Hydrobiologia **287**: 161–178, 1994.

Molluscan cellulolytic activity responses to zinc exposure in laboratory and field stream comparisons

J. L. Farris [1], J. L. Grudzien [2], S. E. Belanger [3], D. S. Cherry [1] & J. Cairns, Jr. [1,*]
[1] *Department of Biology and University Center for Environmental and Hazardous Materials Studies, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA (*author for correspondence);* [2] *Grace Bio-Oncology, Inc., 900 Auburn Road, Pontiac, Michigan 48342, USA;* [3] *Environmental Safety Department, The Procter and Gamble Company, Ivorydale Technical Center, Cincinnati, Ohio 45217, USA*

Received 13 March 1992; in revised form 27 October 192; accepted 19 September 1993

Key words: zinc, cellulolytic activity, molluscs, monitoring, artificial streams

Abstract

Changes in cellulolytic activity of Asiatic clams (*Corbicula fluminea*) and snails (*Mudalia dilatata*) were monitored throughout 30-d exposures to constant additions (0.0, 0.025, 0.05, 0.50, and 1.0 mg l^{-1}) of zinc (Zn). All exposures of 0.05 mg Zn l^{-1} or greater significantly reduced enzyme activity (exo- and endocellulase) in both molluscs as early as 10 d following exposures in outdoor laboratory streams incorporating New River water as diluent. More sterile laboratory stream exposures were less consistent in yielding quantifiable differences that could be attributed to metal induced stress apart from effects of nutritional stress. Tests conducted under natural field conditions during all seasons did not differ significantly with respect to changes in annual energetics of either clams or snails. However, evidence of differing uptake routes, with respect to two ecologically and physiologically distinct molluscs, was apparent in bioaccumulation, growth, and enzyme activity throughout exposure and following 60-d recovery.

Introduction

Efforts to monitor the impacts of heavy metals on aquatic environments have included the examination of metals in aqueous and suspended particulate phases, in sediment phases, and the availability of these phases to organisms qualifying as biomonitors. The present data base is reflective of the large variability in organismic response to differing routes of exposure and uptake. This variability has been shown to be influenced by test species selection, methodology in testing, water quality parameters, and various factors known to affect the range of effect levels (O'Donnel *et al.*, 1985; Giesy *et al.*, 1988). The apparent lack of cohesion between toxicity and bioavailability of metals demands more consideration be given to those tests that include end points that can be reliably measured prior to the onset of mortality. For this reason, the use of indigenous biota (specifically molluscs) to monitor metal contamination in water and sediments has gained recognition for the ability of sedentary or sessile organisms to estimate actual concentrations

realized by organisms (Phillips, 1977; Goldberg *et al.*, 1978). Body burdens of metals may be correlated with ambient water concentrations, but these do not require information on all changes occurring with fluctuations in metal complexation or speciation. Rather it examines the relevant forms of each accumulated metal within the organism through suspected uptake routes. However, bioaccumulation has not proven sufficient as a single end point for decision making because some molluscs have a high capacity to accumulate metals without notable toxic effects (Poulsen *et al.*, 1982).

Although molluscs have been extensively used as monitors of heavy metal pollution (Phillips, 1980; Goldberg *et al.*, 1978; Graney *et al.*, 1983; Doherty & Cherry, 1988), there is a paucity of information on the effects that metal accumulation may exert upon their metabolism and physiology (Bayne, 1976; Viarengo *et al.*, 1982). Efforts to quantify stress during the accumulation of metals that exceed their abilities to effectively store or depurate them have led to the development of general and specific stress indices for molluscs (Viarengo *et al.*, 1982). Stress has been defined by Bayne (1975) as a measurable alteration of a physiological steady state induced by an environmental change that renders the individual or the population more vulnerable to further environmental changes. The use of these indices can support the understanding of the nonspecific processes (including mucus binding, endocytosis, and diffusion [Simkiss & Mason, 1983]) involved with metal uptake in molluscs. Phillips (1980) noted that proposed indices for stress in indicator species should be sufficiently sensitive to changes in metal availability as dominated by food routes, differences in water quality, and even behavioral alterations that might affect indicator ability to reflect exposure accurately.

The studies described in this discussion were undertaken to investigate cellulolytic responses in two molluscs (filter-feeding bivalve and phytophagous gastropod that are ecologically and physiologically distinct) to long-term exposure and recovery to zinc (Zn). The Asiatic clam, *Corbicula fluminea*, has been adequately qualified as a bioindicator of pollutants (Johnston & Hartley, 1981; Foe & Knight, 1985, 1987) and has recently been given increased attention for use in biomonitoring and toxicity testing (Harrison *et al.*, 1984; Belanger, 1991; Belanger *et al.*, 1986a, b; Farris, 1986; Farris *et al.*, 1988; Doherty, 1990). In addition to examining Zn accumulation and cellulolytic activity in clams, comparisons were made between the clam and a freshwater gastropod (*Mudalia dilatata*) to evaluate accumulation of metals through differing uptake routes and behavioral alterations. Stress indices from these two potential monitors are examined seasonally in both laboratory and field-oriented artificial stream systems.

Materials and methods

Seven, 30-d exposures of Zn to the Asiatic clam (*C. fluminea*) and a snail (*M. dilatata*) were carried out at the Glen Lyn field laboratory (GL) and the Virginia Polytechnic Institute and State University (Virginia Tech) Ecosystem Simulation Laboratory (ESL) (Table 1) using New River water (60 mg l^{-1} hardness) as diluent. Invertebrates were analyzed for variations in cellulolytic activity and Zn accumulation in response to duration and degree of exposure in artificial streams.

Description of artificial streams

Four of the chronic exposures were carried out at an outdoor field laboratory (Glen Lyn, Virginia) with untreated New River water (pH 8.1 ± 0.2) and three at the ESL with dechlorinated tap water (controlled pH 7.0). Measured total recoverable Zn was evaluated in all studies. Experimental units consisted of a series of oval, 20-l paddle-driven streams adapted for both field and laboratory situations (Fig. 1). This design was similar to the 'McIntire' artificial stream that has been noted for its economical approach for extent of replications, estimated interactions, and independence of treatments (Kosinski, 1989). Further description of these laboratory and field systems can be found in Farris *et al.* (1989).

Table 1. Dates for analysis using artificial stream systems with untreated New River water at Glen Lyn (GL) and municipal treated, laboratory water at Virginia Tech (ESL).

Site and code	Date	Exposures
GL 1 spring 1984	28April–28May1984	30-d
GL 2 summer 1984	23 June–23 July1984	30-d
GL 3 fall 1984	7 Sept–70 Oct1984	30-d + clam 30-d recovery
GL 4 spring 1985	26April–26May1985	30-d + clam and snail 30-d recovery
ESL 1 spring 1984	27 May–26June1984	30-d
ESL 2 winter 1984	26 Nov–26 Dec1984	30-d
ESL 3 spring 1985	9 April–9May1985	30-d

Glen Lyn streams were filled to a depth of 2 cm with coarse sand sediment (83% of the sediment was 2.5–9.0 mm in particle size); no substrate was added to the ESL streams. A 14L:10D light regime was used in the ESL streams.

Artificial streams at both the ESL and Glen Lyn locations were dosed with $ZnSO_4.7H_2O$ at concentrations ranging from 0.025 to 1.0 mg l^{-1} at a pump flow rate of 0.5 ml min^{-1} from Cole-Parmer variable speed peristaltic pumps. Stock

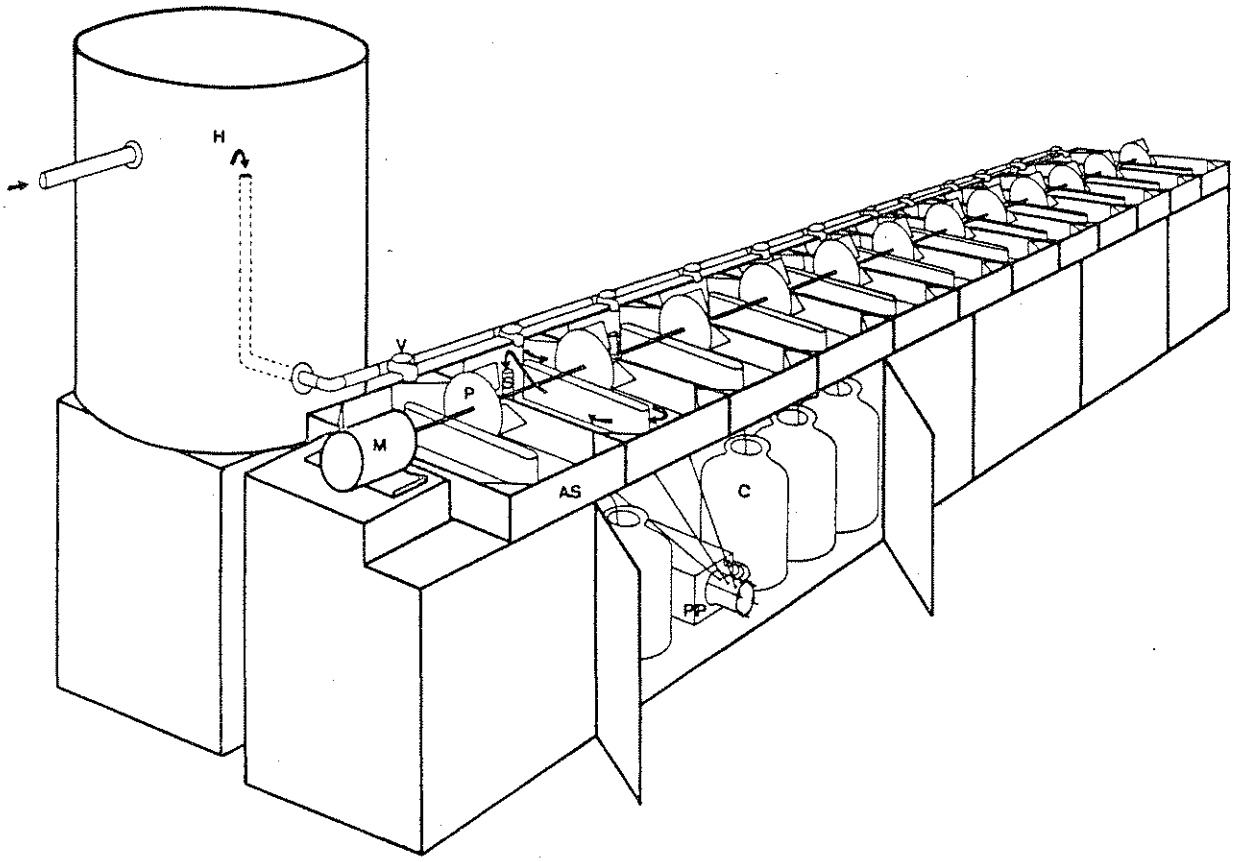

Fig. 1. Schematic representation of artificial stream and metal dosing system showing headbox (H), peristaltic pump (PP), carboys (C), containing Zn pumped (PP) to streams (AS) with paddlewheels (P), motor (M), standpipes (S), and valves (V) to control diluent flow. Arrows indicate stream incurrent and excurrent flow.

solutions held in 25-l carboys were changed every second day. To provide food for *Corbicula*, 250 ml of *Chlamydomonas rheinhardti* cultured in Bold's Basic medium (625 cells l^{-1}, final in-stream density) were added to ESL streams every day; Glen Lyn streams were colonized by resident algae from the New River (Genter *et al.*, 1987) so no food supplements were needed. During acclimation, precolonized rocks transferred from the New River to ESL streams provided substrate and food for *Mudalia* grazing.

Water samples were collected from ESL and Glen Lyn and returned on ice to Virginia Tech for water chemistry analysis on each sample day (Tables 2, 3). The total recoverable Zn fraction was determined (American Public Health Association [APHA] *et al.*, 1980) using a Perkin-Elmer 640 atomic absorption spectrophotometer for each water sample. Alkalinity and hardness were determined titrimetrically (APHA *et al.*, 1980), and pH was determined using a Model 650 Fisher Scientific pH meter. Anions (NO_3^-, PO_4^{-3}, SO_4^{-2} Cl^-) were determined by column ion chromatography using a Dionex Model 10 ion chromatograph.

Exposures at Glen Lyn used three replicate streams at three to four concentrations (Tables 2, 3). Early range-finding studies documented that replicate streams were not significantly different in actual Zn concentrations (Farris, 1986); consequently, the spring 1985 Glen Lyn study and all formal laboratory studies were conducted with single streams per target concentration.

Table 2. Mean (± 1 SE) of selected water chemistry parameters analyzed during the Glen Lyn studies ($n = 15$).

Season	Target concentration (mg l^{-1})	Actual zinc concentration (mg l^{-1})	Temp (°C)	pH	Hardness (mg l^{-1} as $CaCO_3$)	Alkalinity (mg l^{-1} as $CaCO_3$)
GL 1	0	0.020	25.1	8.39	71.1	49.8
(spring 1984)		(± 0)	(± 1.8)	(± 0.30)	(± 13.5)	(± 7.1)
	0.05	0.043	–	8.31	70.7	49.5
		(± 0.060)		(± 0.38)	(± 14.6)	(± 5.9)
	1.0	0.819	–	8.06	72.3	49.8
		(± 1.014)		(± 0.26)	(± 10.6)	(± 6.1)
GL 2	0	0.028	25.5	8.44	70.2	47.6
(summer 1984)		(± 0.016)	(± 0.8)	(± 0.10)	(± 3.65)	(± 1.5)
	0.05	0.035	–	8.42	70.5	48.5
		(± 0.012)		(± 0.09)	(± 4.2)	(± 1.3)
	1.0	1.101	–	8.08	70.8	48.4
		(± 0.955)		(± 0.08)	(± 2.7)	(± 1.4)
GL 3	0	0.094	20.6	8.31	88.8	56.2
(fall 1984)		(± 0.228)	(± 1.9)	(± 0.12)	(± 2.3)	(± 0.7)
	0.05	0.087	–	8.27	88.3	55.5
		(± 0.109)		(± 0.09)	(± 2.6)	(± 0.8)
	0.50	0.504	–	8.20	87.1	59.7
		(± 0.286)		(± 0.05)	(± 2.6)	(± 1.1)
	1.0	0.975	–	8.14	88.3	56.1
		(± 0.299)		(± 0.02)	(± 2.3)	(± 0.7)
GL 4	0	0.020	17.2	8.17	68.0	44.4
(spring 1985)		(± 0)	(± 0.4)	(± 0.26)	(± 5.8)	(± 1.9)
	0.025	0.020	–	8.12	66.6	38.6
		(± 0)		(± 0.05)	(± 5.1)	(± 3.2)
	0.10	0.12	–	8.39	69.0	41.2
		(± 0.056)		(± 0.20)	(± 6.7)	(± 2.3)

In the fall 1984 and spring 1985 studies, the recovery of clams exposed to Zn for 30 d was determined. This recovery analysis also included snails in spring 1985. After the fall 1984 exposure period, clams were removed to flow-through fish hatchery troughs fed with New River water (Clark *et al.*, 1980) that had previously been colonized by algae to be used as a food source. The response parameters (cellulolytic activity and bioconcentration) were analyzed after 10, 20, and 30 d of recovery in clean water. After the spring 1985 exposure period, both snails and clams were allowed to recover in the same artificial streams that had previously been dosed with Zn for 30 d. Response parameters were analyzed as in the previous study.

Experimental design of enzyme analysis

Corbicula were collected from a population in the New River, Virginia (River mile 100) from fine sand sediment. *Mudalia* were removed from rocks 0.5 km upstream and across the river of the Glen Lyn Power Plant. Both clams and snails were immediately transferred to artificial stream systems following collection. After an acclimation period of 10–14 d, six clams and snails from each treatment were randomly chosen and transferred to the laboratory for dissection and weighing on days 0, 2, 5, 10, 20, and 30. Days 0, 2, and 5 were alleviated and 30 recovery days were added in later studies at Glen Lyn. Enzyme extracts from individuals were prepared from whole body homogenates. Samples were homogenized in 0.15 M phosphate buffer pH 6.0 at a wet mass to buffer ratio 0.2 g ml^{-1}. Homogenates were centrifuged for 15 min at $15000 \times g$. Supernatants (extracts) were decanted and the final extract volume recorded. Pellets were recovered for dry mass measurements. Two cellulase assays were used – a viscometric assay (Almin & Eriksson, 1967) and reducing sugar assay (Miller, 1959) – both using carboxymetholycellulose (CMC; Hercules type 7H3SF) as substrate. Details of these assays are reported by Sinsabaugh (1980) and Sinsa-

Table 3. Mean (± 1 SE) of selected water chemistry parameters analyzed during the ESL studies ($n = 4$).

Season	Target zinc concentration ($mg\ l^{-1}$)	Acutal total zinc concentration ($mg\ l^{-1}$)	Temp (°C)	pH	Hardness ($mg\ l^{-1}$ as $CaCO_3$)	Alkalinity ($mg\ l^{-1}$ as $CaCO_3$)
ESL 1 (spring 1984)	0	0.02 (± 0)	16.5 ± 0.8	7.88 ± 0.03	57.5 ± 1.1	27.6 ± 0.9
	0.05	0.092 (± 0.019)	–	7.87 ± 0.03	55.0 ± 1.8	28.7 ± 0.8
	1.0	0.984 (± 0.114)	–	7.82 ± 0.03	55.8 ± 2.4	29.1 ± 1.0
ESL 2 (winter 1984)	0	0.020 (± 0)	11.0 ± 0.03	7.7 ± 0.03	56 ± 1.8	27.0 ± 0.3
	0.05	0.090 (± 0.02)	–	7.7 ± 0.02	62 ± 2.4	28.8 ± 0.12
	0.50	0.531 (± 0.05)	–	8.05 ± 0.0	60 ± 1.2	28.2 ± 0.2
	1.0	0.980 (± 0.12)	–	7.3 ± 0.03	58 ± 2.4	30 ± 0.02
ESL 3 (spring 1985)	0	0.021 (± 0)	16.25 ± 0.23	8.05 ± 0.01	60 ± 1.2	23.1 ± 1.7
	0.025	0.020 (± 0.01)	–	8.1 ± 0	65 ± 2.4	32.0 ± 0.02
	0.10	0.070	–	8.06 ± 0.34	65 ± 0.0	33.3 ± 0.44

baugh *et al.* (1985). The viscometric assay measured endocellulase (1–4, endoglucanase) activity in units proportional to absolute activity. Reducing sugar production (mg glucose equivalents h^{-1}) reflects the synergistic action of all the enzymes responsible for cellulolysis. Soluble protein in extracts was measured by a colorimetric procedure (Bradford, 1976; Kley & Hale, 1977) using Coomassie blue dye (Bio-Rad Laboratories Technical Bulletin 1051, 1977). All assays were performed at 20 °C, and activity measurements for endo- and exo-products were reported as units/dry mass. Assays were conducted in triplicate on each of six individual snail and clam extractions. One unit of the enzyme is defined as the amount of enzyme required to liberate 1 mg of reducing sugar equivalent per hour using CMC as a substrate. Cellulase product indices were standardized to control activity levels on each day of examination and represent the mean of 18 separate measurements per individual.

Enzyme activity data (the cellulase product index) was analyzed by the Kruskal-Wallis Test, a one-way analysis of variance rank analogue (Hollander & Wolfe, 1973), to determine the effect of Zn on enzyme inhibition or activation for each sample day. If significant differences were indicated ($\alpha = 0.05$), a rank-like Least Significant Differences procedure was employed as a multiple range test to detect significantly different means.

Results

Artificial stream dosing

Zinc target concentrations were met for 0.05, 0.1, 0.5, and 1.0 mg l^{-1} in both field (Table 2) and laboratory (Table 3) studies. The 0.025 mg l^{-1} target was met in laboratory streams but fell below detection limits in field studies at Glen Lyn. At Glen Lyn, measured concentrations in the

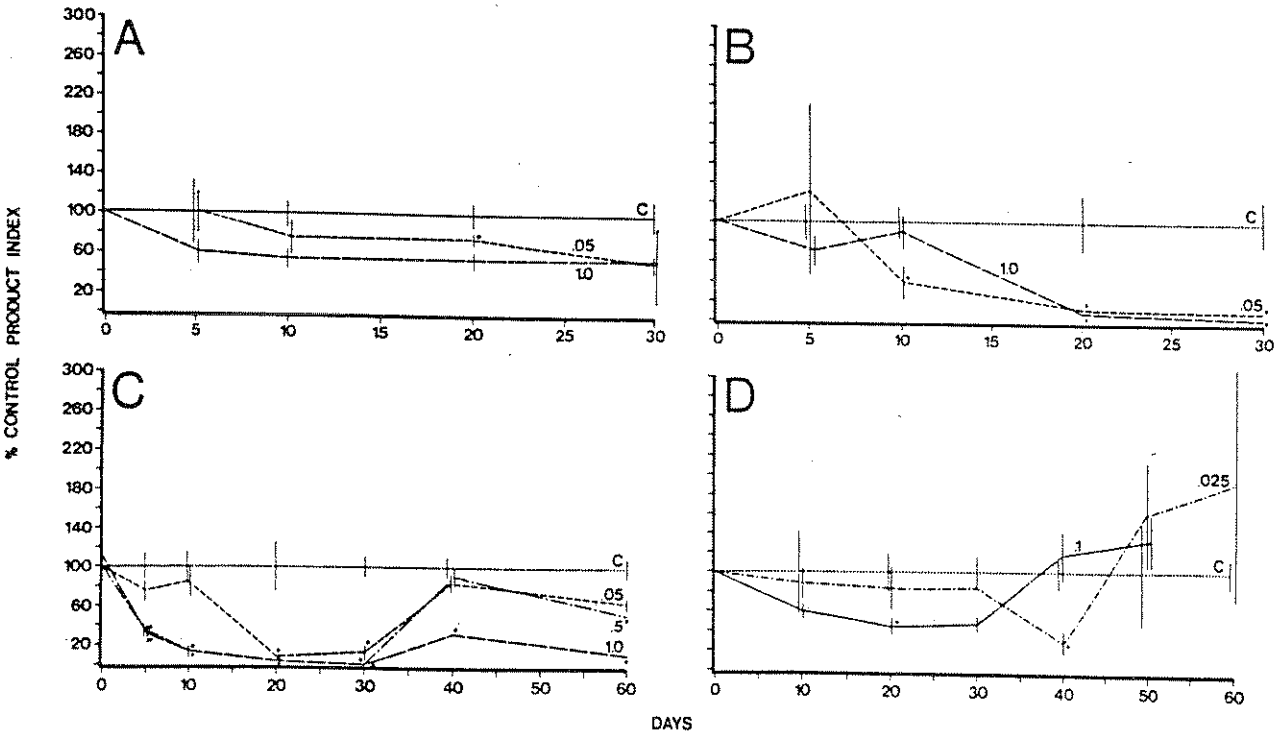

Fig. 2. Response of *Corbicula* cellulolytic complex to Zn in (A) spring 1984, (B) summer 1984, (C) fall 1984, and (D) spring studies at Glen Lyn field-laboratory. Data are relativized to the control product index on each day. Means ± 1 SE are given on each day. Means that are significantly different from the controls are indicated by an asterisk (*).

control and 0.05 mg l^{-1} treatments were not significantly different, although Zn measurements of the 0.05 mg l^{-1} target were within 70% of the calculated concentration (*i.e.*, 0.035 ± 0.012 [SE] and 0.038 ± 0.013 mg l^{-1} for Glen Lyn *vs* ESL streams, respectively). We assumed, for purposes of calculation, that 0.012 mg l^{-1} was present at all times if the concentration was below detection limits where 0.02 is one-half the detection limit. The effect of presuming a 0.012 mg l^{-1} baseline concentration always present in controls altered the outcome of the Kruskal-Wallis test. The high dose (1.0 mg l^{-1}) target had an average concentration of 1.101 ± 0.955 mg l^{-1}. The realized Zn concentrations at the ESL (1.130 ± 0.478 mg l^{-1}) were more consistent than those at Glen Lyn. All ESL treatments (0, 0.05, 1.0 mg l^{-1}) were significantly different from each other.

Dosing reproducibility for each Zn target concentration at Glen Lyn was established according to the Kruskal-Wallis test (Table 2). No significant differences within treatments (control, 0.05, and 1.0 mg l^{-1}) existed. The pattern was consistent for all experiments; therefore, for the purpose of citing realized Zn concentrations, all replicates were pooled for analysis.

Cellulolytic activity in Corbicula

Total clam enzyme activities as represented by the relativized exo- and endocellulase product indices declined both with high Zn doses and time over the course of most 30-d exposures (Fig. 2, 3). Declining cellulolytic activity in *Corbicula* was exposure-dependent. All Zn exposures were significantly different during summer 1984 and fall 1984 studies at Glen Lyn. During the spring 1984 and spring 1985 exposures at Glen Lyn, cellulolytic activity was significantly reduced by 0.1 and 1.0 mg l^{-1} Zn only on day 20 of exposure (Fig. 2). A decline in cellulolytic activity for exposure periods during all seasons was most often evident after 20 d of exposure and especially during fall 1984 for all days of exposure at 1.0 mg l^{-1}. Only in fall 1984 did high Zn exposures (0.5 and 1.0 mg l^{-1}) significantly reduce cellulolytic activity of clams as early as day 5, and their activity failed to recover to levels displayed for controls following 30 d in control water. Clams exposed to Zn levels ranging from 0.025 to 0.1 mg l^{-1}, however, did respond with increased cellulolytic activities following 10 to 30 d of recovery.

Patterns of decline in cellulolytic activity were less consistent in *Corbicula* exposed to Zn in the ESL compared to studies conducted at Glen Lyn (Fig. 3). Only in exposures conducted during spring 1984 did activities show any exposure-dependent response and only after 30 d of exposure. Product indices measured during the winter 1984 study had no discernible patterns of response to Zn. In the spring 1985 study, activity levels were significantly greater than that of controls in clams exposed to both 0.05 and 1.0 mg l^{-1} Zn.

Cellulolytic activity in Mudalia

Product indices for cellulolytic activity in *Mudalia* had consistent patterns of exposure-dependent responses to Zn in all exposures conducted at Glen Lyn (Fig. 4). Zinc exposures at 0.05–1.0 mg l^{-1} in the four seasonal tests caused cellulolytic activity to decline significantly below that of controls by day 10. However, 0.025 mg l^{-1} did not result in reduced activity. Enzyme activity levels of snails exposed to 1.0 mg l^{-1} Zn in the spring and summer 1984 studies had abrupt and significant declines prior to the onset of complete mortality by the following test day (days 20 and 30, respectively).

A significantly lower cellulolytic index prior to the onset of mortality was evident on day 10 for snails in the spring 1984 (ESL) study (Fig. 5). Enzyme activity was difficult to discern in the winter 1984 study due to the high mortality that occurred at all levels tested by day 20 with no surviving controls by day 30. Variations in cellulolytic activity during the spring 1985 study in *Mudalia* were similar to responses of *Corbicula* for that same study where no consistent patterns of increase or decline were evident.

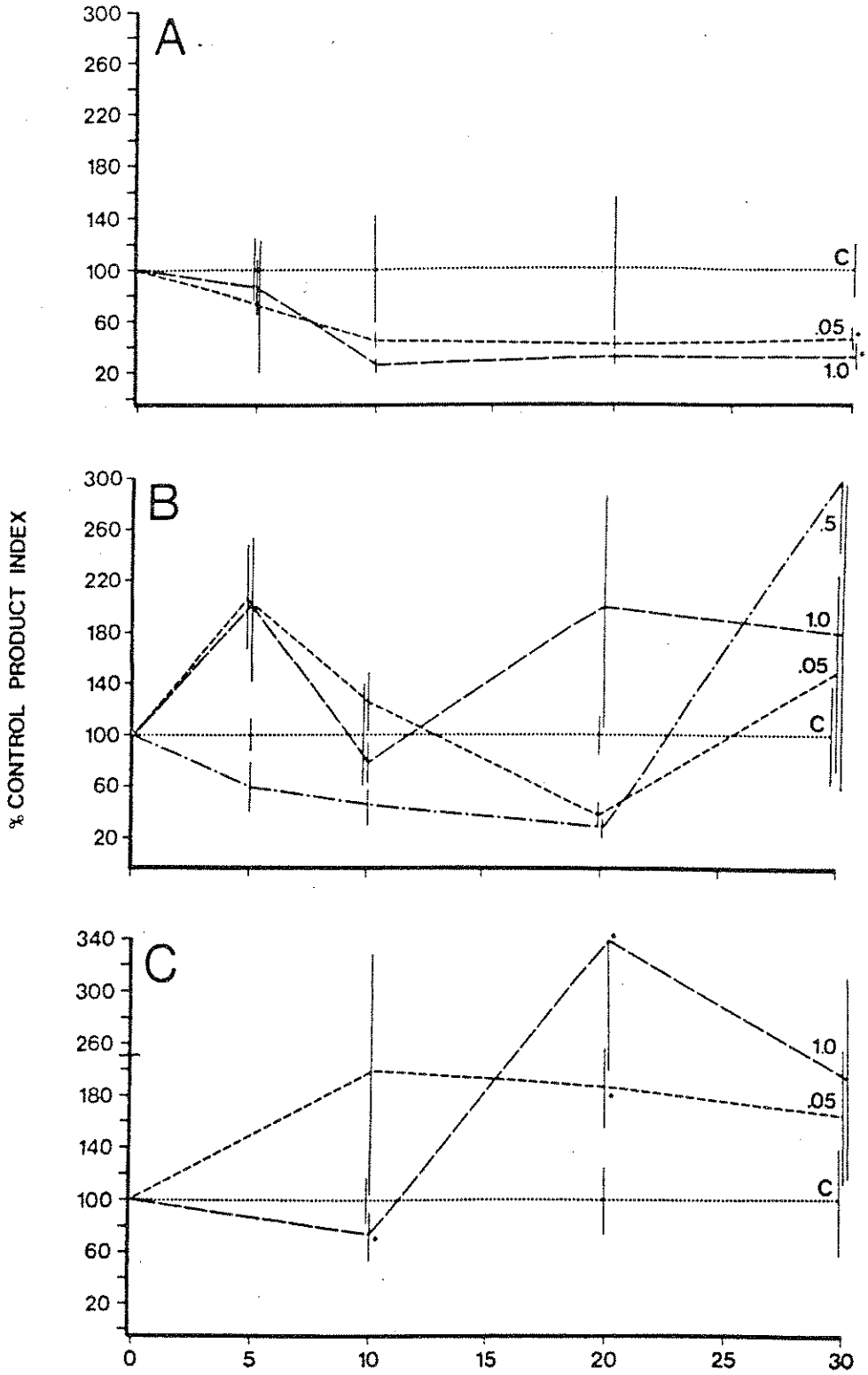

Fig. 3. Response of *Corbicula* cellulolytic enzyme complex to Zn in (A) spring 1984, (B) winter 1984, and (C) spring 1985 Ecosystem Simulation Laboratory studies. Data are relativized to the control product index on each day. Means ± 1 SE are given on each day. Means that are significantly different from the control are indicated by an asterisk (*).

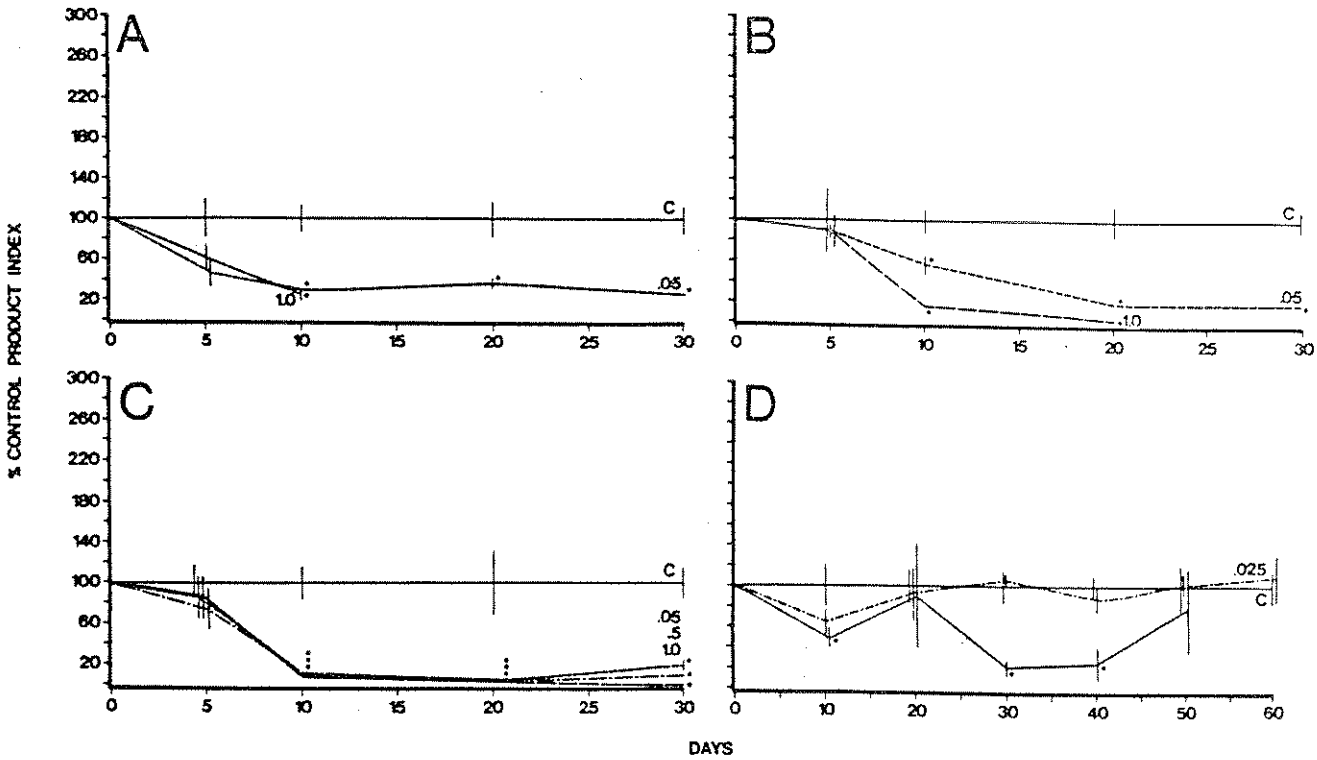

Fig. 4. Response of *Mudalia* cellulase enzyme complex to Zn in (A) spring 1984, (B) summer 1984, (C) fall 1984, and (D) spring 1985 exposures at Glen Lyn field-laboratory. Data are relativized to the control product index on each day. Means ± 1 SE are given on each day. Means that are significantly different from the control are indicated by an asterisk (*).

Zn accumulation

Accumulation of Zn in clams exposed to low level exposures (0.025 and 0.05 mg l^{-1}) was similar to that occurring in clams at higher exposures during the first 10 d of any study at Glen Lyn (Fig. 6). By days 20–30, high dosed clams (1.0 and 0.5 mg l^{-1}) continued to accumulate Zn when low-dosed clams (0.025 and 0.5 mg l^{-1}) failed to display continuing accumulation. This pattern was especially evident in the fall 1984 study for both 1.0 and 0.5 mg l^{-1} exposures. Background body burdens of controls were generally between 150–250 μg Zn g^{-1} dry weight. The highest accumulations observed were 1940 μg Zn g^{-1} at 1.0 mg l^{-1} on day 30 in fall 1984. Maximum accumulations for clams exposed to 0.05 mg l^{-1} concentrations ranged from 440 to 890 μg Zn g^{-1} (summer and spring 1984, respectively).

Accumulation patterns in snails at Glen Lyn showed that Zn uptake was more rapid than in *Corbicula*, with maximum total body burdens at higher exposures most often occurring by day 10 (Fig. 7). Snails failed to accumulate the metal at high Zn doses throughout the 30-d exposures while, at lower concentrations (0.025–0.1 mg l^{-1}), they most often had uptake patterns similar to *Corbicula*. Background body burdens for controls ranged from 108 to 391 μg Zn g^{-1}. The highest accumulations observed were 1403 μg g^{-1} at 1.0 mg l^{-1} on day 10 in fall 1984. Snails exposed to lower (0.05 mg l^{-1} Zn) concentrations accumulated Zn at moderately low levels, 401 to 516 μg g^{-1} (spring and fall 1984, respectively).

In the fall 1984 study at Glen Lyn, *Corbicula* quickly depurated accumulated Zn body burdens in 17 d at all levels of exposure after being transferred to streams not contaminated with Zn (Fig. 8). This depuration pattern was again evident at lower Zn exposures (0.025 and 0.1 mg l^{-1}) as early as 10 d following renewal with undosed river water into previously dosed artificial

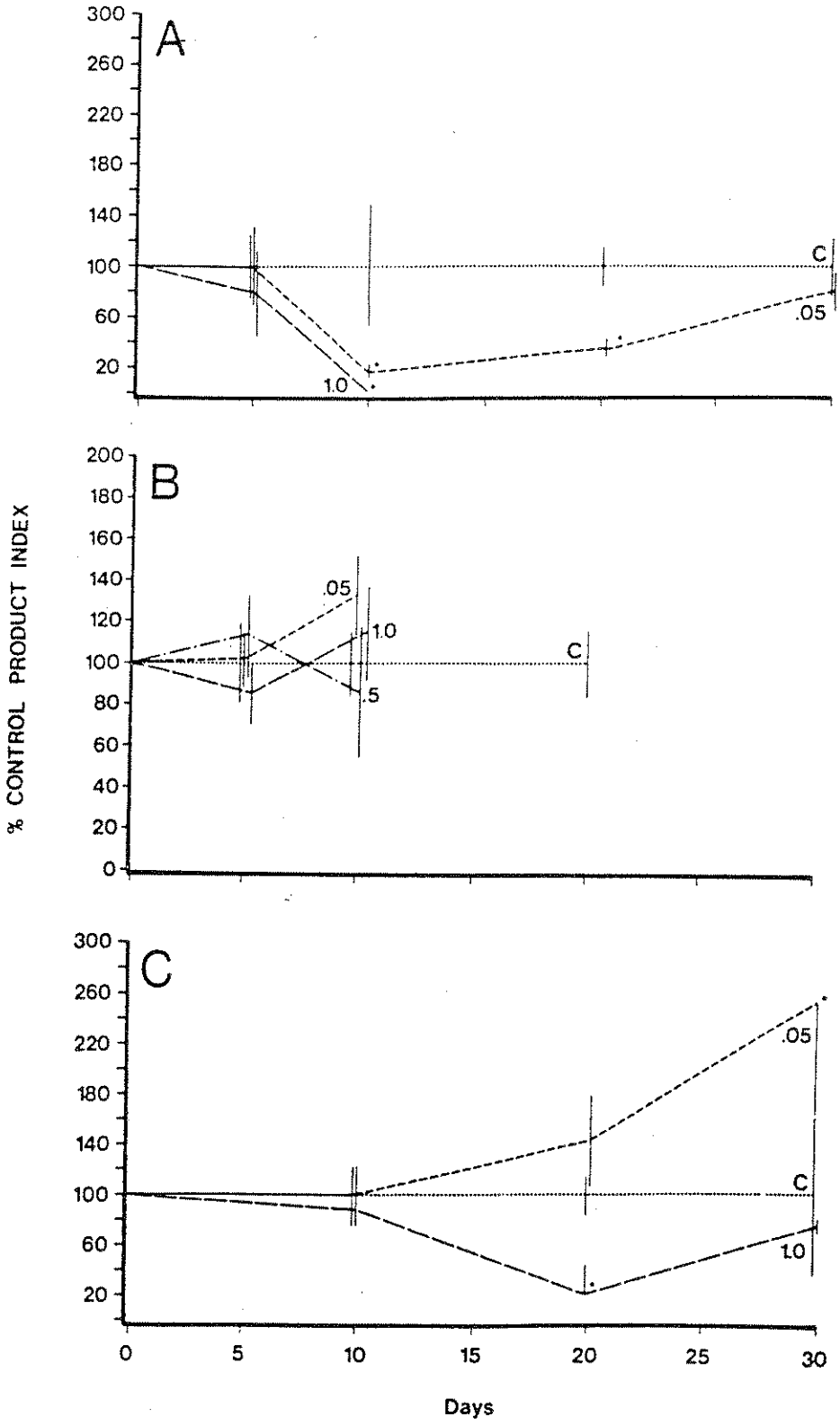

Fig. 5. Response of *Mudalia* cellulase enzyme complex to Zn in (A) spring 1984, (B) winter 1984, and (C) spring 1985 exposures at the Ecosystem Simulation Laboratory. Data are relativized to the control product index on each day. Means ± 1 SE are given on each day. Means that are significantly different from the control are indicated by an asterisk (*).

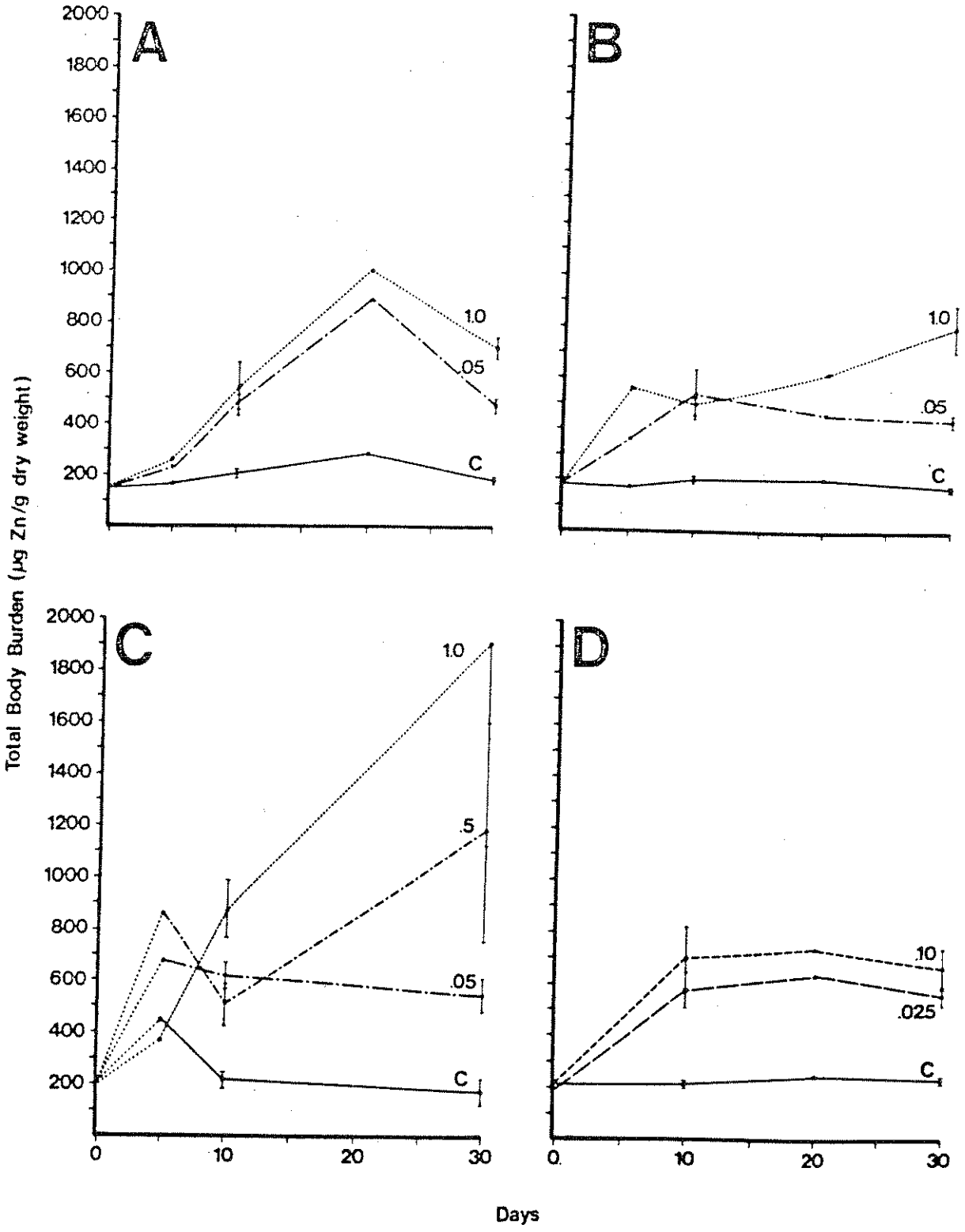

Fig. 6. Total body burden of Zn for *Corbicula* during (A) spring, (B) summer, and (C) fall 1984 and (D) spring 1985 Glen Lyn studies. Means ± 1 SE are given on days 10 and 30 of Zn exposures.

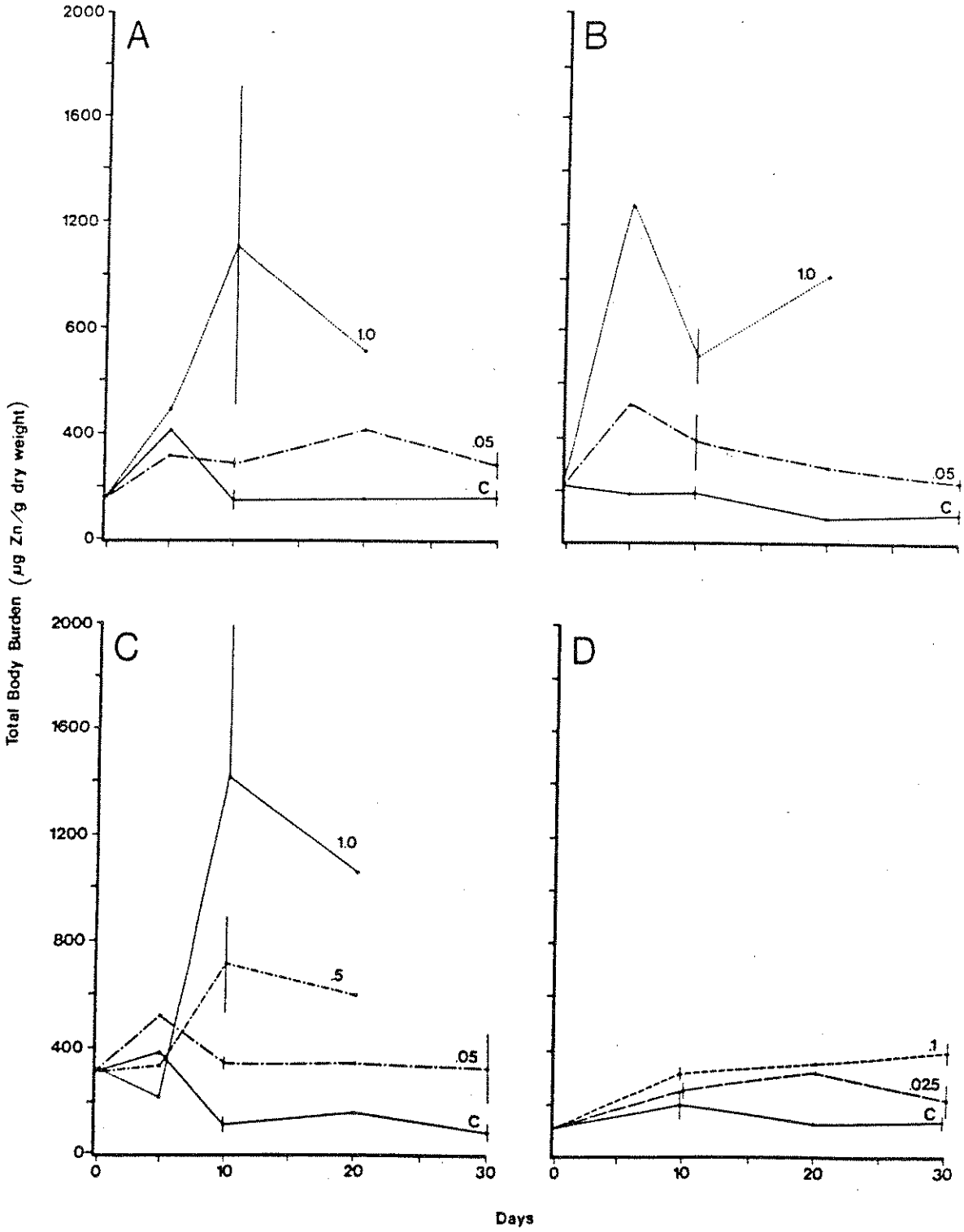

Fig. 7. Total body burden of Zn for *Mudalia* during (A) spring, (B) summer, and (C) fall 1984 and (D) spring 1985 studies. Means ± 1 SE are given on days 10 and 30 of Zn exposures.

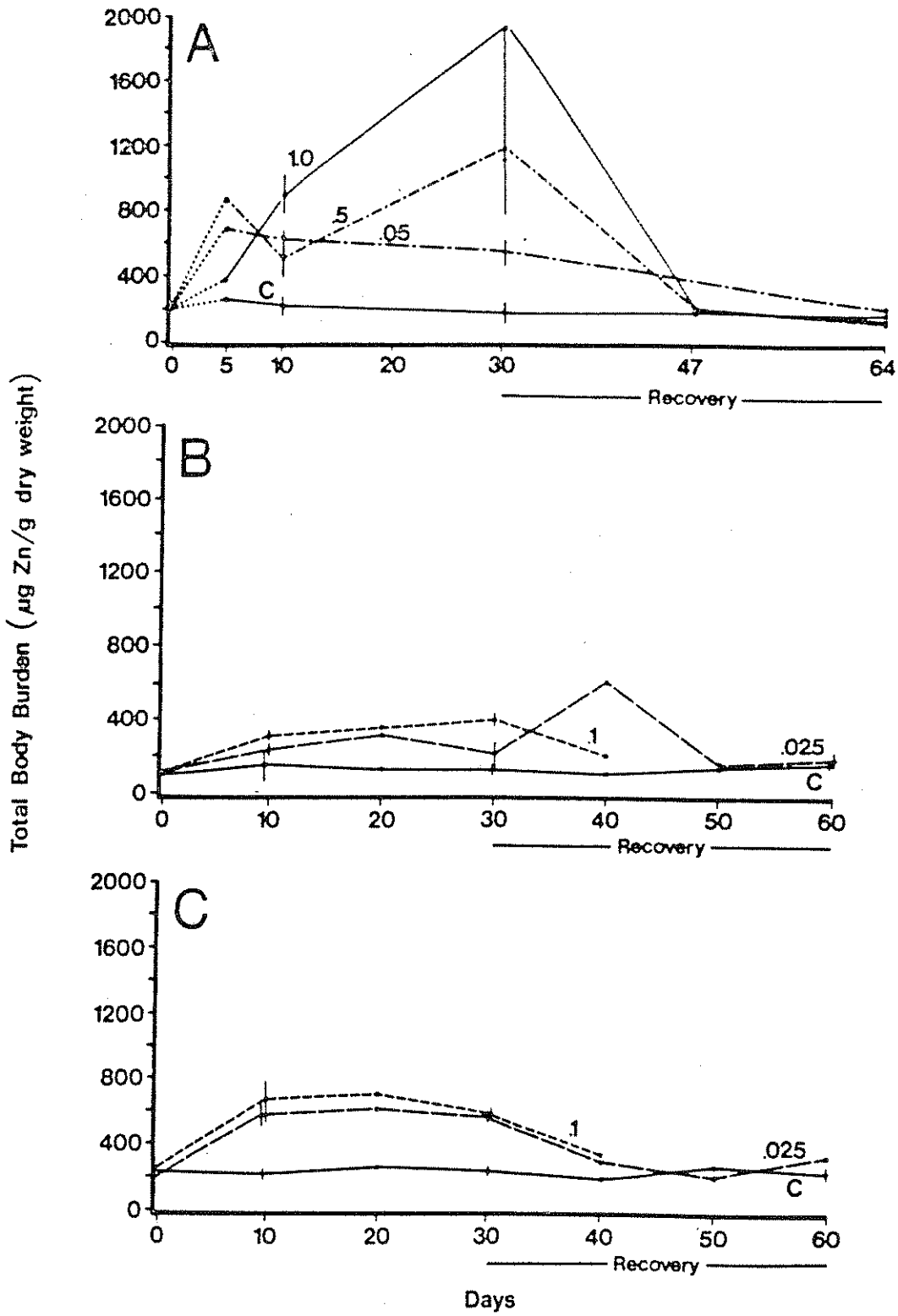

Fig. 8. Total body burden of Zn for *Corbicula* during recovery following exposures in (A) fall 1984 and (B) spring 1985 at Glen Lyn; total body burden of Zn for *Mudalia* during recovery following exposure in (C) spring 1985 at Glen Lyn. Means ± 1 SE are given on days 10, 30, and 60.

streams in spring 1985. *Mudalia* also depurated or removed Zn accumulation within 10 d following cessation of Zn exposure and subsequent addition of clean water. Depuration was considered complete after 20 d of recovery in the 0.025 mg l^{-1} concentration exposed snails.

Discussion

Cellulolytic activity

The utility of the cellulolytic product index as a general stress indicator was substantiated by the reduced enzyme activity found in both *Corbicula* and *Mudalia* as they responded to long-term Zn exposures in both laboratory and field-oriented studies. Both molluscs had significantly reduced enzyme activity at 0.05 mg l^{-1} and greater Zn exposures during all seasons tested. The clam enzyme activity response to this level has been compared to measurements of growth reported by Belanger *et al.* (1986b). While significant changes in enzyme activity were often apparent after 10 d of exposure, differences in growth at similar Zn exposure were comparable by 30 d.

From these field exposures, the calculated no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) were 0.025 and 0.050 mg Zn l^{-1}, respectively. These levels of Zn exposure bracket those considered to be protective of aquatic life during chronic exposure (0.047 mg l^{-1}) by the U.S. Environmental Protection Agency [USEPA] (1987). The first level of observed effect in our studies (0.05 mg l^{-1}) was comparable to the lowest values reported in the USEPA ambient water quality criteria for Zn (USEPA, 1987). Chronic toxicity data provided for nine freshwater species include two for invertebrates, ranging from 46.73 μg l^{-1} for *Daphnia magna* to >5000 μg l^{-1} for the caddisfly, *Clistoronia magnifica*.

Laboratory artificial stream exposures generally were inconclusive for indicating any direct correlations with Zn induced stress except to show that cellulase activity responded, suggesting stress from starvation in snail and clam populations by day 30 during long-term holding (Farris *et al.*, 1989). As data are relativized to the control product to aid in comparing days 0–30, reaction to exposure in ESL experiments appeared as an increase in enzyme activities in both *Mudalia* and *Corbicula* at sublethal levels. However, as in all ESL experiments, unrelativized activity of both endo- and exocellulase activity of control organisms showed very inconsistent trends. The cellulase assay was sensitive to relative health of control organisms in long-term studies and related the false impression of increased activities observed in exposed molluscs to the realized response of starved controls.

Dietary requirements

Dietary requirements were obviously met in the field-laboratory experiments where phytoplankton and suspended periphyton were transported to streams via river water. Consistent trends in cellulolytic activity were observed for control organisms of both species, but also agrees with simultaneous growth analysis performed upon *Corbicula*. Belanger *et al.* (1986b) found tissue and shell degrowth (as defined by Russell-Hunter, 1985) in control populations for the spring 1984 ESL study. Their work was able to show that algal growth within artificial streams, together with transported suspended phytoplankton, was able to support growth in *Corbicula* held in artificial streams at Glen Lyn. Dauble *et al.* (1985) has reported that a constant input of algae at 1000 cells ml^{-1} is required to support tissue and shell growth of *Corbicula* under flow-through laboratory conditions.

Researchers who have investigated growth responses of *Corbicula* in the laboratory (Dauble *et al.*, 1985; Foe & Knight, 1985, 1986; Belanger *et al.*, 1986a) have shown that dietary requirements for *Corbicula* are more specific than was once thought. Studies using *Corbicula* to examine toxicity in the laboratory (Hartley & Johnston, 1983; Harrison *et al.*, 1984; Belanger *et al.*, 1986a) or bioindicator potential (Graney *et al.*, 1983, 1984) may have failed to adequately quantify rela-

tive organismic health throughout all phases of long-term exposure. Effects on cellulolytic activity due to starvation in the laboratory, which complicated interpretation of effects from Zn exposure, were not apparent until days 20 to 30. This duration of exposure does not include the consideration of the initial state of unexposed *Corbicula* health that could occur at different periods of the year. For example, differences in energy allocations occurring in *Corbicula* tested in winter and summer have been shown to affect asbestos uptake as reflected in growth rates (Belanger *et al.*, 1986b).

Metal accumulation

Metal uptake and variations in cellulolytic activity in our studies were clearly more pronounced during certain seasons at comparable Zn exposures (0.05 and 1.0 mg l^{-1}). These differences may be due not only to the relative condition of the organisms tested at that time of year, but also to the seasonal variations in the trace metal uptake rates exhibited by the algae. A filter-feeder, such as *Corbicula*, accumulates metals not only from solution but also from inorganic particulates. The seasonal fluctuation in bioavailability is likely to be a composite of the changes occurring in both phases (Phillips, 1980). Further complications in interpreting toxicity data may be found in *Corbicula* that have reduced siphoning and growth when presented inorganic particulates (Foe & Knight, 1985). Doherty *et al.* (1987) have also warned that interpretation of valve closure behavior in relation to observed bioaccumulation processes should consider not only exposure concentrations and duration, but their effect on threshold phenomenon.

Although it is reported that most filter-feeders, such as the bivalve molluscs, will take up metals rapidly from solution or from food, the latter route is noted as most important for metals not having preference for particulate association (Phillips, 1976). This may in part help explain the striking contrast in responses occurring in laboratory Zn exposure systems in which dechlorinated water does not offer a continual renewed particulate or food resource, and in field-oriented systems where these phases most likely dominate uptake routes. The cellulolytic index was sufficiently sensitive to detect this difference in indicator species of differing trophic strategies. In addition, it supports the selection of these organisms and an enzymatic test so specific to feeding responses known to be a dominant uptake mechanism for Zn in molluscs (Phillips, 1980; Simkiss & Mason, 1983).

There is now obvious evidence that body loads of metals among molluscan herbivores, suspension feeders, carnivores, and detritivores are obtained via various routes (Pentreath, 1973; Young *et al.*, 1977). Forstner & Whittman (1979) have endorsed filter-feeding bivalves as the most suitable indicator among molluscs; however, they did stress that the gastropods can relate the uptake of heavy metals within a food chain in which the species can be at any of the various trophic levels (phytophagous, deposit-feeding, and carnivorous). In comparing the uptake of Zn and accompanying cellulolytic activity in a grazer (e.g., *Mudalia*) with that of a suspension filter-feeder (e.g., *Corbicula*), it was apparent from Glen Lyn exposures that effects from uptake via attached algae were more pronounced earlier in the studies. In these systems, algae accumulated up to 5000 μg Zn g^{-1} dry weight at 0.05 mg l^{-1} and 60 000 μg Zn g^{-1} dry weight at 1.0 mg l^{-1} (Farris *et al.*, 1989). Graney *et al.* (1983), in field studies that exposed *Corbicula* to Zn concentrations from 0.2–0.8 mg l^{-1} for 30 d, indicated that a strong relationship between accumulation and exposure level did not exist. No consideration in their study and others was given to alternative exposure routes where algae, particulates, or sediment may have served as factors affecting exposure levels.

Belanger *et al.* (1986a) have noted that algae played a role in the observations of total Zn in water, growth responses, and calculated nominal Zn concentrations. Algal communities in their field studies accumulated Zn and increased in biomass through exposure periods to the point that it was difficult to detect Zn in water. Growth rates of *Corbicula*, however, were able to provide

evidence in this study that Zn was available to clams, although not as measured in the water.

Metal depuration

Accumulations of Zn by algae at Glen Lyn most likely contributed to the maximum Zn accumulations that occurred by day 10 in snails exposed to high concentrations (0.5–1.0 mg l^{-1}). Accompanying significant declines in cellulolytic activity always accompanied those periods of maximum accumulation. Periods following maximum uptake in snails reflected a trend in depuration or loss of metal accumulation prior to the onset of mortality. However, the cellulolytic activity during these periods continued to remain depressed. This trend also existed for both snails and clams at lower level Zn exposures (0.05 mg l^{-1}) during all seasons. Only during those examined recovery periods were both clams and snails able to sustain recovered cellulolytic activity following metal depuration and only at lower Zn exposure concentrations tested (0.025 and 0.05 mg l^{-1}).

Graney *et al.* (1983) found no significant differences in the body burdens of clams exposed to 0.2 and 0.4 mg l^{-1} Zn. At these levels, they predicted that *Corbicula* was effectively able to regulate Zn uptake and/or excretion. Clams and snails in our studies also effectively regulated Zn body burdens at lower levels of Zn exposure (0.025 and 0.05 mg l^{-1}). The false impression of regulation of Zn accumulation being directly related to relative health of an organism was portrayed by snails as depuration and/or excretion often occurred before the onset of high mortality and declined cellulolytic activity in three Glen Lyn studies (spring and summer, 1984 and fall, 1985). However, depuration of Zn in clams on day 20 in the spring 1984 and spring 1985 studies corresponded with nonsignificant differences in cellulolytic activity occurring on day 30 following earlier periods of significant decline.

The inability of snails to depurate Zn effectively in the first 10 d in comparison to the clams delayed responses of uptake and declining cellulolytic activity most often occurring later by day 20 has suggested that snails acted as more suitable indicators of short-term stress, while clams were better suited for examination of long-term effects. These differences can in part be attributed to the ability of molluscs to isolate their tissue from the environment for extended periods of time. Snails have been shown to cease feeding, slough tentacles, initiate fleeing responses, retract the foot, close the operculum, or become paralyzed in response to toxicant presence (Burris *et al.*, 1990). This behavioral avoidance mechanism can be affected by a whole host of natural parameters (Bayne, 1976). In addition, Phillips (1980) notes that bivalves most often react to toxicants by incorporating changes in valve adduction with adjustments in filtration rates, as well as depressing normal burrowing behavior. All these adjustments are pertinent to affecting the accumulations of metals by the different routes previously mentioned. These behavioral modifications could have also affected the inconclusive results obtained from our laboratory exposures with clams.

Foster-Smith (1975) and Davis (1964) have demonstrated that suspended algae will increase filtering rates of bivalves. The absence of sufficient available food in laboratory experiments not only removed an uptake route but may have also reduced exposure frequency in *Corbicula*. This again reinforces the requirement for adequate feeding regimes for accumulation studies using molluscs as well as a better understanding of those levels of metals known to elicit these behavioral modifications through various uptake routes.

In conclusion, the cellulolytic product index was able to effectively serve as a general stress indicator for Zn exposure studies using both *Corbicula* and *Mudalia* at all Zn levels and seasons tested. The effect of uptake routes, behavioral modifications, and selection of specific species for use as bioindicators for the examination of Zn uptake in molluscs are all of critical importance to the qualification of effective monitoring of heavy metal discharges. Our study further supports the use of site-specific field systems together with *in situ* or laboratory studies for evaluating more sensitive functional tests to qualify indicator potential of selected organisms.

Acknowledgements

We gratefully acknowledge the help of Darla Donald and Teresa Moody for editorial and preparation duties rendered. This research was supported by the American Electric Power Service Corporation, Columbus, Ohio.

References

Almin, K. E. & K. E. Eriksson, 1967. Enzymatic degradation of polymers: I-Viscometric method for the determination of enzymatic activity. Biochem. Biophys. Acta 139: 238–247.

American Public Health Association, American Water Works Association & Water Pollution Control Federation, 1980. Standard methods for the examination of water and wastewater. Washington, D.C., 1193 pp.

Bayne, B. L., 1975. Aspects of physiological condition in *Mytilus edulis* with special reference to the effects of oxygen tension and salinity. In H. Barnes (ed.), Proceedings 9th European Marine Biology Symposium. Aberdeen University Press, Aberdeen: 213–238.

Bayne, B. L., 1976. Marine mussels: Their ecology and physiology. Cambridge University Press, Cambridge, 506 pp.

Belanger, S. E., 1991. The effect of dissolved oxygen, sediment, and sewage treatment plant discharges upon growth, survival and density of Asiatic clams. Hydrobiologia 218: 113–126.

Belanger, S. E., D. S. Cherry & J. Cairns, Jr., 1986a. Uptake of chrysotile asbestos fibers alters growth and reproduction of Asiatic clams. Can. J. Fish. aquat. Sci. 43: 43–52.

Belanger, S. E., J. L. Farris, D. S. Cherry & J. Cairns, Jr., 1986b. Growth of Asiatic clams (*Corbicula* sp.) during and after long-term zinc exposure in field-located and laboratory artificial streams. Arch. envir. Contam. Toxicol. 15: 427–434.

Bio-Rad Laboratories Technical Bulletin 1051, 1977. Bio-Rad protein assay. Bio-Rad Laboratories, Richmond, CA.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248–254.

Burris, J. A., M. S. Bamford & A. J. Stewart, 1990. Behavioral response of marked snails as indicators of water quality. Envir. Toxicol. Chem. 9: 69–76.

Clark, J. R., J. H. Rodgers, Jr., K. L. Dickson & J. Cairns, Jr., 1980. Using artificial streams to evaluate perturbation effects on aufwuchs structure and function. Wat. Res. Bull. 16: 100–104.

Dauble, D. D., D. S. Daly & C. S. Abernathy, 1985. Factors affecting growth and survival of the Asiatic clam, *Corbicula* sp., in controlled laboratory conditions. In R. D. Cardwell, R. Purdy & R. C. Bahner (eds), Aquatic Toxicology and Hazard Assessment. American Society for Testing and Materials, Philadelphia, PA: 134–144.

Davis, C., 1964. The influence of suspensions of microorganisms of different concentrations on the pump and retention of food by the mussel (*Mytilus edulis* L.). Neth. J. Sea Res. 2: 233–249.

Doherty, F. G., 1990. The Asiatic clam, *Corbicula* spp., as a biological monitor in freshwater environments. Envir. Monit. Assess. 15: 143–181.

Doherty, F. G. & D. S. Cherry, 1988. Tolerance of the Asiatic clam, *Corbicula* spp., to lethal levels of toxic stressors – A review. Envir. Pollut. 51: 269–313.

Doherty, F. G., D. S. Cherry & J. Cairns, Jr., 1987. Valve closure responses of the Asiatic clam *Corbicula fluminea* exposed to cadmium and zinc. Hydrobiologia 153: 159–167.

Farris, J. L., 1986. Cellulolytic responses to heavy metal accumulation in *Corbicula fluminea* and *Mudalia dilatata*. Ph.D. Thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Farris, J. L., J. H. Van Hassel, S. E. Belanger, D. S. Cherry & J. Cairns, Jr., 1988. Application of cellulolytic activity of Asiatic clams (*Corbicula* sp.) to in-stream monitoring of power plant effluents. Envir. Toxicol. Chem. 7: 701–713.

Farris, J. L., S. E. Belanger, D. S. Cherry & J. Cairns, Jr., 1989. Cellulolytic activity as a novel approach to assess long-term zinc stress to *Corbicula*. Wat. Res. 23: 1275–1283.

Foe, C. & A. Knight, 1985. The effect of phytoplankton and suspended sediment of the growth of *Corbicula fluminea* (Bivalvia). Hydrobiologia 127: 105–115.

Foe, C. & A. Knight, 1986. Growth of *Corbicula fluminea* (Bivalvia) fed artificial and algal diets. Hydrobiologia 133: 165–174.

Foe, C. & A. Knight, 1987. Assessment of the biological impact of point source discharges employing Asiatic clams. Arch. envir. Contam. Toxicol. 16: 39–51.

Forstner, U. & G. T. W. Whittman, 1979. Metal pollution in the aquatic environment. Springer-Verlag, New York, 486 pp.

Foster-Smith, R. L., 1975. The effect of concentration of suspension on the filtration rates of pseudofaecal production for *Mytilus edulis*, and *Cerastoderma eduli* (L.) and *Venerupis pullastra*. J. exp. mar. Biol. Ecol. 17: 1–22.

Genter, R. B., D. S. Cherry, E. P. Smith & J. Cairns, Jr., 1987. Algal-periphyton population and community changes from zinc stress in stream mesocosms. Hydrobiologia 153: 261–275.

Giesy, J. P., R. L. Graney, J. L. Newsted, C. J. Rosiu, A. Benda, R. G. Kreis, Jr. & F. J. Horvath, 1988. Comparison of three sediment bioassay methods using Detroit River sediments. Envir. Toxicol. Chem. 7: 483–498.

Goldberg, E. W., U. T. Bowen, J. W. Farrington, G. Harvey, J. H. Martin, P. L. Parker, R. W. Risebrough, W. Robertson, E. Schneider & Gamble, 1978. The mussel watch. Envir. Conserv. 5: 1–25.

Graney, R. L., D. S. Cherry & J. Cairns, Jr., 1983. Heavy metal indicator potential of the Asiatic clam (*Corbicula fluminea*) in laboratory artificial streams. Hydrobiologia 102: 81–88.

Graney, R. L., D. S. Cherry & J. Cairns, Jr., 1984. The influence of substrate, pH, diet and temperature upon cadmium accumulation in the Asiatic clam (*Corbicula fluminea*) in laboratory artificial streams. Wat. Res. 18: 833–842.

Harrison, F. L., J. P. Knezovich & D. W. Rice, Jr., 1984. The toxicity of copper to the adult and early life stages of the freshwater clam, *Corbicula manilensis*. Arch. environ. Contam. Toxicol. 13: 85–92.

Hartley, D. M. & J. B. Johnston, 1983. Use of the freshwater clam *Corbicula manilensis* as a monitor for organochlorine pesticides. Bull. envir. Contam. Toxicol. 31: 33–40.

Hollander, M. & D. A. Wolfe, 1973. Nonparametric statistical methods. J. Wiley & Sons, New York.

Johnston, J. B. & D. M. Hartley, 1981. Vivalves as monitors for persistent pollutants in marine and freshwater environments. In S. M. Somani & F. L. Cavender (eds), Environmental Toxicology. Charles C. Thomas Publishers, Springfield, IL: 184–198.

Kley, H. V. & S. Hale, 1977. Assay for protein by dye binding. Anal. Biochem. 81: 485–487.

Kosinski, R. J., 1989. Artificial streams in ecotoxicological research. In A. Boudou & F. Ribeyre (eds), Aquatic Ecotoxicology: Fundamental Concepts and Methodologies, Volume I. CRC Press, Boca Raton, FL: 297–316.

Miller, G. L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analyt. Chem. 31: 426–428.

O'Donnel, J. R., B. M. Kaplan & H. E. Allen, 1985. Bioavailability of trace metals in natural waters. In R. D. Cardwell, R. Purdy & R. C. Bahner (eds), Aquatic Toxicology and Hazard Assessment. American Society for Testing and Materials, Philadelphia, PA: 485–501.

Pentreath, R. J., 1973. The accumulation from water of ^{65}Zn, ^{54}Mn, ^{58}Co and ^{59}Fe by the mussel *Mytilus edulis*. J. mar. biol. Ass. U.K. 53: 127–143.

Phillips, D. J. H., 1976. The common mussel *Mytilus edulis* is an indicator of pollution by zinc, cadmium, lead and copper. II. Relationship of metals in the mussel to those discharged by industry. Mar. Biol. 38: 71–80.

Phillips, D. J. H., 1977. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments – A review. Envir. Pollut. 13: 281–317.

Phillips, D. J. H., 1980. Quantitative aquatic biological indicators: Their use to monitor trace metal and organochlorine pollution. Applied Science Publishers Ltd, London, 488 pp.

Poulsen, E., H. V. Rijsgard & F. Molenberg, 1982. Accumulation of cadmium bioenergetica in the mussel *Mytilus edulis*. Mar. Biol. 68: 25–29.

Russel-Hunter, W. D., 1985. Physiological, ecological and evolutionary aspects of molluscan tissue degrowth. Am. Malacol. Bull. 3: 213–222.

Simkiss, K. & A. Z. Mason, 1983. Metal ions: Metabolic and toxic effects. In P. W. Hochachka (ed.), The Mollusca: Environmental Biochemistry and Physiology. Academic Press, New York: 101–164.

Sinsabaugh, R. L., 1980. Distribution of Microbial and Macroinvertebrate Cellulolytic Activity in Relation to Leaf Processing in a Headwater Stream. M. S. thesis, Virginia Polytechnic Institute and State University, Blacksburg, 119 pp.

Sinsabaugh, R. L., A. E. Linkins & E. F. Benfield, 1985. Cellulase digestion and assimilation by three leaf-shredding aquatic insects. Ecology 66: 1464–1471.

U.S. Environmental Protection Agency, 1987. Ambient water quality criteria for zinc – 1987. EPA-440/5-87-006, Washington, D.C.

Viarengo, A., M. Pertica, G. Mancinelli, S. Palmero, G. Zanicchi & M. Orunesu, 1982. Evaluation of general and specific stress indices in mussels collected from populations subjected to different levels of heavy metal pollution. Mar. Envir. Res. 6: 235–243.

Young, D. R., D. McDermott-Ehrlich & T. C. Heesen, 1977. Sediments as sources of DDT and PCB. Mar. Pollut. Bull. 8: 254–257.