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Valve closure responses of the Asiatic clam *Corbicula fluminea* exposed to cadmium and zinc

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Abstract

The valve movement patterns of immobilized Asiatic clams (*Corbicula fluminea*) were monitored during exposure to constant concentrations of cadmium (0.0, 0.1, 0.2, 0.3, and 0.4 mg l^{-1}) or zinc (0.0, 0.1, 0.3, 0.5, and 0.9 mg l^{-1}) for 24 h following a 24-h acclimation period. Data indicate that the duration of response was concentration dependent and toxicity related. Durations of periods with valves parted declined as the concentration of heavy metal increased. Behavior was consistent for both mean time to first closure following the initial exposure and mean time per valve parting episode over a 24-h exposure period. Mean time per valve parting episode during the 24-h exposure period ranged from ≃ 600 minutes for control trials to 36 and 69 minutes for the highest concentrations of cadmium and zinc tested, respectively. There was no association between durations of periods with valves sealed and solutions of cadmium; however, lengthening durations of periods with valves sealed coincided with exposure of clams to progressively more concentrated zinc solutions. In addition, Asiatic clams demonstrated a greater rate of response (decline in the duration of periods with valves parted) to progressively more concentrated solutions of cadmium than to comparable increases in the concentrations of zinc solutions.

Introduction

The Asiatic clam *Corbicula fluminea* has a natural northerly distribution in the United States that reaches 40°N latitude (Britton & Morton, 1982; McMahon, 1982). Within this range, the clam can persist and thrive in waters of both pristine and reduced quality. Its resistance to the toxic effects of common biocidal control agents such as chlorine has permitted the establishment of extensive populations of *C. fluminea* in industrial cooling water systems (Goss & Cain, 1977; Cherry *et al.*, 1980; Harvey, 1981). The extent of the distribution of this organism and its resistance to the lethal effects of aquatic pollutants have prompted its use as a biomonitoring organism in freshwater environments similar to efforts with bivalves (*Crassostrea gigas, Mya arenaria, Mytilus edulis*, and *Rangia cuneata*) in brackish and marine water systems (Butler, 1969; Goldberg *et al.*, 1978; Popham *et al.*, 1980). Graney *et al.* (1983) demonstrated that the Asiatic clam accumulates heavy metals such as cadmium (Cd), copper (Cu), and zinc (Zn). They reported bioconcentration factor (BCF, the ratio of tissue concentration to water concentration) ranges of 358 to 631 for Zn and 17720 to 22571 for Cu when clams were exposed to these metals in laboratory artificial streams. The BCF values for Cd were intermediate to those for Zn and Cu at 1752 to 3770. Johnston & Hartley (1981) reported BCF values of 1000 to 10000 for caged *C. fluminea* individuals placed in a chlorinated pesticide polluted river in central Illinois.

Numerous studies have demonstrated that bivalves exhibit daily and seasonal cycles of physiological and behavioral activity (Kuwatani, 1963; Imlay, 1968; McCorkle *et al.*, 1979; Higgins, 1980). Exposure of bivalves to toxicants (e.g., metals and halogens) though produces alterations in behavior (e.g., cessation of siphoning and valve closure) that result in isolation of the soft tissues from the toxic effects of the pollutant (Davenport, 1977; Akberali & Black, 1980; Cherry *et al.*, 1980). Consequently, these responses may interrupt the bioaccumulation of metals. This study was undertaken to determine if exposure to Cd or Zn would result in changes in the valve movement patterns of the Asiatic clam and possibly interfere with bioaccumulation processes operating within *C. fluminea*.

Materials and methods

Specimens for study were collected from the New River in Narrows, Virginia at various times in spring and fall, 1985. Clams were collected from a fine sand substrate with a 3-m handled dredging cage (mesh size = 7.0 mm) at water temperatures ranging from 17 to 22 °C. Clams were returned to the laboratory and held in artificial streams (Farris, 1986) while slowly acclimating ($\simeq$ 1.5 °C/d) to a temperature of $\simeq$ 25 °C. Clams were fed daily with a green alga, *Chlamydomonas* sp., from a highly concentrated stock culture.

Durations of periods with valves parted or valves sealed were determined by monitoring the movements of the left-hand valve of immobilized individuals. The lower (right-hand) valve was secured to a polypropylene strip (10 × 2 × 0.5 mm) with a cyanoacrylate ester bonding agent. The upper valve was connected to the lever of a heart/smooth muscle transducer (Harvard Apparatus Co., Inc.) by thread cemented to the upper valve. This positioning always directed the sensory possessing siphons toward the rear of the test enclosure away from the activities of the experimenter. A continuous record of valve movements was traced over paper loaded on a Harvard Apparatus multi-speed transmission chart recorder (McCorkle *et al.*, 1979). Three test strips per trial were secured with plastic screw clamps to a table within the 30-l capacity exposure tank (35 × 30 × 30 cm). Metallic items were not present in the tank during testing to eliminate fluctuations in metal concentration due to leaching of metal ions from metallic test equipment. The exposure chamber was placed on a magnetic stir plate to provide homogeneous test solutions and was isolated from the laboratory by a plywood enclosure (2.5 × 1.5 × 1.0 m). Access to the test equipment was afforded by hinged doors on the front of the enclosure. Timer-controlled fluorescent lighting provided a 14L:10D diurnal cycle. Temperatures were maintained between 24 and 26 °C by heat generated from test equipment within the enclosure.

Water for each study was obtained by filtration of the laboratory tap supply through activated charcoal. Chemical characteristics of the test water were generally consistent. Typical ranges for alkalinity, hardness (as $CaCO_3$), and conductivity were 40 to 50 mg l^{-1}, 70 to 80 mg l^{-1}, and 140 to 155 μmhos, respectively. The concentration ranges for chlorides, nitrates, phosphates, and sulfates were 9.5 to 12.5, 4 to 6, 0.2 to 1.0, and 13 to 17 mg l^{-1}, respectively. Incoming test water pH generally ranged from 8.0 to 8.3 and was adjusted to a test pH of 7.0 by addition of dilute H_2SO_4 with peristaltic pumps (Cole-Parmer) under the control of a pH meter/controller (Fisher Scientific). Water samples (20 ml) from test solutions were collected frequently (4/d) for analysis of metal content. Samples were acidified with three drops of ultrapure HNO_3 at the time of collection. Samples were not filtered prior to analysis by atomic absorption spectrophotometry (Perkin-Elmer Model 560) with an air: acetylene flame.

The study design consisted of consecutive 24-h observation periods. The initial 24-h period served as a control and acclimation phase in which the valve movement patterns of each individual were recorded in the absence of heavy metal. Subsequently, a 24-h exposure period was initiated by draining the exposure tank to a level below the surface of the table on which the clams were located, temporarily resulting in aerial exposure of the test clams. A predetermined volume of stock solution was added (28 to 250 ml of 100 mg metal l^{-1} from solutions of either $CdSO_4$ or $ZnSO_4 \cdot 7H_20$) to the exposure tank to facilitate dilution of the metal stocks, and the tank was refilled to a volume of 28 l with fresh diluent. Clams were neither physically handled nor offered food (algae) during the consecutive 24-h observations periods. Trials were conducted at Cd concentrations of 0.0, 0.1, 0.2, 0.3, and 0.4 mg l^{-1} and Zn concentrations of 0.0, 0.1, 0.3, 0.5, and 0.9 mg l^{-1}. In general, metal concentrations varied by no more than $\pm$ 0.03 mg metal l^{-1} during the exposure period. Replicate trials were arbitrarily conducted with certain concentrations of metal (0.2 mg l^{-1} Cd and 0.1 and 0.9 mg l^{-1} Zn). Triplicate trials were conducted with other concentrations of metal (0.0 mg l^{-1} heavy metal and 0.5 mg l^{-1} Zn). All trials were conducted with previously unexposed organisms.

Evidence for a change in valve movement patterns resulting from exposure to solutions of either Cd or Zn was determined by regressing the mean lengths of time to first closure following initial exposure or the average duration of periods with valves parted or sealed during the 24-h exposure phase against heavy metal concentration. Mean lengths of time to first closure and durations of periods with valves parted or sealed were obtained by pooling observations from all individuals within a trial and between trials conducted at the same concentration. Sample sizes for calculation of mean time to first closure (= number of individuals monitored) ranged from 3 to 8. Sample sizes for calculation of the average duration of periods with valves parted or sealed ranged from 2 to 21. Data collected were subjected to least squares (linear) regression analysis (SAS Institute Inc., 1982).

Results

Exposure of Asiatic clams to irritant and possibly toxic concentrations of heavy metals results in valve closure. This response, as measured by both the time to first closure and the duration of periods with valves parted, is concentration dependent and toxicity related (Fig. 1). Exposure to increasingly

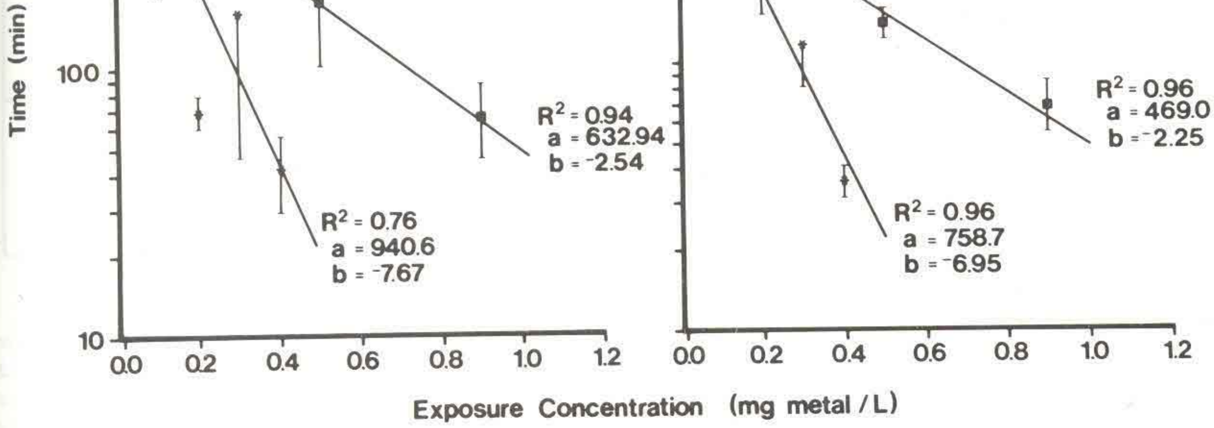

Fig. 1. Mean time with valves parted (± SE) for *Corbicula fluminea* individuals exposed to various concentrations of cadmium (Cd) and zinc (Zn). (A) Mean time to first closure following initial exposure. (B) Mean time for all valves parted episodes during the 24-h exposure period. R^2 = coefficient of determination, a = Y-intercept, b = slope.

concentrated solutions of either metal resulted in a reduction in the mean duration of periods with valves parted experienced by each clam. The relationship was observed to a varying degree in the time between initial exposure and first closure (Fig. 1A) but more obviously expressed in the mean time with valves parted for all valve parting episodes during the 24-h exposure period (Fig. 1B). Mean time to first closure during the exposure phase of the 2-d trials ranged from ≃ 860 minutes for control trials to ≃ 42 and 66 minutes for the highest concentrations of Cd and Zn tested (0.4 and 0.9 mg l^{-1}, respectively). Least squares regression analysis of mean time to first closure and metal concentration generated lines of best fit with coefficients of determination (R^2) of 0.76 and 0.94 for Cd and Zn exposure trials, respectively. Slopes of the regression lines demonstrated that clams responded more quickly on exposure to Cd (−7.67) than on exposure to Zn (−2.54).

A better fit relationship was observed between mean time with valves parted for all exposure phase valve parting episodes and heavy metal concentration ($R^2 = 0.96$ for trials with either metal) than mean time to first closure. The observed rates of response by *C. fluminea* though were similar between the two approaches. Slopes of the lines describing the relationship between mean time with valves parted and metal concentration were −6.95 and −2.25 for Cd and Zn, respectively. Mean time per valve parting episode for the duration of the 24-h exposure phase ranged from ≃ 600 minutes for control trials to 36 and 69 minutes for the highest concentrations of Cd and Zn tested, respectively. Mean time with valves parted during the initial 24-h observation period (340 minutes) was considerably lower than that observed during control trials of the exposure phase (600 minutes).

Mean time with valves sealed during periods of exposure to Cd was independent of the Cd concen-

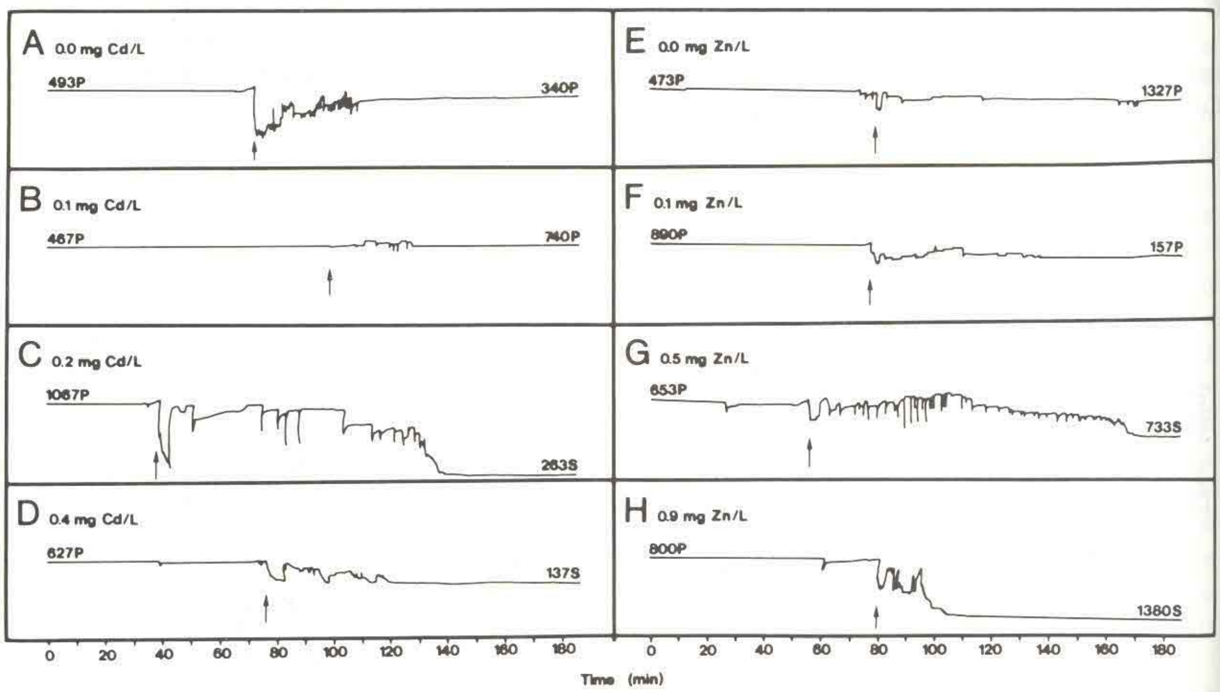

Fig. 2. Representative valve movement tracing patterns of individual *Corbicula fluminea* test subjects exposed to minimal (A, E), intermediate (B, C, F, G), and maximal (D, H) concentrations of cadmium (Cd) and zinc (Zn) following a 24-h control observation period. Numbers appearing on either end of each tracing within each subfigure represents the length of time in minutes spent by that individual with valves either parted (P) or sealed (S). Arrows beneath each tracing represent the point in time at which valve closure was elicted because of tank drainage prior to the addition of metal and refilling of the tank. The tank was not drained before adding Cd to a final concentration of 0.1 mg l^{-1} (B).

tration ($R^2 = 0.01$, slope $= -0.22$). Control trials were characterized by an average duration of $\simeq 325$ minutes versus times of 140, 240, 170, and 260 minutes during exposure to 0.1, 0.2, 0.3, and 0.4 mg l^{-1} solutions of Cd, respectively. Mean durations of time with valves sealed during exposure to 0.9 mg l^{-1} Zn increased $\simeq$ 3-fold in comparison with control periods and $\simeq$ 2-fold in comparison with that observed for clams exposed to 0.1 mg l^{-1} solutions of Zn ($R^2 = 0.62$, slope $= 0.99$). Mean durations of time with valves sealed were 450, 880, 530, and 970 minutes in 0.1, 0.3, 0.5, and 0.9 mg l^{-1} solutions of Zn, respectively.

The relationship between time to first valve closure and metal concentration is presented in Fig. 2. These valve movement tracings provide a respresentative depiction of the rapidity with which valve closure occurs in response to exposure to various concentrations of heavy metal. Tank drainage prior to the addition of either Cd or Zn stock solutions resulted in a momentary valve closure response (indicated by the black arrows) during aerial exposure. Exposure of test clams to either metal-free diluent (Fig. 2A, 2E) or low concentrations of metal (Fig. 2B, 2F) did not produce any readily discernible change in valve position once the valves returned to the positions held prior to drainage of the exposure tank. Exposure to higher concentrations of metal though resulted in increased valve adduction frequency and shorter durations of periods with valves parted (Fig. 2C, 2D, 2G, 2H).

Subsequent to the initial valve closure event during the 24-h heavy metal exposure period, highly distinct patterns of valve movement were observed that were indicative of the quality of the test solution (Fig. 3). Control trials and tests with low con-

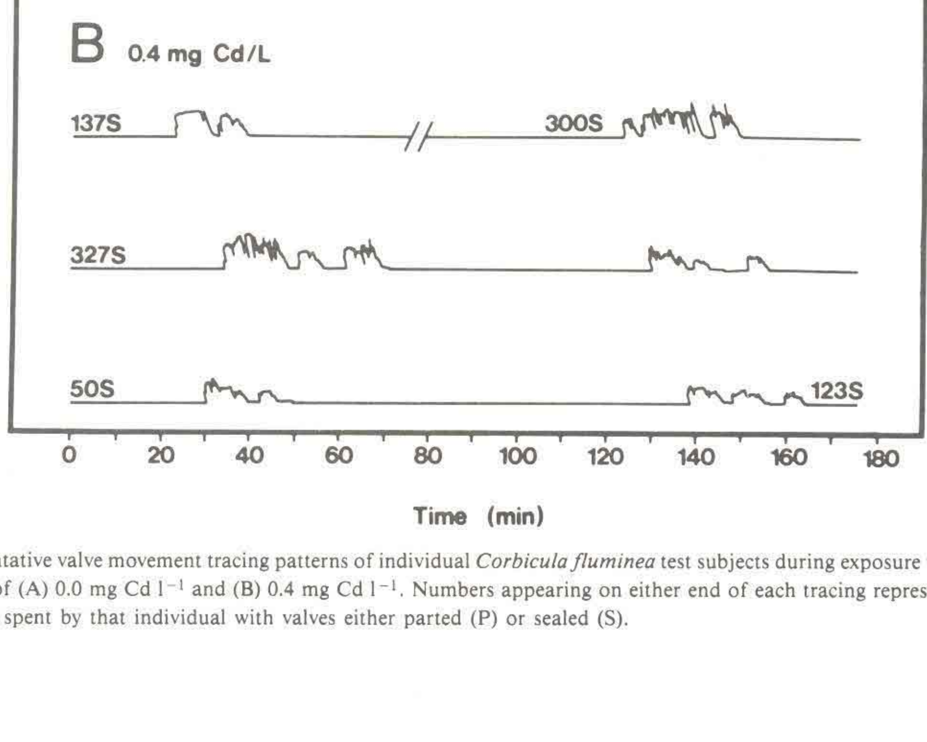

Fig. 3. Representative valve movement tracing patterns of individual *Corbicula fluminea* test subjects during exposure to cadmium (Cd) concentrations of (A) 0.0 mg Cd l^{-1} and (B) 0.4 mg Cd l^{-1}. Numbers appearing on either end of each tracing represent the length of time in minutes spent by that individual with valves either parted (P) or sealed (S).

centrations of metal were typified by tracings revealing extended periods of time with valves either parted or sealed (Fig. 3A). Alternatively, levels of metal that were severely irritating to the clams produced patterns of activity typified by rapid and frequent adduction of the valves, "fluttering"; and alternating periods with valves parted and sealed, "testing behavior" (Fig. 3B; Davenport & Manley, 1978).

Discussion

It has been demonstrated that exposure of Asiatic clams to increasingly concentrated solutions of Cd and Zn results in a decrease in the duration of periods with valves parted and possibly results in an increase in the durations of periods with valves sealed. Asiatic clams exposed to Cd at a concentration of 0.4 mg l^{-1} demonstrated shorter durations of valve parting episodes than that exhibited by a marine bivalve. Sunila (1981) found that exposure of *Mytilus edulis* to Cd did not result in valve closure until the test specimens experienced a concentration of 3 mg l^{-1}. Responses exhibited by another marine bivalve though were similar to those observed with *C. fluminea* exposed to comparable concentrations of Zn. Akberali *et al.* (1981) reported that test specimens of the marine bivalve *Scrobicularia plana* interacted freely with their environment contaminated with Zn at concentrations of 0.1 and 0.5 mg l^{-1}. Behavior of *S. plana* exposed to 1.0 mg l^{-1} demonstrated little similarity with that of individuals in weaker solutions. Valves were rarely parted during 6-h exposures to 5, 10, and 20 mg l^{-1} Zn solutions. Changes in the behavior patterns of a pulmonate snail exposed to solutions of Cd and Zn were observed at concentrations comparable to those affecting the Asiatic clam. Harry & Aldrich (1963) reported that *Taphius glabratus* demonstrated distressed reactions to Cd at concentrations of 0.05 and 0.10 mg l^{-1} and sustained retraction of the animal into its shell at 1.0 mg l^{-1}. Test specimens displayed distress and body retraction to Zn at concentrations of 0.1 to 1.0 and 5.0 mg l^{-1}, respectively.

A number of studies addressing the behavior of bivalves exposed to other pollutants has also been reported. Davenport & Manley (1978) noted an association between valve adduction, testing behavior, valve closure, and Cu concentration in *M. edulis*. Valve adduction, testing behavior, and valve closure were initially noted at concentrations of 0.01, 0.02, and 0.02 mg l^{-1} in 44, 56, and 22% of the test specimens, respectively. A 100% response for each behavior component was obtained at concentrations of 0.05, 0.05, and 0.20 mg l^{-1}, respectively. Manley & Davenport (1979) reported that the threshold of detection (interruption of normal valve movements) for Cu by representatives of five marine bivalve genera ranged from 0.02 to 0.14 mg l^{-1}. Akberali & Black (1980) reported that specimens of *S. plana* interacted with their environment in the presence of Cu at concentrations of 0.01 and 0.05 mg l^{-1}. A reduction in activity was observed at 0.1 mg l^{-1} while exposure to Cu at a concentration of 0.5 mg l^{-1} resulted in rapid (< 1 minute) retraction of the siphons and closure of the valves. In a study assessing the behavioral response of *M. edulis* to chlorine, James (1967) reported that low doses (0.05 mg l^{-1}) prompted test specimens to detach their byssal threads from the substrate and migrate from the fouled site. He also reported that mortality rates were increased at lower doses of chlorine. Exposure periods for complete eradication were prolonged at higher chlorine concentrations. Doherty (1986) discovered that exposure of *C. fluminea* to total residual chlorine (TRC) concentrations ranging from 0.09 to 0.43 mg l^{-1} resulted in valve closure. Subsequently, Doherty *et al.* (1986) demonstrated that exposure of Asiatic clams to low levels of chlorine (0.25 to 0.35 mg l^{-1} TRC) for 14 to 20 d resulted in extended periods with valves sealed that weakened clams and enhanced biofouling control efforts with molluscicides.

Other investigators have used discontinuous visual observations to ascertain the effects of toxicants on changes in siphoning and valve closure. Rodgers *et al.* (1980) reported an inverse relationship between Cu concentrations and visually assessed filtering activity by Asiatic clams. They observed that $> 50\%$ of the test specimens exposed to Cu at a concentration of 0.01 mg l^{-1} were filtering after 72 h of exposure. No specimens were active in Cu solutions of 0.05 mg l^{-1}. Similar observations were reported for exposure to Zn at concentrations of 0.50 and 1.0 to 5.0 mg l^{-1}, respectively. Belanger *et al.* (1986a, b) observed significant reductions in siphoning by adult and juvenile *C. fluminea* exposed to chrysotile asbestos concentrations ranging from 10^2 to 10^8 fibers l^{-1}.

It has also been demonstrated that the rate of behavioral response by *C. fluminea* may be a function of the toxicity of the pollutant. Exposure of Asiatic clams to Cd resulted in valve closure at a rate approximately three times faster than occurred on exposure to Zn. While there have been no studies conducted with the Asiatic clam in which the toxicities of these metals have been determined concurrently, numerous studies with other organisms demonstrate that Cd is one to two orders of magnitude more toxic than Zn under comparable test conditions. This relationship has been established for the ciliated protozoan *Tetrahymena pyriformis* (Carter & Cameron, 1973), the crustacean *Daphnia magna* (Biesinger & Christensen, 1973), the mayfly *Ephemerella subvaria* (Warnick & Bell, 1969), and various species of fish (Spehar, 1976; Chapman, 1978). While Graney (1980) did not conduct standard toxicity tests with Cd and Zn against the Asiatic clam, he did report $\simeq$ 15 and 6% mortality among adults exposed to 0.055 and 0.835 mg l^{-1} solutions of Cd and Zn, respectively, after 30 d. A definitive statement on the relationship between stimulation of valve closure responses by bivalves exposed to pollutants and the toxicity of those pollutants would require comparisons between the behavior altering capacity and toxicity of three or more pollutants in a single species.

The mechanisms by which *C. fluminea* and other bivalves detect the presence of toxic materials have not been fully elucidated. Kraemer (1983) noted an atypical clustering of cilia on the highly innervated epithelial surface of the anal siphon of *C. fluminea*. She suggested that these ciliary tufts may be the sensory organelles responsible for the high degree of sensitivity of the anal siphon. Akberali *et al.* (1983) studied the influence of external calcium (Ca) on Cu induced contraction of the isolated siphon of the estuarine bivalve *S. plana*. They found that siphonal contraction in the presence of Cu was strongly dependent on the presence of Ca in the bathing medium. They hypothesized that Cu facilitates release of Ca from intracellular stores causing contraction either through effects on transmitter release in presynaptic nerve terminals or excitation contraction in muscle cells.

This study has demonstrated that the valve closure response of *C. fluminea* is a phenomenon dependent on the exposure concentration of dissolved Cd and Zn. Specifically, durations of periods with valves parted decline as metal concentration increases. By design, biomonitoring organisms are employed for the detection of low levels of environmental contamination by the accumulation of that contaminant in the tissues of the biological monitor. Based on the results of this study, it does not appear that the behavioral (valve closure) responses of the Asiatic clam will interfere with the bioaccumulation processes in operation at low levels of Cd or Zn contamination ($<$ 0.1 mg metal l^{-1}). These observations, however, should not be extrapolated to define the valve closure behavior of individuals with elevated body burden levels of heavy metal resulting from chronic exposure to low levels of environmental contamination or from exposure to particulate metal. It is not known if durations of periods with valves parted would decline due to an internal threshold phenomenon, remain unchanged, or increase because of a habituation or acclimation process. Conclusions of a more definitive nature will be possible only after behavior monitoring studies that extend to weeks in duration rather than days are conducted.

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