoma and Jenne 1976). The presence or absence of food may affect both feeding rates and the rate of water transport across gill epithelia, which can affect the uptake of metals (Janssen and Scholz 1979). The limited data on nutritional requirements of freshwater mussels preclude recommendations on feeding regimes at this time.

Cellular and subcellular distribution. There is little information on the cellular or subcellular distribution of metals in mussels (Pauley and Nakatini 1968, Cassini *et al.* 1986, Hemelraad and Herwig 1988). Epithelial cells generally store more Cd relative to muscle cells (Hemelraad and Herwig 1988). Pauley and Nakatini (1968), who studied the distribution of ^{65}Zn in *Anodonta californiensis*, found that haemocytes, the outer mantle epithelium, renal epithelial cells and the renal lumen had high concentrations of

Zn, whereas the inner mantle epithelium contained little Zn. Lysosomes in marine organisms contain high metal concentrations, thus, lysosomal formation may detoxify metals through chelation (Walker 1977, George and Pirie 1979, Viarengo *et al.* 1981). Furthermore, metals that concentrate in the cell nucleus could cause mutagenesis. Double-stranded deoxyribonucleic acid, for example, readily binds *in vitro* with Cd (Waalkes and Poirier 1984).

Cassini *et al.* (1986) found that the distribution of metals between the particulate and cytosolic fractions differed among metals (Cu, Cd and Zn), between mussel species (*A. cygnea* and *Unio elongatulus*) and among tissues (gill, digestive gland and remainder). This variation in distribution suggests that multiple-metal detoxification or elimination mechanisms may exist. In contrast, Hemelraad *et al.* (1986a) found that the cytosolic and particulate distribution (including the nuclear, mitochondrial–lysosomal and microsomal fractions) of Cd did not differ between species (*A. anatina* and *A. cygnea*) or among organs (gill, mantle, digestive gland and kidney) during a 16 week exposure period.

Detoxification mechanisms and metal elimination

Detoxification. Many freshwater organisms possess mechanisms that protect against toxic metals. These mechanisms include inhibition of transfer across biological barriers, elimination from the organism and detoxification by binding of the toxic metal into complexes. Perhaps the most widely studied binding mechanism is the binding of divalent metal ions by the group of proteins termed metallothioneins (MT) (Hamilton and Mehrle 1986, Steinert and Pickwell 1988, Garvey 1990). These proteins contain sites that can bind metals, such as Cd, Cu, Hg and Zn, that have an affinity for sulfhydryl groups.

An MT-like protein with a molecular weight of 11 kDa has been shown to bind Cd in freshwater mussels (Hemelraad *et al.* 1986a). In a laboratory experiment, these authors found that the fraction of MT-bound Cd (relative to unbound Cd) in the gills, mantle and digestive gland of *A. anatina* and *A. cygnea* increased with exposure time, especially during the first 4 weeks; this suggests that MT synthesis is induced by exposure to Cd. Couillard *et al.* (1993) recently provided field evidence that MT is involved in Cd detoxification in *A. grandis*. These authors sampled mussels from 11 lakes along a Cd contaminant gradient and documented that MT synthesis can be induced at metal concentrations encountered in polluted environments and that tissue concentrations of MT respond in a dose-dependent relation.

Another possible metal-binding and detoxification system in freshwater molluscs involves the incorporation of metals in inorganic crystalline concretions assembled on inorganic matrices (Abolins-Krogis 1958, Simkiss 1981, Silverman *et al.* 1987b). Such concretions in freshwater mussels appear to be calcium dominated, whereas concretions in marine invertebrates consist mostly of lead carbonate (Marshall and Talbot 1979), ferric phosphate (Buchanan *et al.* 1980) or zinc phosphate (Walker *et al.* 1975). The concretions in freshwater mussels store calcium for the construction of the glochidial shells during reproduction (Silverman *et al.* 1985, 1987a). In addition, gill concretions may bind Zn, Cd and Mn; however, the strength of the binding depends on the ionic content of the blood (Silverman *et al.* 1987b). Similar concretions in snails (Simkiss 1981), crustaceans (Becker *et al.* 1974, Guary and Negrel 1981) and scallops (Carmichael *et al.* 1979) also seem to have a detoxifying capacity.

The gills of *A. anatina*, *A. cygnea*, and *Unio pictorum* contain large amounts of calcium concretions; up to 55% of the total tissue dry weight in *A. cygnea* (Pynnonen *et al.* 1987). Furthermore, 75% of the total body concretion in *A. cygnea* was in the gills. Pynnonen *et al.* (1987) concluded that up to 20% of the Cd accumulated during a 3 week exposure to 40 $\mu g\,Cd\,l^{-1}$ was associated with the calcium concretions, but the proportion of Cd bound to the concretions decreased during the exposure period. This suggests either that concretions serve only a temporary role in metal detoxification or that the rate of metal uptake greatly exceeded that of concretion formation under these laboratory conditions.

Silverman *et al.* (1987b) suggested that calcium concretions in freshwater mussel gills are a short-term storage site for metals. These concretions are mobilized annually for glochidial shell formation during reproduction (Silverman *et al.* 1985) and may not be particularly useful for detoxification. In marine mussels, Viarengo *et al.* (1981) found that Cu bound to thionein-like proteins was eliminated within 12 days, which suggests that MT-like proteins may be an even more short-term repository than calcium concretions. More information will be needed on the functions and turnover rates of calcium concretions and MT-like proteins in both marine and freshwater mussels before generalizations can be made as to the primary detoxification mechanism.

Elimination. Many metals do not appear to be readily eliminated from freshwater mussel tissues. *Velesunio ambiguus* were exposed to aqueous Zn concentrations of 1, 5 and 10 $mg\,l^{-1}$ for 21 days and transferred to clean water for 21 days; no significant depuration of Zn occurred during this 42 day period (Millington and Walker 1983). Cadmium is characterized by its long retention time within mussel tissue, the rate of elimination being much slower than the rate of uptake (Holwerda *et al.* 1988). *Dreissena polymorpha* exposed to 3.3 $\mu g\,Cd\,l^{-1}$ for 10 days retained 56% of the accumulated Cd after 34 days in clean water; the only significant elimination of Cd was from the shell (Bias and Karbe 1985).

The elimination of Cd may occur in distinct stages and be influenced by differential metal release rates from organs (Holwerda *et al.* 1988). Cadmium was eliminated from *A. anatina* in three phases. Elimination first occurred from the total soft parts and gills, then ceased between 19 and 42 days after primary exposure to 16 $\mu g\,l^{-1}$ and, finally, increased again from the total soft parts and gills. This multi compartmented system for metal release is also seen in marine molluscs (Borchardt 1983).

An effective elimination system depends on metabolic processes within the organism (Borchardt 1983, Bias and Karbe 1985). Test mussels were not fed in the studies by Millington and Walker (1983), Bias and Karbe (1985) and Holwerda *et al.* (1988), even in 150 day tests. In controlled experiments, mussels are seldom fed the quality or quantity of food consumed in nature; this may affect feeding rates and the rate of water passage over the gills. Therefore, the slow elimination rates seen in these studies may reflect undernourishment and a consequent alteration in metabolic activity (Janssen and Scholz 1979).

Toxicity

Acute responses

Standardized toxicity test procedures have been developed for freshwater macroinvertebrates (ASTM, 1988) and for marine molluscs (APHA *et al.* 1989), but these

procedures do not apply well to freshwater mussels. The marine test is primarily for use with larval oysters, for which culture methods are well documented. Even though the *in vitro* culture of freshwater mussel glochidia is possible (Ellis 1929, Isom and Hudson 1982), the procedures are not well established. In addition, oysters achieve sexual maturity in approximately 5 months, much less than the 2–6 years required for freshwater mussels. Full life-cycle tests are consequently much less feasible for freshwater mussels than for oysters.

Several authors have suggested that standardized test methods be developed for freshwater mussels, especially the larval and juvenile stages (Keller and Zam 1991, Lasee 1991). Although adult mussels are often used in acute lethality tests (Rodgers *et al.* 1980, Harrison *et al.* 1984), their ability to close their valves to reduce exposure hinders their usefulness in acute lethality tests (Naimo *et al.* 1992a). Acute lethality tests suggest that juvenile freshwater mussels are more sensitive to Cd than the larval or adult stages (Lasee 1991) and more sensitive to Cu than adults (Jacobson *et al.* 1993). Conversely, larvae in marine systems are frequently more sensitive than juveniles (Ringwood 1990). Keller and Zam (1991) recently showed that juvenile *Anodonta imbecilis* are as sensitive to six metals (Cd, Cr, Cu, Hg, nickel (Ni) and Zn) as several widely used test organisms (e.g. *Daphnia magna*, fathead minnow *Pimephales promelas* and *Chironomus tentans*). Keller and Zam (1991) suggested that culture methods for this species are adequate and propose *A. imbecilis* as the prototype for standardized toxicity testing with mussels. However, it is desirable to have information on culture methods, physiological condition and nutritional requirements of additional genera before toxicity-test procedures are standardized for freshwater mussels.

Toxicity end-points

Toxicity tests require sensitive end-points. Both lethal and sublethal end-points have been used in tests with freshwater mussels. Death, the most common end-point in acute toxicity tests, may not be appropriate for mussels because the time of death is difficult to determine. Gaping valves, which remain open after gentle prodding with a probe, usually indicate mortality. It is not easy to demonstrate if an organism exhibiting these characteristics is dead.

Sublethal end-points in mussel toxicity studies include changes in foot immobilization (Millington and Walker 1983, Doherty and Cherry 1988), filtering activity (Rodgers *et al.* 1980, Doherty and Cherry 1988), oxygen consumption (Naimo *et al.* 1992a), blood osmotic pressure (Doherty and Cherry 1988), bioelectric activity (Morgan *et al.* 1989), ciliary activity (Lasee 1991) and valve activity (Rodgers *et al.* 1980, Millington and Walker 1983, Doherty *et al.* 1987, Jacobson *et al.* 1993).

Lasee (1991) determined both lethal (LC_{50}) and sublethal (EC_{50} – effective concentration affecting 50% of the organisms) end-points in acute toxicity tests with juvenile *Lampsilis cardium*. A stressed individual was defined as having evidence of ciliary activity but no foot movement. The 48 h LC_{50} in 0 day old juveniles was 141 $\mu g\,Cd\,l^{-1}$, but significant reductions in ciliary activity (EC_{50}) were observed at 90 $\mu g\,Cd\,l^{-1}$. This suggests that freshwater mussels become stressed at metal concentrations that are much lower than those reported in acute toxicity tests. Likewise, Jacobson *et al.* (1993) determined that the 24 h LC_{50} for juvenile *V. iris* was 83 $\mu g\,Cu\,l^{-1}$, but the 24 h EC_{50} (percentage that were gaped and dead or ungaped) was 27 $\mu g\,Cu\,l^{-1}$.

Freshwater mussels are sometimes considered to be insensitive to metals because they seem to withstand high concentrations within their tissues. Yet, data on the acute toxicity of metals to freshwater mussels are few (Table 2). The variations among test methods, mussel species, life history stages, metals and water hardness permit few generalizations and the influence of such variables should be examined thoroughly. Results from chronic toxicity tests with freshwater mussels should provide more ecologically relevant assessments than acute toxicity tests.

Chronic responses

There is scant information on the chronic effects of metals on freshwater mussels. Yet, the effects of metals on feeding, growth and reproduction could significantly affect mussel populations. Changes in valve movement patterns have been associated with contaminant exposure (Imlay 1968, Doherty *et al.* 1987). The mean time to first valve closure in *Corbicula fluminea* was 860 min with no exposure, 42 min after a 24 h exposure to 0.4 mg $Cd\,l^{-1}$ and 66 min after a 24 h exposure to 0.9 mg $Zn\,l^{-1}$ (Doherty *et al.* 1987). The lowest nominal concentrations causing extended valve closure in *D. polymorpha* were 0.37 mg $Cd\,l^{-1}$ and 0.030 mg $Cu\,l^{-1}$ (Sloof *et al.* 1983). The latter value for Cu is near the acute water quality criterion of 34 $\mu g\,Cu\,l^{-1}$ for water hardness of 200 mg l^{-1} as $CaCO_3$ (US Environmental Protection Agency 1984).

There are few data on the effects of low-level metal exposure on growth. Lasee (1991), who exposed juvenile *L. cardium* to 0–100 $\mu g\,Cd\,l^{-1}$ in a 7 day static renewal test, found that concentrations as low as 10 $\mu g\,Cd\,l^{-1}$ significantly reduced anterior shell growth. Histological observations of these juveniles revealed that concentrations greater than 30 $\mu g\,Cd\,l^{-1}$ inhibited or caused dissolution of the crystalline style. At concentrations of 100 $\mu g\,Cd\,l^{-1}$, extreme vacuolar degeneration of most organs was observed. A reduction in total weight gain has also been observed with 30 day exposure of *Corbicula* sp. to Zn (Belanger *et al.* 1986).

Physiological responses. Physiological studies of freshwater mussels have been used to assess metal effects on the mussel community. Periodic activity of adductor muscles in unionids have been used as physiological end-points of exposure. Periodic activity is the time interval between the open and closed positions of the valves (Salanki and Varanka 1976). In *A. cygnea*, exposure to $CuSO_4$ (nominal concentrations in the range 10^{-3}–10^{-9} g l^{-1}) decreased the durations of periodic activity by 10% at 0.1 $\mu g\,Cu\,l^{-1}$ and by 50% at 1.0 $\mu g\,Cu\,l^{-1}$, but exposure to $PbCl_3$ and $PbNO_3$ did not decrease activity at concentrations of 1 mg l^{-1} (Salanki and Varanka 1976). Furthermore, 2 week exposure of *A. cygnea* to 100 $\mu g\,Cd\,l^{-1}$ resulted in a 40% reduction in the mean time the valves were open (Herwig 1989).

Cellulolytic activity, which uses an enzyme group to hydrolyse algal cellulose into short-chain sugars, can be a physiological indicator of stress in freshwater mussels. Cellulolytic activity in *Corbicula* sp. was significantly reduced in artificial streams in 10–20 days at concentrations of 16 $\mu g\,Cu\,l^{-1}$ and 87 $\mu g\,Zn\,l^{-1}$, respectively (Farris *et al.* 1988).

The molar ratio of oxygen consumed to nitrogen excreted (O:N) can be used to assess the physiological condition of a mussel after contaminant exposure. This ratio provides an index of the use of protein in metabolism (Widdows 1978, Russell-Hunter *et al.* 1983). In a study on freshwater mussels, Aldridge *et al.* (1987) reported that O:N ratios less than 20 indicated catabolism of proteins and O:N ratios greater than 100

Table 2. Results of acute toxicity studies exposing freshwater mussels to metals in the laboratory

Organism	Test system	Life stage	Water hardness (as mg l^{-1} $CaCO_3$)	Metal	LC_{50} ($\mu g\,l^{-1}$)	Reference
A. imbecilis	Static	1–2 day juvenile	39	$NiSO_4$	48 h = 240	Keller and Zam (1991)
A. imbecilis	Static	1–2 day juvenile	60–120[a]	$NiSO_4$	48 h = 471	Keller and Zam (1991)
A. imbecilis	Static	1–2 day juvenile	39	$ZnSO_4$	48 h = 355	Keller and Zam (1991)
A. imbecilis	Static	1–2 day juvenile	60–120[a]	$ZnSO_4$	48 h = 588	Keller and Zam (1991)
A. imbecilis	Static	1–2 day juvenile	60–120[a]	$ZnSO_4$	96 h = 438	Keller and Zam (1991)
C. fluminea	Static renewal	Adult	64	$ZnSO_4$	96 h = 6040	Rodgers *et al.* (1980)
C. fluminea	Static renewal	Adult	64	$ZnSO_4$ + $CuSO_4$	24 h = 2410	Rodgers *et al.* (1980)
C. fluminea	Static renewal	Adult	64	$ZnSO_4$ + $CuSO_4$	96 h = 50	Rodgers *et al.* (1980)
C. fluminea	Static renewal	Adult	64	$CuSO_4$	24 h = 590	Rodgers *et al.* (1980)
C. fluminea	Static renewal	Adult	64	$CuSO_4$	96 h = 40	Rodgers *et al.* (1980)
C. manilensis	Static	Juvenile	17	$CuCl_2$	24 h = 600	Harrison *et al.* (1984)
C. manilensis	Static	Veliger	17	$CuCl_2$	24 h = 28	Harrison *et al.* (1984)
C. manilensis	Flow through	Adult	17	$CuCl_2$	96 h = 2600	Harrison *et al.* (1984)
A. imbecilis	Static	1–2 day juvenile	39	$CuSO_4$	48 h = 171	Keller and Zam (1991)
A. imbecilis	Static	1–2 day juvenile	60–120[a]	$CuSO_4$	48 h = 388	Keller and Zam (1991)
A. imbecilis	Static	1–2 day juvenile	60–120[a]	$CuSO_4$	96 h = 199	Keller and Zam (1991)
V. iris	Static	1–2 day juvenile	190	$CuSO_4$	24 h = 83	Jacobson *et al.* (1993)
A. grandis	Static	1–2 day juvenile	70	$CuSO_4$	24 h = 44	Jacobson *et al.* (1993)
A. imbecilis	Static	1–2 day juvenile	39	$CdCl_2$	48 h = 57	Keller and Zam (1991)
A. imbecilis	Static	1–2 day juvenile	60–120[a]	$CdCl_2$	48 h = 137	Keller and Zam (1991)
A. imbecilis	Static	1–2 day juvenile	60–120[a]	$CdCl_2$	96 h = 107	Keller and Zam (1991)
L. cardium	Static renewal	0 day juvenile	149	$CdCl_2$	48 h = 141	Lasee (1991)
L. cardium	Static renewal	7 day juvenile	149	$CdCl_2$	48 h = 166	Lasee (1991)
L. cardium	Static renewal	14 day juvenile	149	$CdCl_2$	48 h = 345	Lasee (1991)
L. cardium	Static renewal	Glochidia	149	$CdCl_2$	48 h = > 1000	Lasee (1991)

[a]Hardness reported only as 'moderately hard reconstituted water'.

indicated catabolism of stored lipids and carbohydrates. Naimo *et al.* (1992a) reported significantly increased O:N ratios in *L. cardium* exposed to 100 $\mu g\,Cd\,l^{-1}$ for 28 days.

Exposure of adult *L. cardium* to 30 $\mu g\,Cd\,l^{-1}$ for 28 days significantly reduced the respiration rate, but the food clearance rate, ammonia excretion rate and assimilation efficiency were highly variable, masking detection of cadmium effects at an acceptable statistical level (Naimo *et al.* 1992a). They concluded that the physiological criteria used to test similar responses to contaminants in marine bivalves (i.e. Bayne *et al.* 1977, Widdows *et al.* 1981) proved highly variable in freshwater mussels, precluding the incorporation of these physiological measures into a bioenergetics model at this time.

Several methods are available to assess the physiological condition of mussels. Physiological processes that involve respiration rate, clearance rate, food absorption efficiency and ammonia excretion rate are components of an energetics equation known as 'scope for growth' (Bayne *et al.* 1985). The scope for growth measurement is based on the balanced energy equation of Winberg (1960):

$$P = A - (R + U)$$

where P is the energy incorporated into somatic and gametic production (in $J\,h^{-1}$), A is the energy absorbed from food ($A = C - F$, where C is food energy consumed and F is energy lost as faeces), R is the energy respired and U is the energy excreted. Incorporation of individual bioenergetic rates into the scope for growth equation is an instantaneous integration of the organism's response to an environmental stimulus. The energy budget is an indirect measurement of growth – subtle effects of environmental change may be demonstrated before a direct alteration in growth is detectable. Scope for growth is useful in assessing the organism's physiological condition in both stressful and non-stressful environments. A positive P indicates that energy above routine metabolic costs is available to support growth and reproduction. A negative P may indicate a stressed organism, because the energy consumed and absorbed is less than the energy lost through respiration and excretion. Scope for growth has been used extensively to assess the physiological condition of marine bivalves (Thompson and Bayne 1974, Bayne *et al.* 1977, Widdows 1978, Widdows *et al.* 1981), but has not yet been applied to freshwater mussels – perhaps because few physiological methods have been developed for freshwater mussels.

Net growth efficiency (K_2), another measure of physiological condition, can be derived from the same physiological tests used in the scope for growth measurement. Net growth efficiency (calculated as P/A) measures the efficiency of food conversion into tissue (Ivlev 1961, Paloheimo and Dickie 1966). A reduced K_2 indicates a stressed individual because a greater proportion of the energy absorbed from the food is being used for maintenance rather than growth. Widdows *et al.* (1981) reported a decrease in K_2 in mussels transplanted along a pollution gradient in Narragansett Bay, Rhode Island.

Conclusions

Exposure of freshwater mussels to high concentrations of Cd, Cu, Hg and Zn in the laboratory has caused mortality, alterations in weight, changes in enzyme activity and filtration rate and behavioural modifications. Analyses of freshwater mussels can indicate metal bioavailability and these organisms may be useful in more sensitive,

sublethal toxicity tests. Most research on the effects of heavy metals on freshwater mussels has concerned bioaccumulation. Experimental studies should incorporate measurements that examine linkages between sublethal toxicity and bioaccumulation in freshwater mussels. However, more immediate information on nutritional requirements, culture methods, exposure concentrations, reproductive strategies and physiological activity of freshwater mussels will be needed before detailed test methods can be standardized.

This review highlighted several areas where information on the effects of metals on freshwater mussels is sparse. The largest data gaps pertain to the effects of sublethal contaminant concentrations on processes such as reproduction and growth. Furthermore, the majority of the data presented in this review are laboratory derived. There are few data on the effects of existing metal concentrations on freshwater mussels in the field.

Attempts to apply standardized toxicity tests to freshwater mussels overlook several important considerations, such as the primary contaminant uptake route(s). The route of accumulation should determine the necessary test conditions (water versus sediment exposure) that are environmentally and biologically realistic.

Data are also needed on appropriate exposure concentrations and test durations. With a life span in the range of 10–50 years, what is an adequate exposure duration for a chronic test? Realistic exposure concentrations are especially critical in freshwater mussels because they can close their valves and avoid exposure to high concentrations. In fact, short-term exposure to high concentrations may actually be less harmful than long-term exposure to low concentrations, especially if mussels close their valves rather than continue to filter water (Naimo *et al.* 1992a).

Techniques that are developed to measure the sublethal effects of metals on freshwater mussels will be useful for studies other than basic toxicity tests. An increase in the database on these organisms and a better understanding of basic physiological processes will facilitate the development of more environmentally relevant tests that can be used to evaluate the modes of action of contaminants and contribute to the development of water quality criteria.

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