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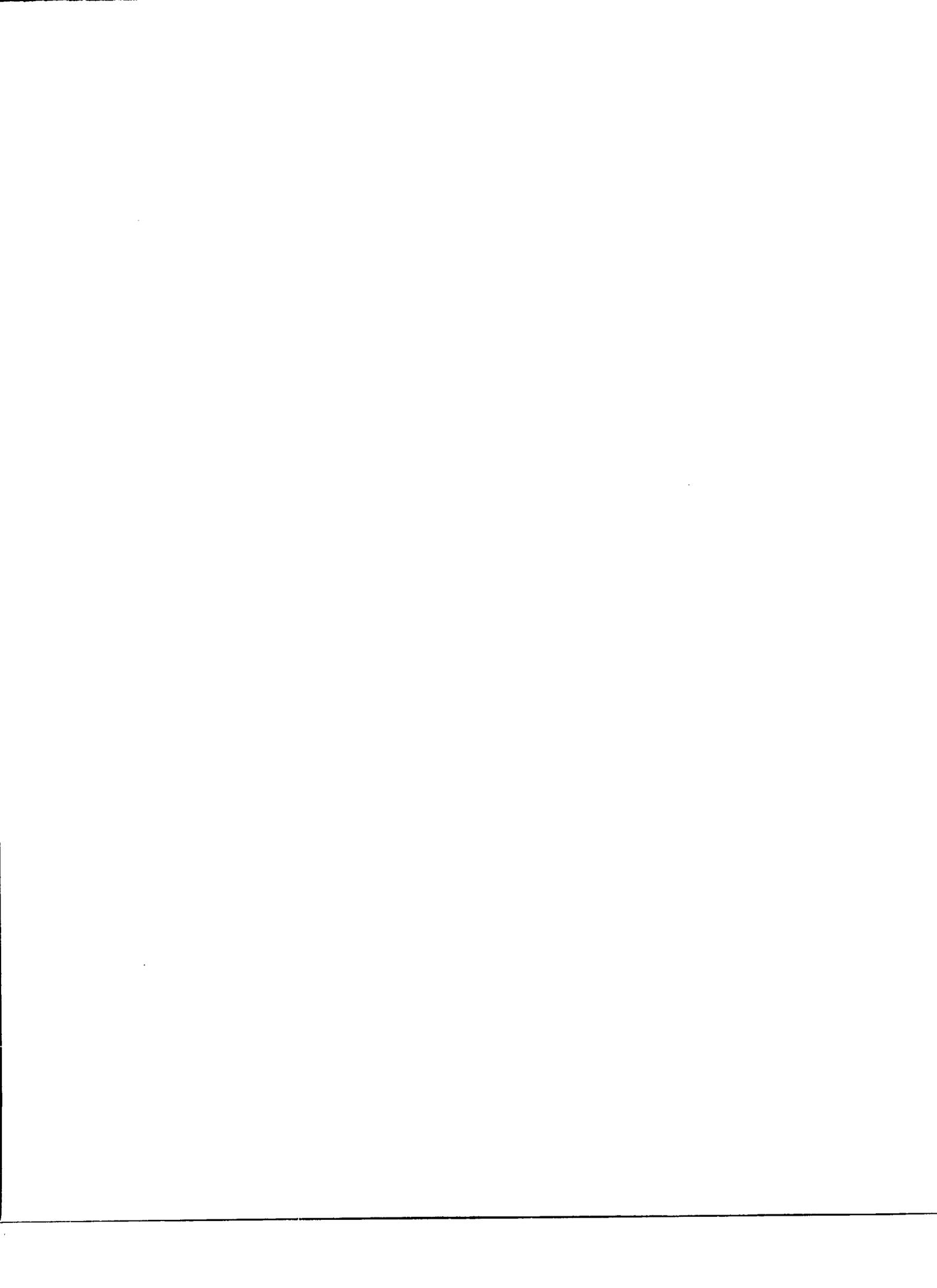
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**A physiological approach to the sublethal effects of cadmium in
*Lampsilis ventricosa***

Naimo, Teresa J., Ph.D.

Iowa State University, 1990

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A physiological approach to the sublethal
effects of cadmium in Lampsilis ventricosa

by

Teresa J. Naimo

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
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1990

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INTRODUCTION

Freshwater bivalves are ecologically and economically important in many large river systems. Recent declines in mussel populations (Family Unionidae) have generated interest in methods to culture these organisms and to elucidate reasons for their decline. One proposed explanation for the decline concerns increasing concentrations of contaminants in aquatic ecosystems. Although acute chemical spills and other point sources of contaminants are easily documented, impacts from non-point sources and low-level contamination in general, are undocumented.

Metal concentrations and chemical forms can vary greatly both spatially and temporally in an aquatic ecosystem (Luoma and Bryan 1979). Generally, water and sediment samples are analyzed for total or filterable concentrations telling relatively little about metal speciation or bioavailability. Indigenous, sessile, benthic organisms can often provide more informative data for monitoring contaminants in water and sediment. The organisms extract metals from their environment and their body burdens integrate exposure levels over time. The use of freshwater mussels as monitoring organisms has been sufficiently demonstrated (i.e., Adams et al. 1981; Bedford and Zabik 1973; Schmitt et al. 1987; Boryslawskyj et al.

1988), but their use could expand to evaluation of biological impacts from contaminant exposure.

Most studies evaluating the effects of heavy metals on freshwater mussels have emphasized the potential of mussels to accumulate contaminants in their tissues (i.e., Manly and George 1977; Tessier et al. 1984; Hemelraad and Herwig 1988). These studies have primarily focused on organ distribution, metal binding and detoxification mechanisms, cellular distribution and species differences. Limited data exist on the biological significance of contaminant effects at individual, population, or community levels. In assessing the impacts of contaminant levels in aquatic ecosystems, aquatic organisms are often used to quantify contaminant effects. Acute and chronic laboratory toxicity tests are the principal methods used to determine contaminant concentrations that are safe to aquatic life.

Although not standardized, acute toxicity tests have been conducted on freshwater mussels (Wurtz 1962; Rodgers et al. 1980; Van Puymbroeck et al. 1982; Millington and Walker 1983; Harrison et al. 1984). However, these tests have been limited primarily to snail species and the Asiatic clam (Corbicula sp.), neither of which have similar life histories to Unionid mussels. Because of the ability of freshwater mussels to close their valves in high contaminant concentrations, several researchers have suggested that

acute lethality tests should have a minimum duration of 96 hours.

The declines in both species diversity and population density of Unionid mussels (Stansbery 1970; Starrett 1971; Coon et al. 1977; Duncan and Thiel 1983; Gordon and Layzer 1989; Williams and Schuster 1989) may be due to the subtle but pervasive impacts from low-level contamination. If this hypothesis is valid, then conducting chronic toxicity tests on freshwater mussels would be more representative of continuous low-level contaminant exposure in the field than would acute toxicity tests.

In chronic toxicity tests, the determination of a suitable endpoint or response variable is crucial. Physiological responses are sensitive and reliable indicators of contaminant exposure in marine mussels (Thompson and Bayne 1974; Widdows 1978; Martin et al. 1984). Furthermore, the physiological responses of respiration rate, excretion rate, clearance rate and food assimilation efficiency can be quantified and used in a bioenergetics model known as scope for growth (Bayne et al. 1985). This model integrates these physiological responses and estimates an individual's instantaneous energy budget. Field application of scope for growth measurements has also been established (Bayne and Widdows 1978; Widdows et al. 1981; Bayne et al. 1985). To date, no known studies have been

conducted which apply these physiological responses to heavy metal exposure in freshwater mussels. The objectives of this study were: 1) to quantify the sublethal effects of cadmium on the physiology of adult Lampsilis ventricosa and 2) to evaluate the application of the scope for growth bioenergetics model to freshwater mussels.

Explanation of Dissertation Format

The three manuscripts of this dissertation represent three manuscripts which will be submitted for publication in scientific journals. Authorship of manuscripts is as follows:

Section 1. Teresa J. Naimo

Section 2. Teresa J. Naimo, Gary J. Atchison and Leslie E. Holland-Bartels.

Section 3. Teresa J. Naimo

**SECTION 1. A REVIEW OF THE EFFECTS OF HEAVY METALS ON
FRESHWATER MUSSELS**

A review of the effects of heavy metals on freshwater
mussels

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ABSTRACT

Freshwater mussels are declining in many areas in both species diversity and population density. Although the causes of this decline are unknown, one contributing cause may be exposure of freshwater mussels to chronic low-level heavy metal contamination. As benthic filter-feeding organisms, freshwater mussels can bioaccumulate heavy metals to concentrations greatly exceeding water concentrations. In adults, the most common uptake site of heavy metals is the gills, followed by the mantle and kidney. Information on cellular and subcellular distribution is scarce, but epithelial cells generally contain larger amounts of heavy metals than muscle cells.

Little information is available regarding toxicity of metals to mussels. Acute toxicity tests have been conducted, but several components of the mussel's life history preclude the use of mussels on a routine basis at this time. Sublethal chronic effects of heavy metals on freshwater mussels are rarely documented. Sublethal effects of contaminant exposure include: decreased weight gain, reduced filtration efficiency, reduced enzyme activity and modifications in behavior. Physiological responses to contaminant exposure have been quantified and used in a bioenergetics model known as scope for growth. This model, along with several other indices of physiological condition,

may provide valuable information regarding the effects of
contaminant exposure on freshwater mussels.

INTRODUCTION

Freshwater mussels are ecologically and economically important in many aquatic ecosystems. Bivalve mussels frequently contribute a significant proportion of the standing crop of freshwater benthic communities (Mann 1964; Negus 1966; Waters 1977; Cameron et al. 1979), are important in the cycling of calcium in lakes (Green 1980), and aid in mixing lake sediments through their movement (McCall et al. 1979). Mussels also serve as food for raccoons, muskrats and otters (Van der Schalie and Van der Schalie 1950; Imlay 1971). Economically, the shells of freshwater mussels are used in the pearl culturing industry.

Declines in population density and species diversity of freshwater mussels are occurring in many areas, but seldom are the causes known. Declines in species diversity and abundance have been documented in many large river systems including the Illinois River (Starrett 1971), Tennessee River system (Stansbery 1964; Isom 1969; Gordon and Layzer 1989), Ohio River (Williams and Schuster 1989) and upper Mississippi River (Coon et al. 1977; Duncan and Thiel 1983).

Declines may be due to increased silt levels (Ellis 1931, 1936; Stansbery 1970), changes in fish host distribution (Isom and Yokley 1968), impoundments (Bates 1982) and creation of wing dams (Fuller 1974). Although not documented, exposure to contaminants may also contribute to

these declines. Although accidental chemical spills and other point sources of contaminants can cause acute lethality, decreases in density and diversity are more likely to result from subtle, pervasive impacts from low-level contamination.

Mussels are exposed to many different organic and inorganic contaminants. This review focuses on metals because they are common, persistent and often toxic constituents of contaminated sites. The chemical form, bioavailability and toxicity of most metals are greatly affected by water and sediment chemistry. The aquatic chemistry and toxicology of metals have been widely studied (Vuceta and Morgan 1978; Nriagu 1979; Nieboer and Richardson 1980; Moore and Ramamoorthy 1984; Leland and Kuwabara 1985; Nriagu and Sprague 1987). However, little is known about the effects of metals on freshwater mussels, especially sublethal chronic effects. Most studies have focused on metal uptake, distribution and elimination; few have examined lethal or sublethal contaminant impacts or contaminant mode of action on mussels.

This review covers the pertinent literature on the use of freshwater mussels as metal bioaccumulators, the uptake and elimination of metals by mussels, the distribution of metals in mussel tissues, and the lethal and sublethal effects of metals on mussels.

BIOACCUMULATION

The availability of metals for uptake by organisms is influenced by sediment composition, metal speciation, water quality characteristics and suspended material (Luoma and Bryan 1979). Metals are often adsorbed onto the surface of fine particles and may be available for uptake by organisms. This may be particularly true in the case of freshwater mussels because they are virtually continuous benthic filter feeders and may be exposed both to dissolved and sediment bound contaminants.

It is often assumed that a relationship should exist between the metal concentration in sediments and that in mussels. This relationship is neither simple nor frequently observed (Heit et al. 1980; Elder and Matraw 1984; Pugsley et al. 1988). Pugsley et al. (1988) found lead (Pb) concentrations in Lampsilis radiata siliquoidea that were one-half the lead concentration in surrounding sediments, while cadmium (Cd) concentrations in L. r. siliquoidea averaged thirty times the concentrations found in corresponding sediments. This lack of a consistent relationship between sediment and mussel metal concentration may be caused by a number of factors. Metal uptake might be primarily from the water and not the sediment. Or, if metals in sediments are in chemical forms not available for uptake, then the metal concentration in the mussels may not

exceed that in the sediment (Schmitt et al. 1987). Tessier et al. (1984) quantified metal concentrations in Elliptio complanata and related them to associated sediment metal concentration. Copper (Cu), zinc (Zn) and Pb levels in mussel tissue could be predicted from the concentration of extractable metals (therefore available for uptake) in the sediments. This correlation was greater for easily extractable metals like those bound to sulfides, carbonates, iron (Fe)-manganese (Mn) oxides and organic matter, compared to those metals which were tightly bound.

Indigenous, sessile, benthic organisms, such as freshwater mussels, are often used as indicators of metal bioavailability. These organisms extract available metals from their environment and their body burden integrates exposure levels over time. Freshwater mussels have been used as indicators of: biological recovery zones (Simons and Reed 1973), mine tailings (Tessier et al. 1984; Czarnezki 1987; Schmitt et al. 1987), radionuclides (Harvey 1969; Maki and Johnson 1976), lampricides (Maki et al. 1975; Maki and Johnson 1976), organochlorine pesticides (Bedford and Zabik 1973; Leard et al. 1980; Pillai et al. 1980; Boryslawskyj et al. 1988) and heavy metals (Jones and Walker 1979; Adams et al. 1981; Graney et al. 1983; Millington and Walker 1983).

Bioaccumulation of toxicants first gained public attention in the 1960s with the high residual levels of DDT and associated metabolites in fish and wildlife. Since then, many studies have examined the contaminant bioaccumulation potential of aquatic organisms. Bioaccumulation occurs when the uptake rate exceeds the elimination rate. Bioaccumulation of metals is often evident in freshwater mussels because they live in sediment, feed by filtering water and eliminate metals slowly (Millington and Walker 1983; Bias and Karbe 1985).

The bioconcentration factor (concentration of a chemical in an organism divided by the concentration in water) is used to assess the potential of chemicals to accumulate (Kenaga and Goring 1980). Examples of bioconcentration factors in freshwater mussels include: mercury: 10,000 (V.-Balogh and Salanki 1984), zinc: 350 to 600 (Graney et al. 1983), lead: 24,000 (Salanki et al. 1982), copper: 20,000 (Graney et al. 1983), and cadmium: 1000 to 6800 (Graney et al. 1983; V.-Balogh and Salanki 1984; Salanki et al. 1982).

Organ Distribution

The most common uptake site of heavy metals in freshwater mussels is the gills, followed closely by the mantle (Manly and George 1977; Salanki et al. 1982; Tessier

et al. 1984; Hemelraad and Herwig 1988). In marine bivalves a similar condition has been observed in which highest metal concentrations are found in the gills and digestive system (Cunningham 1979). Laboratory and field experiments have shown that the ingestion of sediments can contribute to heavy metal uptake in marine bivalves (Bryan and Uysal 1978; Pruell et al. 1987). This has not been sufficiently documented in freshwater bivalves, perhaps because fewer species are deposit feeders compared to marine bivalves.

The most extensive work on metal uptake and distribution in freshwater mussels was with cadmium and Anodonta spp. (Hemelraad et al. 1986a; Hemelraad et al. 1986b; Hemelraad and Herwig 1988; Holwerda et al. 1988; Holwerda et al. 1989). In one series of experiments, Hemelraad et al. (1986a) exposed Anodonta anatina and A. cygnea to 29 ug Cd/l for 16 weeks. No significant differences in overall accumulation rates between the two species were seen until after 11 weeks, at which time Cd accumulation continued only in A. anatina. The total dry weight of soft parts for the two species was similar; however, the gill dry weight of A. cygnea was twice that of A. anatina. When accumulation was expressed on an organ dry weight basis, the differences between the two species were large, but, when expressed on a total dry weight basis, the differences between the two species were minimal.

In addition to analyzing tissues for Cd residues, Hemelraad and Herwig (1988) localized Cd using a histochemical staining procedure called the sulphide-silver technique. They exposed Anodonta anatina and A. cygnea to 25 ug Cd/l for 16 weeks. After three weeks of exposure, reaction products indicating the presence of free or loosely bound Cd were evident in the pallial mantle epithelium of both species. After six weeks, Cd was evident in the gill epithelia of both species and in the kidney epithelia of A. anatina. Reaction products did not appear in the kidney epithelia in A. cygnea until after nine weeks exposure. Furthermore, Cd concentrations in the mantle and gill did not increase, indicating an apparent steady state condition had been reached. These authors concluded that the lag in time before Cd was present in the kidney epithelia supports their hypothesis that mantle and gills are primary accumulation sites while the kidney is an "end accumulator". However, at very low environmental concentrations, Hemelraad et al. (1986b) showed that Cd is preferentially stored in the midgut gland and kidney.

The effects of other metals on Cd uptake by freshwater mussels have also been investigated. Hemelraad et al. (1987) demonstrated that Zn has an antagonistic effect on Cd in freshwater mussels by inhibiting Cd uptake in gills and accelerating Cd transport from gills towards internal

organs. They hypothesized that Zn competes with Cd for binding sites in the gills. Lanthanum, an antagonist of calcium, was applied as a calcium channel blocker to excised Anodonta anatina gills, resulting in the inhibition of Cd uptake (Holwerda et al. 1989). This observation suggests that calcium channels are involved in the Cd uptake by gills of freshwater mussels.

Cellular and Subcellular Distribution

Only limited information on cellular or subcellular distribution of metals in mussels is available (Pauley and Nakatini 1968; Cassini et al. 1986; Hemelraad and Herwig 1988). Epithelial cells have been shown to contain large amounts of cadmium, while muscle cells contain little (Hemelraad and Herwig 1988). Pauley and Nakatini (1968), localized the concentration of ^{65}Zn within tissues in Anodonta californiensis, and found that hemocytes and the base and tip of the outer mantle epithelium contained large amounts of Zn. Inner mantle epithelium contained little Zn. High concentrations of Zn were noted in kidney epithelial cells and in the lumen of the kidney near the surface of the epithelium. Studies on marine organisms have found high metal concentrations in lysosomes (mainly those in the kidney); thus lysosomal degradation may contribute to metal detoxification via metal chelating compounds (Walker 1977;

George and Pirie 1979; Viarengo et al. 1981; George 1983). Furthermore, if metals concentrate in the nucleus, they have the potential to cause mutagenic activity. In vitro, double stranded DNA, for example, has high affinity binding sites for cadmium (Waalkes and Poirier 1984).

Cassini et al. (1986) found that metal distribution between the particulate and cytosolic fractions differed with metal (Cu, Cd and Zn), species (Anodonta cygnea and Unio elongatulus) and tissue (gill, hepatopancreas and remainder). This suggests that a mechanism to detoxify or eliminate the metals may be present. In contrast, Hemelraad et al. (1986a) found the subcellular and molecular distribution of Cd to be similar between species (Anodonta anatina and A. cygnea) and organs (gill, mantle, midgut gland and kidney) over a sixteen-week exposure period.

Metal Binding

At least some freshwater organisms have evolved mechanisms that enable them to tolerate high concentrations of metals. These mechanisms include a reduced uptake rate, an increased elimination rate, and detoxification by binding of the toxic metal into insoluble complexes. Perhaps the most widely studied binding mechanism is the binding of divalent cations by the metallothionein (MT) class of proteins (Hamilton and Mehrle 1986; Steinert and Pickwell

1988; Baksi et al. 1988). These proteins contain sites capable of binding metals with an affinity for sulfhydryl groups such as Cd, Cu, mercury (Hg), arsenic (As) and Zn. A MT-like protein with an apparent molecular weight of 11 kD has been shown to bind Cd in freshwater mussels (Hemelraad et al. 1986a). In the gills, mantle and midgut gland of Anodonta anatina and A. cygnea, the percent of MT-bound Cd increased with exposure time, especially during the first four weeks, suggesting that MT synthesis may be inducible in freshwater mussels.

Another possible binding system in freshwater mussels consists of inorganic, amorphous, crystalline concretions assembled on inorganic matrices (Abolins-Krogis 1958; Simkiss 1981; Silverman et al. 1987b). These calcium concretions function as storage sites for calcium which is mobilized during reproduction; this mobilized calcium is used in construction of the glochidial shell (Silverman et al. 1985, 1987a). Similar concretions in the hepatopancreas of snails (Simkiss 1981), crustaceans (Becker et al. 1974; Guary and Niegrel 1981) and scallops (Carmichael et al. 1979) may have detoxifying capacity. These concretions in freshwater mussel gills have the ability to bind Zn, Cd and Mn; however, the strength of the binding is dependent on the ionic content of the blood (Silverman et al. 1987b).

The gills of Anodonta 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, in A. cygnea, 75% of the total body concretions were located in the gills. Pynnonen et al. (1987) concluded that up to 20% of the Cd which had accumulated over a three week exposure to 40 ug Cd/l was associated with the calcium concretions, but the proportion of Cd bound to the concretions decreased over the exposure period. This suggests that the role these concretions play in metal detoxification may be temporary. The Cd binds initially to the concretions but at prolonged exposure more efficient Cd sinks (like MT) may be required.

Elimination

To better understand the accumulation of heavy metals, the kinetics of toxicant uptake, organ distribution and elimination are all essential components. At exposure levels of 1, 5 and 10 mg Zn/l, no significant depuration over 21 days was observed in Velesunio ambiguus (Millington and Walker 1983). Cadmium is characterized by its extremely long retention time (approximately 360 days). After 10-day exposure of Dreissena polymorpha to 3.3 ug Cd/l, 44% of the original Cd concentration was still present in mussel tissue after 34 days in clean water; the only significant

elimination of Cd was from the shell (Bias and Karbe 1985). Metal elimination may occur in distinct stages and be influenced by differential metal release rates from organs (Holwerda et al. 1988). Cadmium elimination in Anodonta anatina proceeded in three phases. The first phase saw a significant depuration in total soft parts and gills. Elimination ceased between 19 and 42 days after primary exposure, then increased once again in 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 active metabolic processes within the organism (Bias and Karbe 1985; Borchardt 1983). In the studies by Millington and Walker 1983, Bias and Karbe 1985, and Holwerda et al. 1988, the mussels were not fed, even over test durations of 150 days. If the organisms were not feeding, their filtration rates, and thus the volume of water passing over their gills, would have decreased substantially over these time periods. The slow elimination rates seen in these studies may have reflected undernourishment and a consequent decreased metabolic activity.

TOXICITY

Standardized toxicity tests have been developed for freshwater macroinvertebrates (ASTM 1988) and a tentative procedure has been developed for marine molluscs (APHA et al. 1990). Neither applies well to freshwater bivalves. The marine test is primarily for use with oyster larvae where 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. Oysters (the principal organisms referred to in the marine larvae test) achieve sexual maturity in about five months compared to 2-6 years in freshwater mussels. Full lifecycle tests in oysters are more realistic than in freshwater mussels which can live upwards of 50 years, of which 45 or more may be reproductively active (Imlay 1971).

An important consideration in any toxicity test is the determination of a suitable and sensitive endpoint. Both lethal and sublethal endpoints have been used in freshwater mussel contaminant studies. Death is the most common endpoint for acute toxicity tests (Table 1). For freshwater mussels this may not be the most appropriate endpoint because the point of death is difficult to determine. It is often assumed that a gaping of the valves, which remain open after gentle prodding with a glass rod, indicates mortality.

Table 1. Acute lethality tests conducted on freshwater bivalves

Organism (reference ^a)	Testing Conditions	Metal	LC ₅₀
<u>Corbicula fluminea</u> (1)	static renewal	Cu	24hr = 0.59 ppm
<u>Corbicula fluminea</u> (1)	static renewal	Cu	96hr = 0.04 ppm
<u>Corbicula fluminea</u> (1)	static renewal	Zn	96hr = 6.04 ppm
<u>Corbicula fluminea</u> (1)	static renewal	Cu + Zn	24hr = 2.41 ppm
<u>Corbicula fluminea</u> (1)	static renewal	Cu + Zn	96hr = 0.05 ppm
<u>Velesunio ambiguus</u> (2)	static renewal	Zn	336hr = 66 ppm
<u>Helisoma companulata</u> (3)	hard water	Zn	96hr = 3 ppm
<u>Helisoma companulata</u> (3)	soft water	Zn	96hr = 0.87 ppm
<u>Corbicula manilensis</u> (4)	static, juvenile stage	Cu	24hr = 600 ppm
<u>Corbicula manilensis</u> (4)	static, veliger stage	Cu	24hr = 28 ppb
<u>Corbicula manilensis</u> (4)	flow through, adult	Cu	96hr =>2600 ppb
<u>Lymnaea stagnalis</u> (5)	static renewal	Cd	7d = 200 ppb
<u>Lymnaea stagnalis</u> (5)	static renewal	Cd	14d = 120 ppb

^a(1) Rodgers et al. (1980); (2) Millington and Walker (1983); (3) Wurtz (1962); (4) Harrison et al. (1984); (5) Van Puymbroeck et al. (1982).

This lack of response results from a failure of the adductor muscles to contract. Whether or not the animals are actually dead at this time is not easily demonstrated. Sublethal endpoints include foot immobilization (Millington and Walker 1983; Doherty and Cherry 1988), filtering activity (Rodgers et al. 1980; Doherty and Cherry 1988), oxygen consumption (Radhakrishnaiah 1988), blood osmotic pressure (Doherty and Cherry 1988), bioelectric activity (Morgan et al. 1989) and valve activity (Rodgers et al. 1980; Millington and Walker 1983; Doherty et al. 1987). The valve activity response closely mimics the 96-hr LC_{50} value for both Cu and Zn (Rodgers et al. 1980) and may be a suitable EC_{50} (effective concentration causing 50% of the organisms to gape) endpoint for toxicity tests. Rodgers et al. (1980) found that in most cases few individuals were still filtering at the observed LC_{50} value, indicating that this EC_{50} may be a sensitive endpoint. A possible EC_{50} endpoint may be the time from gentle prodding with a glass rod until valve closure. This response time is rapid in healthy individuals and slower in stressed individuals. Whether this indicator would show a dose-response relationship is not known.

Chronic effects of metals on freshwater mussels are rarely documented. To our knowledge, no chronic tests on growth, reproductive effects or the sensitivity of various

life stages have been conducted. Information on the effects of contaminants on the relationship between glochidia and their fish hosts is also lacking. Sublethal effects of contaminants, such as alterations in feeding, growth, and reproduction may lead to significant long-term impacts on mussel populations. Changes in valve movement patterns have been associated with contaminant exposure (Imlay 1968; Davenport and Manley 1978; Doherty et al. 1987; Higgins 1980). A dose-response relationship was established on exposure of Corbicula fluminea to Cd or Zn and the length of time the valves remained parted. The mean time to first valve closure in C. fluminea with no contaminant exposure was 860 minutes, while 24-hr exposure to 0.4 mg Cd/l and 0.9 mg Zn/l resulted in response times of 42 and 66 minutes, respectively (Doherty et al. 1987). The lowest test concentrations (nominal) causing extended valve closure in Dreissena polymorpha were 0.37 mg Cd/l and 0.030 mg Cu/l (Sloof et al. 1983). This value for Cu is near the acute water quality criterion of 34 ug Cu/l for water hardness of 200 mg CaCO₃/l (U.S. Environmental Protection Agency 1984). A reduction in total weight gain has also been observed with 30-day exposure of Corbicula sp. to Zn (Belanger et al. 1986).

Physiological effects of contaminant exposure in freshwater mussels have recently been used to assess

contaminant impacts. Heavy metals decreased filtration activity in Anodonta cygnea, perhaps inhibiting both feeding and growth (V.-Balogh and Salanki 1984). Both rhythmic and periodic activities in freshwater mussels have been used as physiological endpoints after contaminant exposure.

Rhythmic activity is defined as the fast, rhythmic adductor activity occurring during the open position of the valves; periodic activity refers to the open and closed positions of the valves each lasting for several hours (Salanki et al. 1970). Copper sulphate at 0.1 and 1.0 ug/l caused a decrease in duration of active periods in Anodonta cygnea by 10 and 50%, respectively, while lead chloride and lead nitrate did not decrease activity at concentrations of 1 mg/l (Salanki and Varanka 1976).

Radhakrishnaiah (1988) exposed Lamellidens marginalis to 6 mg/l cadmium nitrate for three days and found significant decreases in oxygen consumption, heart rate and ciliary action. Exposure to 2 mg/l CdNO₃ resulted in an initial decline in the same physiological responses, but all responses returned to near control levels by day 10. Experimental design problems and insufficient information on methods make it difficult to interpret these results, however.

Cellulolytic activity can be used as another physiological indicator of stress. The enzyme group

involved catalyzes the hydrolysis of algal cellulose into short chain sugars. Corbicula sp. cellulolytic activity was significantly reduced after 10-20 days in artificial streams at concentrations of 16 ug Cu/l and 87 ug Zn/l (Farris et al. 1988).

Several methods are available to assess the physiological condition of a freshwater mussel. Physiological processes involving 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 = the energy incorporated into somatic and gametic production (in Joules/hour); A = energy absorbed from food (A = C - F; where C = food energy consumed and F = energy lost as feces); R = energy respired and U = energy excreted. Incorporation of individual bioenergetic rates into the scope for growth equation represents an integration of the whole organism's response to an environmental stimulus. The energy budget is an indirect measurement of growth by which subtle effects of environmental change may be perceived before a direct alteration in growth. Scope for growth is useful in assessing the organism's physiological condition

in both stressful and non-stressful environments. A positive scope for growth (positive P) indicates that energy is available in excess over routine metabolic costs, to support growth and reproduction. A negative scope for growth (negative P) may be indicative of a stressed organism, because the energy consumed and absorbed is less than the energy lost through respiration and excretion. The 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; Martin et al. 1984), but has yet to be applied to freshwater mussels.

Net growth efficiency (K_2) is another measure of physiological condition and can be derived from these same physiological tests. Net growth efficiency (calculated from scope for growth equation as P/A) is a measure of the efficiency with which food is converted into tissue (Ivlev 1961; Paloheimo and Dickie 1966). A reduced K_2 is indicative of stress since a greater proportion of the energy absorbed from the food is being utilized for maintenance instead of growth. Widdows et al. (1981) reported a decrease in K_2 in mussels transplanted along a pollution gradient in Narragansett Bay, Rhode Island.

The third test used to assess the physiological condition of a mussel after contaminant exposure is the

ratio of oxygen consumed to nitrogen excreted (O:N). This provides an index of the use of protein in metabolism (Ikeda 1977; Widdows 1978; Russell-Hunter et al. 1983). In a study on freshwater mussels, Aldridge et al. (1987) reported O:N ratios of less than 20 to be indicative of catabolism based on proteins while O:N ratios greater than 100 to be the result of catabolism of stored lipids and carbohydrates.

CONCLUSIONS

The effects of heavy metal exposure on freshwater mussels may include acute lethality, decreased weight gain, reduced enzyme activity, reduced filtering activity and behavioral modifications. Freshwater mussels can be used as indicators of metal availability and they may have utility in more sensitive, sublethal toxicity tests. Most of the research concerning freshwater mussels and heavy metals has been on bioaccumulation. While this information is important to aquatic toxicology, the data base on these organisms needs to develop beyond accumulation studies, and into more studies which demonstrate the biological significance of contaminant effects on freshwater mussels. However, more information on such basic questions as nutritional requirements, culture methods, reproductive strategies and physiological activity are required before conducting more detailed toxicity tests.

The development of techniques for measuring contaminant effects on freshwater mussels has implications which far exceed basic toxicity tests. With an increase in the data base on these organisms and a better understanding of basic physiological processes, more complex tests may be feasible. Toxicity tests can be used as tools to evaluate the modes of action of contaminants and can contribute to the development of water quality criteria and risk assessment. In order to

evaluate the potential harm of contaminants to freshwater mussels, methodology to accurately measure sublethal responses is critical. Once the methods are established, measurements such as the "no adverse biological effect" and the "highest predicted environmental concentration" can be made. Only then can accurate estimates of contaminant risk be made.

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SECTION 2. CHRONIC TOXICITY OF CADMIUM ON PHYSIOLOGICAL
RESPONSES IN THE POCKETBOOK MUSSEL, LAMPSILIS
VENTRICOSA

Chronic toxicity of cadmium on physiological responses in
the pocketbook mussel, Lampsilis ventricosa

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ABSTRACT

The upper Mississippi River contains one of the most numerous and diverse assemblages of freshwater mussels in the United States. Recent studies indicate that both the density and diversity of these organisms are declining. One proposed explanation is the potential subtle, pervasive impacts from low level contamination in the upper Mississippi River. A study was conducted to evaluate the physiological responses of adult Lampsilis ventricosa to sublethal cadmium exposure. Physiological processes studied included respiration rate, food clearance rate, ammonia excretion rate and food absorption efficiency. Mussels were exposed to Cd (0, 30, 100 and 300 ug/l) for 28 days in a proportional diluter. Analyses indicate that respiration rate was the most sensitive and least variable indicator of Cd exposure, while food absorption efficiency was the most variable response. Respiration rates in mussels exposed to cadmium were significantly ($p < 0.05$) depressed compared to respiration rates in mussels with no Cd exposure. Although food clearance rates and ammonia excretion rates showed no statistical differences among Cd treatments, ammonia excretion rates in mussels exposed to 300 ug Cd/l fell from 22 to 5 ug/hr/g dry tissue weight by day 28. By day 28, food clearance rates also fell to one-third of their original values in mussels exposed to 300 ug Cd/l.

Assimilation efficiencies increased over the test duration in all treatments. Freshwater mussels can be sensitive indicators of sublethal contaminant exposure. However, due to large variability in some physiological rates, care must be taken in selecting appropriate physiological indicators of contaminant effects.

INTRODUCTION

Mussels are unique freshwater organisms; they are large, long-lived invertebrates that inhabit bottom sediments and obtain food by filter feeding. They are exposed to contaminants that are: 1) dissolved in water; 2) associated with small suspended particles; and 3) deposited in bottom sediments. Therefore, mussels may bioaccumulate contaminants such as heavy metals (Foster and Bates 1978; Adams et al. 1981; Hemelraad et al. 1986b) and pesticides (Bedford and Zabik 1973; Pillai et al. 1980; Boryslawskyj et al. 1988), to greater levels than many other aquatic organisms.

This study was initiated because of the concern that contaminated sediment in the upper Mississippi River may contribute to the decline of certain mussel populations. The river has been modified into a series of locks and dams, creating diverse habitats, many of which (especially backwater and main channel border) are subject to fine sediment deposition. Some sediments are contaminated, especially with heavy metals, due in part to municipal and industrial discharges near the Twin Cities Metropolitan Area, Minnesota. Although the upper Mississippi River is not highly contaminated, subtle biological impacts from continuous low-level contaminant exposure need to be examined.

Cadmium, one of the major contaminants of the upper Mississippi River, is toxic to some aquatic organisms in the low ug/l range (Eisler 1985). During 1979, the Twin Cities Metropolitan Wastewater Treatment Plant discharged an estimated 5710 kg of cadmium into the river (Sprafka 1981). This discharge level has declined substantially over the past decade due to improvements in treatment technology, but surficial sediments in the river are still contaminated with heavy metals (Rada et al. 1990). Although our original interest was in sediment related effects, we found that more background information on the effects of dissolved cadmium was necessary before the more complex issues of cadmium in sediment were studied.

Cadmium effects studies on freshwater mussels have focused on uptake and distribution (Hemelraad et al. 1986a, 1986b, 1987; Hemelraad and Herwig 1988), lethality (Van Puymbroeck et al. 1982), detoxification mechanisms (Pynnonen et al. 1987; Silverman et al. 1987) and sublethal effects (Belanger et al. 1986; Doherty et al. 1987; Farris et al. 1988). However, most of the toxicity tests have been confined to research on the Asiatic clam, Corbicula sp. No similar studies have been done on mussels in the family Unionidae. The freshwater mussel Lampsilis ventricosa (fat pocketbook) was chosen because it is abundant in the upper Mississippi River and some of its life history

characteristics have been documented (Holland-Bartels and Kammer 1989).

Several physiological responses of an organism to a stressor have been used in evaluating contaminant effects in marine bivalves (Thompson and Bayne 1974; Widdows et al. 1980; Martin et al. 1984). Respiration rate, food clearance rate, ammonia excretion rate, and food assimilation efficiency can be quantified and incorporated into a bioenergetics model known as scope for growth (Bayne et al. 1985). This model uses the rates of several physiological measurements to estimate an individual's instantaneous energy budget, and allows quantification of the amount of energy available for growth and reproduction.

Application of these techniques to contaminant exposure and sublethal effects in freshwater mussels may demonstrate subtle environmental impacts. Although the focus of this research was on the upper Mississippi River, the principles presented here can be used to assess sublethal contaminant effects on freshwater mussels in a wide variety of lotic and lentic environments. The objectives of this study were: 1) to quantify the sublethal effects of cadmium on the physiology of adult L. ventricosa and 2) to evaluate the application of the scope for growth bioenergetics model to freshwater mussels.

MATERIALS AND METHODS

Mussel Collection

L. ventricosa (also known as L. cardium; Turgeon et al. 1988) were collected on 21 July 1989 from Pool 7 of the upper Mississippi River (River Miles 704.5-710.5). Length and height ranged from 95.9-109.0 mm and 71.8-88.9 mm for 50 males, and 88.0-106.6 mm and 70.7-92.0 mm for 50 females, respectively. Average bottom water temperature for all collection sites was 23°C. Mussels were transported in wet burlap on ice to the laboratory, then placed in 57-L flow-through aquaria, without substrate, at a temperature of 8°C (the temperature of the mussels on ice). Water temperature was gradually increased (1°C/day) to 20°C, the temperature for experimentation. Every third day during the acclimation period (maximum of 17 days) and throughout subsequent experiments each aquarium received 120 ml of a 12 g/l blended solution of Ziegler Microfeast Plus L-10 aquatic food. During the acclimation period, aquaria were covered with black plastic to minimize human disturbance.

Exposure System

Dilution water was Ames, Iowa, tap water to which 52 g/l sodium bicarbonate and 10% hydrochloric acid were added by peristaltic pumps to simulate the water quality of upper Mississippi River water at Dresbach, MN. The dilution water

had the following characteristics (mean \pm SE; n=18): temperature ($^{\circ}$ C) 20.5 ± 1.3 ; dissolved oxygen (mg/l) 8.3 ± 0.3 ; pH 8.1 ± 0.4 ; alkalinity (mg CaCO_3 /l) 159 ± 6.2 ; hardness (mg CaCO_3 /l) 165 ± 3.4 and conductivity (umhos/cm) 653 ± 12.1 . Tap water was analyzed for the following metals by graphite furnace atomic absorption spectrophotometry before the study (mean \pm SE; n=3): Na 17 ± 2.1 ppm; Pb <10 ppb; Cr <10 ppb; Cd <1 ppb; Hg <2 ppb; Zn 0.6 ± 0.3 ppm; As <10 ppb; Se <10 ppb; Cu <10 ppb; Fe <0.5 ppm and Al 38 ± 1.6 ppb.

A proportional diluter delivered cadmium (as CdCl_2) to a series of 8-57 L glass aquaria. Target cadmium concentrations were chosen after conducting a preliminary range finding toxicity test to determine the upper limits of cadmium's sublethal toxicity to adult L. ventricosa over a 28-day exposure. The concentrations chosen were 0, 30, 100 and 300 ug Cd/l with two replicates per treatment. Measured mean (\pm SE; n=12) cadmium concentrations were: 0 (not detectable); 20.0 ± 2.5 , 95.2 ± 6.4 and 305 ± 2.8 ug Cd/l. The controls were below detection limits by flame atomic absorption spectrophotometry, but the graphite furnace method indicated Cd levels below 1 ug/l in the tap water.

Once cadmium levels in the test aquaria stabilized, ten mussels were randomly chosen and placed into each treatment tank (20 mussels per treatment). If a mussel died during

the test, it was removed and replaced with a new mussel from a holding tank. This additional mussel was used to maintain similar biomass among tanks but was not used in any of the physiological measurements. Because it was not possible to measure physiological responses of all 80 mussels on any one day, the start of exposure and the days of testing were staggered. Each of two randomly chosen tanks were staggered over four time periods.

Test duration was 28 days with respiration rate, ammonia excretion rate, food clearance rate and assimilation efficiency measurements made on each individual mussel at day 0 (before Cd exposure), day 14 and day 28. The first three rates were determined in an environmental chamber at a temperature of 20°C and subdued lighting. On exposure day 28, all mussels were sacrificed and separated into shell and tissue to obtain the dry weights for each component. Estimates of respiration, ammonia excretion, and clearance rates were expressed on a dry tissue weight basis.

Physiological Measurements

Because L. ventricosa is principally an ammonotelic organism (excretes primarily ammonia), excretion rates were determined by measuring the total ammonia nitrogen (TAN) excreted, following the methods of Aldridge et al. (1987). Each mussel was isolated in a 1-L beaker of dilution water

for one hour, at which time the TAN content of the water was determined with an Orion model 95-12 ammonia electrode coupled to an Orion specific ion meter (model 407A). The TAN concentration of the dilution water prior to placing the mussel into the beaker was subtracted from the final TAN concentration in experimental beakers to give the excretion rate reported as mg TAN/hr/g dry weight.

Respiration rates were monitored in 1.5-L glass canning jars; the snap on lids were modified for insertion of a dissolved oxygen probe (YSI model 5739 connected to a YSI model 51-B dissolved oxygen meter). The respirometers were placed in a water bath and maintained at 20°C. The bottom of each respirometer contained a stir bar under a perforated plastic platform. Each mussel was cleaned with a toothbrush to remove aufwuchs which could have contributed to oxygen consumption and placed into the respirometer. After a mussel began siphoning water, oxygen concentration was measured every 10 minutes until the percent saturation fell below 65%. Respiration rates were recorded as mg O₂/hr/g dry weight.

A mussel's clearance rate is defined as the volume of water cleared of particles per unit time (Widdows et al. 1979). For measurements of clearance rate over short periods of time, the use of a static system has been shown to yield similar results to a flow-through design (Bayne et

al. 1985). Consequently, each mussel was isolated in a 1-L beaker of dilution water and 30 mg/l of microfeast plus L-10 was added. After 15 minutes, 3 30-ml samples were removed from each beaker and the remaining particle concentration was measured by a Hach Ratio Turbidimeter (model 18900). Calculation of liters of water cleared per hour were made by measuring the decline in particle concentration in experimental beakers compared to control beakers.

The efficiency of food absorption by the digestive system was calculated as the ratio of organic matter in the food to the ratio of organic matter in the feces. The organic content of microfeast plus L-10 was determined by obtaining the ratio of ash-free dry weight (muffle sample at 550°C for 4 hours) to dry weight (dry sample at 100°C for 48 hours). The ratio of twenty microfeast plus L-10 samples (mean \pm SE) was 0.883 ± 0.001 . Since this measurement is a ratio, quantitative collection of mussel feces was not necessary. A system was developed which delivered a constant food supply for 18 hours into multiple containers with food inflow at the bottom and outflow at the top. After 18 hours, feces (including pseudofeces) were collected and the majority of water removed from the sample with capillary tubes. The feces sample was dried and muffled. The percent assimilation (e) was estimated using the formula

developed by Conover (1966): $e = (F - E)/(1 - E)F$; where F = ash-free dry weight: dry weight ratio of food and E = ash-free dry weight: dry weight ratio of feces.

Tissue condition index (TCI) is often used as a measure of physiological condition in bivalves (Baird 1958; Payne and Miller 1987). The index was determined by dividing the tissue dry mass by shell dry mass and multiplying the quotient by 100. Values for test animals were compared to a sample of 33 *L. ventricosa* that were collected from Pool 7 upper Mississippi River and processed immediately.

The final test used to assess the physiological condition of a mussel after cadmium exposure was the oxygen to nitrogen (O:N) ratio. This ratio of moles of oxygen consumed to moles of nitrogen excreted is an index of protein utilization in metabolism (Ikeda 1977; Widdows 1978; Russell-Hunter et al. 1983). In a study on freshwater mussels, Aldridge et al. (1987) reported O:N ratios of less than 20 to be indicative of catabolism based on proteins, and O:N ratios greater than 100 due to catabolism of lipids and carbohydrates.

Metal Analysis

Cadmium concentrations in the dilution water were analyzed weekly. Water samples were taken from each tank, acidified with instra-analyzed nitric acid to pH <2 and

analyzed by direct aspiration into an atomic absorption-flame ionization spectrophotometer. Every sampling period, each tank was sampled in triplicate and three samples were randomly spiked. The percent recovery of the spiked samples was 97.7% (mean; n=12).

Statistical Analyses

The physiological responses of the organisms represent a split-plot experimental design with each experimental unit (treatment tanks) measured repeatedly over time (Winer 1971; Diggle 1988). Because time is a factor that cannot be randomized, a repeated measures analysis was used, allowing incorporation of data from several time periods without assuming that sampling dates are independent of each other. Another assumption of this analysis is that errors for differences between tanks receiving the same treatment should be larger than errors made measuring the same tank on different occasions. Differences among cadmium treatments were tested using the variance of the treatment tanks nested within cadmium as the main-plot error term (F with 3,4 df). The sub-plot contained the variance of time by treatment tanks within cadmium as the error term for testing either the effects of time (F with 2,8 df) or time by cadmium effects (F with 6,8 df). All analysis of variance (ANOVA) tests were done using the General Linear Models (GLM)

procedure in the Statistical Analysis System (SAS Institute, Inc. 1985). Null hypotheses were rejected at $P < 0.05$.

RESULTS

Physiological responses were measured on days 0, 14 and 28 to determine changes over time. With the exception of food assimilation efficiency and O:N ratio, no statistical differences in responses over time were found among mussels exposed to different cadmium treatments (Table 1). Graphs of respiration rates, clearance rates, and ammonia-nitrogen excretion rates represent the average response of individuals measured on days 14 and 28. For graphical purposes, the day 0 measurements were not included because they represent the baseline physiological rate for a given cadmium treatment. There were no statistically significant differences among the two replicate tanks at a given cadmium treatment for any of the physiological responses measured (Table 1).

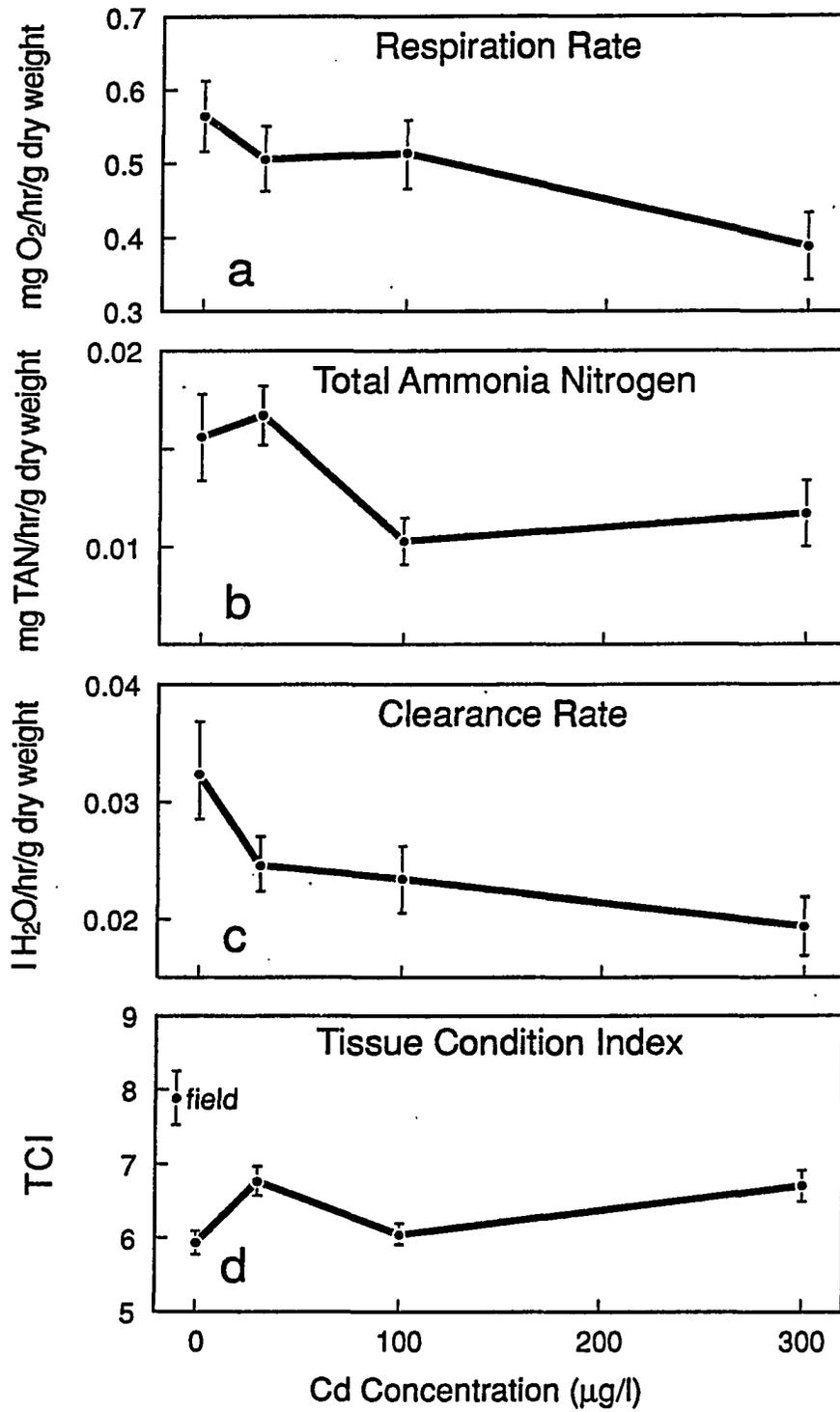
Respiration rates in mussels exposed to cadmium were significantly reduced compared to control mussels. Mean respiration rates (\pm SE) decreased from 0.563 ± 0.046 mg O₂/hr/g dry weight in control mussels to 0.388 ± 0.045 mg O₂/hr/g dry weight in mussels exposed to 300 ug Cd/l (Figure 1a). This physiological measurement was the most sensitive and least variable of all measurements made.

Ammonia excretion rates in mussels exposed to 30 ug Cd/l were slightly higher than excretion rates in control mussels, but in the 100 and 300 ug Cd/l treatments,

Table 1. Summary of cadmium effects on the physiology of the pocketbook mussel. Differences between cadmium exposed mussels and control mussels were significant at $p < 0.05$. R = respiration rate (mg O_2 /hr/g dry weight); A = ammonia excretion rate (mg TAN/hr/g dry weight); C = clearance rate (l H_2O /hr/g dry weight); AE = assimilation efficiency (percent assimilation); and O:N = moles oxygen consumed to moles nitrogen excreted. TAN = total ammonia nitrogen

Statistical Question?	P value				
	R	A	C	AE	O:N
Were there treatment differences?	0.022	0.824	0.749	0.182	0.396
Were there differences between the two tanks at each treatment level?	0.921	0.078	0.072	0.099	0.063
Were there differences over time?	0.207	0.052	0.980	0.003	0.003
Was the average slope equal to zero?	0.410	0.040	0.929	0.004	0.075
Was there an interaction between time and treatment?	0.922	0.207	0.467	0.720	0.007
Were the mean treatment slopes different?	0.959	0.080	0.162	0.360	0.058

Figure 1. Physiological responses of L. ventricosa to cadmium. Data points represent the mean physiological responses (\pm SE) for individuals measured on days 14 and 28. Figure 1d field samples represent a sample of 33 L. ventricosa mussels collected from the field and processed immediately for TCI measurements. $TCI = [(tissue\ dry\ weight/shell\ dry\ weight) \times 100]$



excretion rates were reduced by 34% and 25%, respectively, compared to controls (Figure 1b). Even though a dose response relationship was evident, ammonia excretion rates were not statistically significant in cadmium exposed mussels compared to controls (Table 1).

Clearance rate measurements exhibited a dose-response relationship, with clearance rates decreasing with increasing cadmium concentration (Figure 1c). The average clearance rate (\pm SE) in mussels exposed to 300 ug Cd/l was 0.019 ± 0.002 l H₂O/hr/g dry weight. Even though this represented a 41% reduction compared to clearance rates in unexposed mussels, there were no statistical differences between the clearance rate of mussels exposed to cadmium and the clearance rate of control mussels. In this measurement, as well as ammonia excretion, an excessive mucus production was observed in most mussels exposed to cadmium.

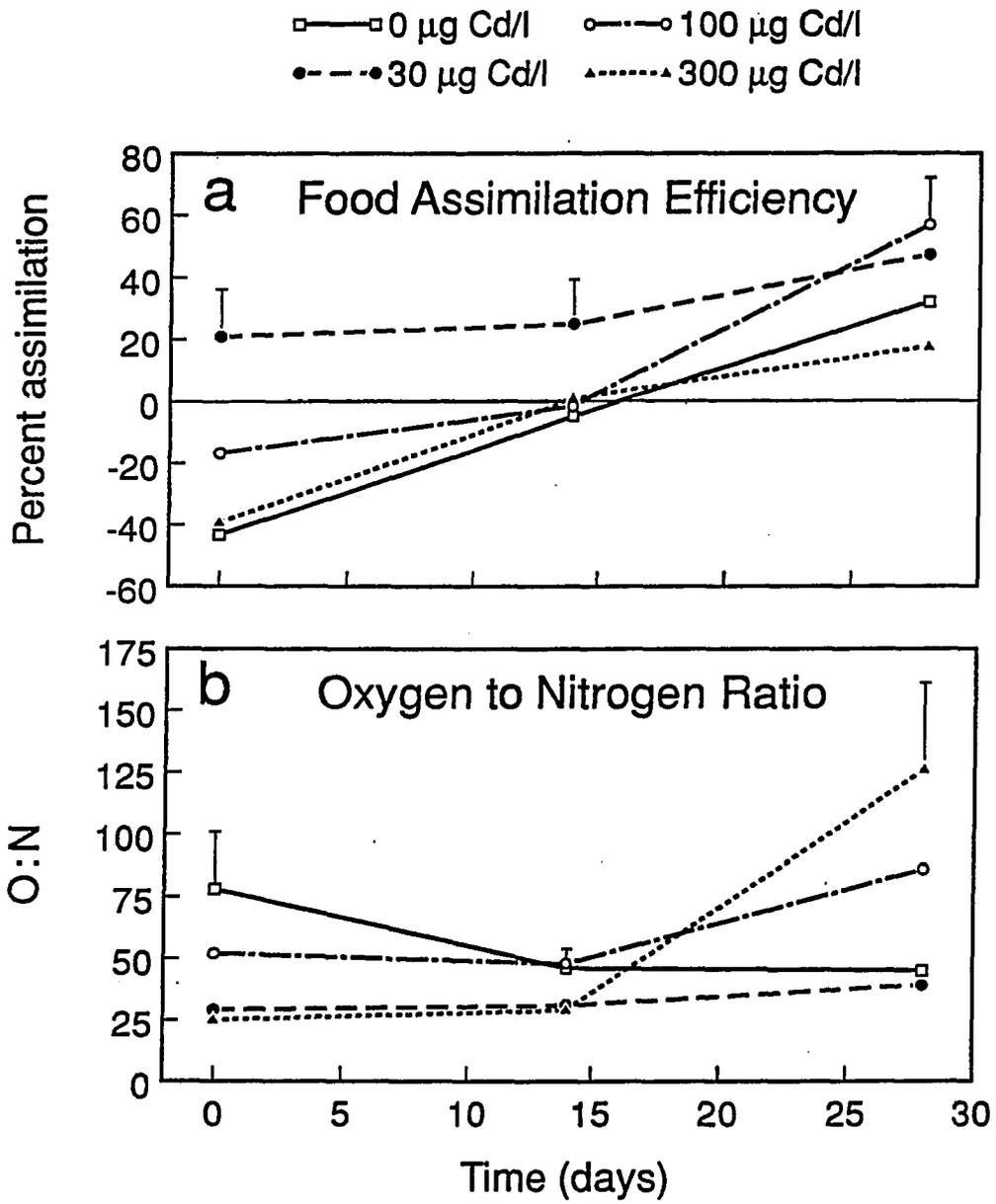
The tissue condition index (TCI) was not subjected to the repeated measures analysis because it could only be calculated on day 28 for all treatments. Although not a highly variable measurement, the TCI did not exhibit a dose-response relationship. The TCI of all mussels remained constant over all cadmium exposure levels (Figure 1d). However, the TCI of all mussels used in the toxicity test were statistically lower compared to the mean TCI of a sample of 33 Lampsilis ventricosa mussels collected from

Pool 7 and processed immediately. The mean TCI (\pm SE) for these mussels was 7.91 ± 0.37 .

Food assimilation efficiency was the most variable physiological response. Assimilation efficiencies in mussels measured on day 0 were, on average, negative, indicating a higher percentage of organic matter in the feces than in the food (Figure 2a). Food assimilation efficiencies increased significantly over time (Table 1), indicating that the percentage of organic matter in the feces declined with time.

The oxygen to nitrogen ratios (O:N) of all mussels were not significantly different at days 0 and day 14, but by day 28, those mussels exposed to either 100 ug Cd/l or 300 ug Cd/l had O:N ratios that were significantly greater than O:N ratios in unexposed mussels (Figure 2b).

Figure 2. Changes in food assimilation efficiency and O:N ratios over time and differing cadmium exposures. Means represent the average response of 20 mussels/treatment/time. Food assimilation efficiency measured as percent of food assimilated. O:N ratios measured as moles of oxygen consumed to moles of nitrogen excreted



DISCUSSION

The overall response of Lampsilis ventricosa to sublethal cadmium exposure was a modification in physiological rates. Respiration rates, food clearance rates, and ammonia-nitrogen excretion rates were all reduced after cadmium exposure.

Mean respiration rates (mg O₂/hr/g dry weight; ± SE) in control mussels in this study (0.563 ± 0.046) were within the ranges encountered by other investigators. Respiration rates in fed Helisoma trivolvis averaged 0.500 mg O₂/hr/g dry weight during a 124-day experimental period (Russell-Hunter et al. 1983). The mean (± SE) respiration rates in mussels exposed to 300 ug Cd/l in this study (0.388 ± 0.045 mg O₂/hr/g dry weight) were similar to the mean respiration rates (0.399 mg O₂/hr/g dry weight) in Quadrula pustulosa after exposure to infrequent turbulence plus turbidity (Aldridge et al. 1987).

During all experiments an excessive production of mucus was observed in cadmium exposed mussels. An accumulation of mucus on the gill surfaces could reduce the efficiency of gas exchange across the gills, resulting in lower oxygen uptake rates. If not enough oxygen is available, mussels could respond by either increasing oxygen uptake rate or decreasing metabolic rate. Lower rates of oxygen uptake are often an indication of lower metabolic rates in aerobic

organisms (Prosser, 1973); therefore, it is possible that mussels exposed to cadmium responded by decreasing their metabolic rates.

A decrease in clearance rates is a common response to environmental stressors in bivalves (Widdows et al. 1979; Bayne et al. 1982; Aldridge et al. 1987). If mussels decrease their metabolic activity in response to cadmium, the amount of water passing over their gills would be reduced. Furthermore, if gills are coated with mucus, their functional capacity as a sorting and transporting device for food would be reduced. With less water flowing over gill surfaces, the amount of food obtained by organisms would likewise be reduced. The decrease in TAN excretion rates can be partially explained by the reduction in food intake and lowered metabolic activity of the mussel. Aldridge et al. (1987) reported significant decreases in nitrogen excretion rates in three species of freshwater mussels (Q. pustulosa, Fusconaia cerina and Pleurobema beadleanum) exposed to frequent turbulence (7 minutes every half hour) compared to infrequent turbulence (7 minutes every 3 hours).

Assimilation efficiency measurements in freshwater mussels are highly variable. The negative assimilation efficiencies in mussels before cadmium exposure was the result of a higher organic content in the feces compared to the food, although the reasons for this are unclear. By day

28, assimilation efficiencies significantly increased in all treatments, indicating that the mussels were assimilating a greater proportion of the organic content of the food because the organic content of their feces had decreased. However, by day 28, estimates of clearance rates in mussels exposed to 300 ug Cd/l dropped by 41% compared to control mussels, representing a substantial reduction in food intake by the mussel. The higher assimilation efficiencies in these mussels could have been the result of breakdown of the large glycogen stores from complex organic materials to simpler inorganic molecules, increasing the percentage of inorganic material in the feces and therefore increasing the assimilation efficiencies. So even though the assimilation efficiencies appeared to increase with time, if food intake had ceased, the mussels may have shifted substrates and catabolized their glycogen stores. Because a reduction in clearance rate is a common response to an environmental stressor, and because the measurement of food assimilation depends on an actively feeding mussel, the utility of the food assimilation efficiency measurement is questionable. Different methods, such as the use of radiotracers, may provide more information on the fate of the organic portion of the food.

Any alteration in the utilization of carbohydrates and proteins as energy sources should be reflected in the O:N

ratio. In Mytilus edulis, starvation resulted in a shift from catabolism based primarily on carbohydrates (high O:N ratio) to catabolism based on proteins (low O:N ratio; Widdows 1978). While researchers working in marine environments have suggested that low O:N ratios are indicative of a stressed individual (Widdows 1978; Bayne et al. 1985), studies on freshwater molluscs have not supported this. O:N ratios in Helisoma trivolvis increased from 16.02 in control snails to 84.60 in unfed snails after 124 days (Russell-Hunter et al. 1983). Exposure of Q. pustulosa to turbidity resulted in a mean O:N ratio of 233.5 compared to a mean O:N value of 17.2 in the reference individuals (Aldridge et al. 1987). In the present study, O:N ratios likewise increased upon exposure to cadmium after 28 days, indicating the predominance of carbohydrate catabolism. The large glycogen stores (Dietz 1974) in freshwater mussels were a likely source of this carbohydrate material. As suggested by Bayne and Scullard (1977), the O:N ratio can be indicative of physiological condition only if seasonal and gametogenic cycles are taken into account.

There are many problems inherent in the use of freshwater mussels in toxicity tests. One concern is the ability to detect a biological effect amongst natural variability. A common way to reduce variability is to increase the number of replications or increase the number

of organisms per treatment. Adult freshwater mussels can be regionally abundant, but once restrictions are placed on species, size, and sex, sample size can become limited. Another problem often encountered in mussel toxicity tests is the tendency of mussels to close their valves during contaminant exposure (Kapkov 1971; Maki and Johnson 1976; Morgan et al. 1989). This represents a type of avoidance behavior and may allow the mussel to avoid acute contaminant exposure. Valve closing may not be a hindrance; possibly, once more information is obtained on the functions of valve opening and closing it could serve as a toxicity test endpoint.

In assessing the impacts of contaminant exposure on the bioenergetics of freshwater mussels, the nutritional status of the mussel has often been overlooked. Information on the nutritional requirements of freshwater mussels is sorely lacking. Because freshwater mussels can exist on body reserves for many months without visible effects, it is often assumed that they are being well fed. The TCI is often used to assess the nutritional status of an individual. The higher the TCI, the greater the proportion of tissue mass to shell mass. In the present study, although cadmium had no effect on the TCI, the mussels in the laboratory had a reduced TCI compared to L. ventricosa mussels in the field. This may have resulted from the

decrease in clearance rates and may be an indication that the food source was unpalatable.

The scope for growth bioenergetics model is based on the balanced energy equation developed by Winberg (1960), as modified by Bayne et al. (1985): $P = A - (R + U)$, where P = the energy incorporated into somatic and gametic production (Joules/hr); A = energy absorbed from food ($A = C - F$; where C = food energy consumed and F = energy lost as feces); R = energy respired and U = energy excreted. Positive scope for growth values (P) indicate that energy is available for growth and reproduction; negative scope for growth values (P) indicate that animals are using their body reserves for maintenance metabolism. The scope for growth model may be useful for freshwater mussel studies when more sensitive and reliable physiological measurements are found and more background information is available on these organisms. There are no data on physiological rates of freshwater mussels in natural environments. Furthermore, information on the effects of nutritional level on physiological processes is crucial, since all physiological measurements in the scope for growth model are dependent upon a nutritionally balanced food source.

In searching for criteria to assess the effects of an environmental contaminant, physiological responses provide an integration of both biochemical and cytological effects.

These physiological responses can be used to assess contaminant impacts on the population. The present research indicated that certain physiological responses can be sensitive sub-lethal indicators of contaminant exposure.

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SECTION 3. SOME CONSIDERATIONS IN THE USE OF FRESHWATER
MUSSELS IN TOXICITY TESTS

Some considerations in the use of freshwater mussels in
toxicity tests

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ABSTRACT

Freshwater mussels have been used to monitor contaminant exposure in the field environment. However, before freshwater mussels are used in laboratory toxicity tests, several points should be considered. These include the availability of the species, feasibility of determining sex, reproductive condition of test individuals, nutritional requirements, reduced physiological condition in laboratory acclimated individuals and the functions of valve closure as a toxicant escape response. These considerations should be viewed as unique aspects of a mussel's life history and not as reasons to preclude the use of mussels in toxicity tests. Tests must be designed which incorporate the unique features of a mussel's life history so that ecologically relevant information can be obtained.

INTRODUCTION

The life history of freshwater mussels makes them unique indicator organisms of contaminant exposure. They are benthic filter feeders, live directly associated with sediment, are locally abundant, are long-lived and they bioaccumulate contaminants to levels greatly exceeding water concentrations. They have been used to monitor contaminant levels in the field (Maki and Johnson 1976; Adams et al. 1981; Schmitt et al. 1987; Boryslawskyj et al. 1988). However, their use in the laboratory as toxicity test organisms has been limited. Although no standardized acute toxicity tests are available, acute toxicity tests have been conducted on freshwater mussels (Wurtz 1962; Rodgers et al. 1980; Van Puymbroeck et al. 1982; Millington and Walker 1983; Harrison et al. 1984).

CONSIDERATIONS

Before freshwater mussels can be used in the laboratory to assess the biological impacts of contaminant exposure, a good knowledge of the biology of the test organism is essential. The choice of mussel species to use in a toxicity test can be confounded by sex, size, availability and differences in the reproductive biology of the species. When assessing the feasibility of incorporating these species into standardized testing procedures, their parasitic lifestyle requires consideration. The reproductive biology of freshwater mussels (Family Unionidae) is highly variable; some females are gravid for all but a few months. The reproductive condition of the mussel is important in deciding when to conduct a test, especially if the added stress of being gravid compounds the effects of the contaminant on females. We have observed that gravid female mussels often release their glochidia in high concentrations of cadmium. Morgan et al. (1989) noted this same effect when Potamilus purpuratus were exposed to 0.66 mg/l of the fungicide thiobencarb. If a bradytictic (long-term breeder) species, is used, the toxicity test should be conducted with only gravid females (compared to non-gravid females), because this is their "normal" condition. However, the females should not be collected just prior to glochidial release or immediately after egg

formation when energy reserves are depleted and the female may be more vulnerable to toxic stress. Unfortunately the optimal time for bradytictic mussels may well be the period between November and March when they are inaccessible (at least in northern latitudes). Running toxicity tests in early spring and keeping the temperature cool (at ambient river temperature when collected) so as not to initiate glochidial release, may work best.

In the foregoing discussion the possibility of sex determination was assumed. Only about 10% of freshwater mussel species are sexually dimorphic, thus sex determination is impossible in most species without sacrificing the individual. In designing toxicity tests that examine contaminant effects on reproductive behavior, total number of glochidia produced, or glochidial viability, the ability to differentiate sexes is important. If the desired test endpoints are reproduction-oriented, then a sexually dimorphic species should be used. However, if the test is designed to evaluate specific contaminant effects and the mussel is chosen simply as a test organism, then the use of a sexually dimorphic species is not as critical.

A critical consideration is the influence that nutritional status has on toxicity tests. Freshwater mussels have large glycogen stores enabling them to live off body reserves for lengthy periods. Because of this, their

nutritional status is often overlooked. The nutritional requirements of freshwater mussels need to be determined before they are incorporated into toxicity tests.

Nutritional requirements are especially critical when conducting chronic, physiological toxicity tests, where most measurements are dependent upon a balanced diet. Recently, Lanno et al. (1989) evaluated the role that nutrition plays in modifying toxicity tests with larval fathead minnows (Pimephales promelas). They found significant changes in the toxicity of sodium pentachlorophenate to starved (96-hr LC_{50} =142 ug/l) and fed (96-hr LC_{50} =427 ug/l) minnows.

Parental diet has profound effects on the susceptibility of Ceriodaphnia dubia neonates to copper. A diet consisting of three algal species (Chlamydomonas reinhardtii, Ankistrodesmus falcatus and Chlorella vulgaris) resulted in a threefold increase in LC_{50} s compared to C. dubia fed synthetic diets (Belanger et al. 1989).

Availability of mussels may be the single most limiting factor in choosing a species. Many species are abundant regionally, however, once restrictions are placed on a single species, one sex, and a particular size range, sample size can become limited. ASTM (1988) suggests using all organisms of approximately the same size and age. Age determination in mussels is difficult; the assumption is often made that similar sized mussels are of similar age.

Kat (1982), however, suggested that mussels inhabiting different substrate types have different growth rates. ASTM (1988) suggests using a geometric series of at least five contaminant concentrations with at least 20 organisms per treatment in a flow through system. If only five treatments are chosen plus a control, 120 mussels would be required. With size, sex and species limitations, this number may be hard to get from a single location. Alternatively, the number of organisms per treatment or the number of treatments could be reduced. How this will affect the statistical ability to show contaminant effects is not known.

The stocking density of mussels may be critical to the toxicity test outcome. The number of mussels per treatment should be standardized because it can influence the LC_{50} s. In static tests, the more mussels per treatment, the lower the toxicity because there may be proportionally less contaminant available for uptake. The number of mussels per treatment may be so critical as to preclude their use in static toxicity tests. In static renewal and continuous flow tests, if an individual dies during the test, it should be replaced with another mussel to maintain a similar biomass among treatments.

Size can also influence the results of toxicity tests. Less than 9 cm Anodonta spp. exposed to the lampricide TFM

had a 96-hr LC_{50} of 8.3 mg/l, significantly different from the 96-hr LC_{50} of 11.7 mg/l in individuals greater than 16 cm (Maki et al. 1975). For toxicity test purposes, mussels of various size intervals could be collected and the same number of a particular size class could be placed into each treatment. This would make collection more feasible but may confound analyses.

There is convincing evidence that freshwater mussels are stressed simply by being brought into the laboratory. A long acclimation period may cause significant decreases in tissue mass (Russell-Hunter 1985; Payne and Miller 1987). This may reflect many factors: inability to feed them properly, lack of appropriate substrate, lack of suitable water, and stress involved in transport. These factors may compromise toxicity tests. Most researchers have noted a decline in total tissue weight over exposure periods as short as five weeks (V.-Balogh and Salanki 1984) and a consistent linear decline for as long as 22 weeks (Hemelraad et al. 1986). Weight loss was greatest in those tissues having the highest glycogen content such as the mantle and visceral mass (Holwerda et al. 1988). However, no change from control in the ratio of tissue volume/shell volume was observed in Corbicula manilensis exposed to Cu for 75 days (Harrison et al. 1984).

Freshwater mussels may close their shell in high contaminant concentrations and avoid complete exposure (Kapkov 1971; Maki and Johnson 1976; Morgan et al. 1989). This response is similar to avoidance behavior in fish (Sprague 1964; Westlake et al. 1974; Giattina et al. 1982). Fish can actively avoid contaminated areas by removing themselves from impacted areas. This response has survival value to the fish and is an ecologically important response to contaminant exposure. Mussels that can isolate their tissues from contaminant exposure for extended periods of time could be avoiding most of the contaminant exposure. In mussels, a high exposure level could actually represent a low contaminant dose and a low exposure level could be more toxic if the mussel maintained its filtering rate and did not close its valves. Whether or not the mussel's valves remain closed or re-open is unclear, but is probably dependent on exposure duration. The length of time a mussel's valves are closed may be a sensitive avoidance behavior response. Exposure of the Zebra mussel (Dreissena polymorpha) to increasing chlorine concentrations resulted in a decrease in the time the valves were open, with virtually no valve activity at a concentration of 500 ug/l (Kramer et al. 1989). However, more information about the functions of valve opening and closing is needed before a judgment on its utility as a toxicity testing endpoint can

be made. Because of the ability of mussels to curtail siphoning upon contaminant addition, Millington and Walker (1983) suggested that acute lethality tests should have a minimum duration of 96 hours. ASTM (1988) states that because both juveniles and adults can avoid toxic exposure, acute lethality tests should not be conducted with them. Instead of obtaining LC_{50} values for mussels, sublethal endpoints or EC_{50} (effective concentration) values may be more appropriate.

CONCLUSIONS

Freshwater mussels would make unique toxicity test organisms due to their benthic nature and filter feeding lifestyle. However, more information on the biology of these organisms is required before they are used in laboratory tests. Specifically, information on the nutritional requirements, functions of valve closure, maintenance of physiological rates and effects of reproductive condition must be developed. These considerations should not limit their use in toxicity tests, but be incorporated into test designs.

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SUMMARY

The upper Mississippi River contains one the most numerous and diverse assemblages of freshwater mussels in the United States. Recent studies indicate that both the density and diversity of these organisms are declining. One proposed explanation is the potential subtle, pervasive impacts from low level contamination in the upper Mississippi River. As indigenous, benthic filter-feeding organisms, freshwater mussels can bioaccumulate heavy metals to concentrations greatly exceeding water concentrations. In adults, the most common uptake site of heavy metals is the gills, followed by the mantle and kidney. Information on cellular and subcellular distribution is scarce, but epithelial cells generally contain larger amounts of heavy metals than muscle cells.

Little information is available regarding toxicity of metals to mussels. Acute toxicity tests have been conducted, but several attributes of mussels preclude their use on a routine basis at this time. Sublethal chronic effects of heavy metals on freshwater mussels are rarely documented. Sublethal effects of contaminant exposure in freshwater mussels include: decreased weight gain, reduced filtration efficiency, reduced enzyme activity and modifications in behavior. Physiological responses to contaminant exposure have been quantified and used in a

bioenergetics model known as scope for growth. This model, along with several other indices of physiological condition, may provide valuable information regarding the effects of contaminant exposure on freshwater mussels.

A study was conducted to evaluate the physiological responses of adult Lampsilis ventricosa to sublethal cadmium exposure. Physiological processes studied included respiration rate, food clearance rate, ammonia excretion rate and food absorption efficiency. Mussels were exposed to Cd (0, 30, 100 and 300 ug/l) for 28 days in a proportional diluter. Analyses indicate that respiration rate was the most sensitive and least variable indicator of Cd exposure, whereas food absorption efficiency was the most variable response. Respiration rates in mussels exposed to cadmium were significantly ($p < 0.05$) depressed compared to respiration rates in mussels with no Cd exposure. Although food clearance rates and ammonia excretion rates showed no statistical differences among Cd treatments, ammonia excretion rates in mussels exposed to 300 ug Cd/l fell from 22 to 5 ug/hr/g dry tissue weight by day 28. By day 28, food clearance rates also fell to one-third of their original values in mussels exposed to 300 ug Cd/l. Assimilation efficiencies increased over the test duration in all treatments. Freshwater mussels can be sensitive indicators of sublethal contaminant exposure. However, due

to large variability in some physiological rates, care must be taken in selecting appropriate physiological indicators of contaminant effects.

Application of the scope for growth bioenergetics model to freshwater mussels is not feasible until more sensitive physiological measurements are found and more background information is available on these organisms. Specifically, information on baseline physiological rates in mussels is needed. In attempting to compare the physiological results of this study to physiological rates of freshwater mussels in the natural environment, data were generally not available. Furthermore, information on the effects of nutritional level on physiological processes is crucial, since all measurements in the scope for growth model are dependent upon a balanced diet. Only after these types of questions are answered, should the scope for growth bioenergetics model be applied to freshwater mussels.

Freshwater mussels have been used to monitor contaminant exposure in the field environment. However, before freshwater mussels are used in laboratory toxicity tests to evaluate the effects of contaminant exposure, several points should be considered. These include the availability of mussel species, sex determination, influence of reproductive condition on toxicity test results, lack of adequate information on nutritional requirements, reduced

physiological condition in laboratory acclimated individuals and the functions of valve closure as a toxicant escape response. These considerations should not necessarily preclude the use of mussels in toxicity testing. Tests must be designed which incorporate the unique features of a mussel's life history so that ecologically relevant information can be obtained.

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