CELLULOLYTIC RESPONSES TO HEAVY METAL ACCUMULATION IN CORBICULA FLUMINEA AND MUDALIA DILATATA

by

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(ABSTRACT)

Cellulolytic responses of the Asiatic clam, Corbicula fluminea and a snail, Mudalia dilatata, to selected constituents of power plant effluents (i.e., zinc, cadmium, acidic and alkaline pH, individually and paired) were investigated in 30-day exposures. Exposures were conducted in both laboratory and field-oriented artificial streams and then validated in the river receiving system of a power plant. Cellulolytic activity was reduced by laboratory and field exposures to cadmium and zinc at all levels tested from 0.012 to 0.10 mg cadmium/L and generally at 0.025 to 1.0 mg zinc/L. Clams detected acute lethal levels of metal and used valve closure as an avoidance mechanism for 14 days. Snails, however, did not effectively avoid exposures and were more sensitive to acute stress during all exposures. These behavioral responses were corroborated by both cellulolytic activity and metal accumulation.

Measurements of cellulolytic activity for both test species in laboratory exposures differed from those in field

artificial streams. Reduced enzyme activity in controls by day 30 was attributed to artificially induced stress associated with the laboratory environment. This factor precluded any analysis of laboratory responses for periods of exposure longer than 20 days as well as recovery analysis. Field-oriented artificial streams provided a sufficient environment to adequately assess long-term stress and recovery as measured by cellulolytic activity and metal accumulation in both clams and snails. Enzyme activity responded to metal exposure with respect to both degree and duration of exposure.

Cadmium and zinc combined exposures caused significantly reduced cellulolytic activity at the same concentration as those for cadmium alone. Reduced enzyme activity caused by cadmium and zinc addition at levels that were not detectable suggested that the cellulolytic index was sensitive to sublethal stressors. This was supported by metal uptake patterns in clams and snails. Cellulolytic activity responded to zinc addition at alkaline and acidic pH in a manner that supported pH optima for cellulases and bioavailability of metals.

Effects seen in macroinvertebrate assemblages (diversity, richness, and similarity) were compared with cellulolytic activity of caged Corbicula from a site specific power plant discharge. Enzyme activity inhibition was the most sensitive indicator measured. Reductions in cellulolytic activity at stations monitored for total zinc content were consistent

with effects seen at comparable exposures to zinc in field-located artificial streams. A zinc concentration of 0.05 mg/L consistently caused the first significant reductions in cellulolytic activity. This concentration is comparable to the U.S. Environmental Protection Agency's Water Quality Criteria value (0.047 mg/L zinc) for protection of aquatic life.

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CHAPTER ONE: INTRODUCTION

CRITERIA DEVELOPMENT

A growing number of industries are now being required by state regulatory agencies to implement biological and chemical monitoring programs which will effectively detect ecological impact of their discharges on receiving stream biota. The resulting data must then be evaluated and compared with what one would hope to be scientifically defensible criteria. Criteria are to be quantities and qualities based on scientific determinations that are testable. Standards derived from criteria should result from local or national political, economic and social influences to allow adequate information for implementation. However, it quickly becomes apparent that present water quality criteria offer inadequate quidance for conversion of local standards. This is understandable in that nationwide standards cannot include evaluation of the wide variability in environmental and biological factors known to exist. But until November 1980 the policy of presumptive applicability was applied in the U.S. Environmental Protection Agency review of standards; that is, the EPA water quality criteria were presumably applicable to all waters. This policy further assumed that an organism would receive the measured concentration at chronic or lifelong

exposure and that all the measured forms of the contaminant would be available to cause adverse effects (Lee and Jones 1983). It was further implied that water quality standards adopted by the states were to be at least as strict as the EPA numeric criterion for that parameter. Thus, the National Pollutant Discharge and Elimination System (NPDES) discharge limits, as well as most state water quality standards, are derived using results from a limited number or type of standard laboratory bioassays. These are then being directly applied to field situations without regard to the availability of contaminants or duration of exposure that may affect an organism's response in the field. Utilization of an inflexible approach to criteria development is reason enough for most legal and administrative challenges put forth to the EPA. These challenges question overly conservative standards proposed by state regulatory agencies, as well as demanding more thorough consideration of the EPA's new policy direction toward site-specific conversion.

EPA has since abandoned its presumptive applicability policy, and responsibility for water quality protection has now been placed upon state governments. With this shift from federal to state responsibility comes the expectation that more consideration be given to the appropriateness of single-compound laboratory bioassays as applied in the past. It is also hoped by state agencies that municipalities, industry, and agricultural interests will not only monitor lo-

cal physical, chemical, and biological factors characteristic of their receiving system, but that gathered site specific information can be used to make better application and interpretation of bioassay results. It may then be possible to derive site-specific water quality criteria (U.S. EPA, 1983). Approaches to criteria derivation assume that differences in toxicity values of specific material determined in laboratory water and site water may be attributed to chemical and/or physical factors altering biological availability and/or toxicity of materials. Also, that selected test species can integrate differences in biological availability and/or toxicity of materials and that some sensitive measurement of their physical, biochemical or behavioral response can reflect a direct measure of the capacity of a site water to increase or decrease toxicity values relative to values obtained in laboratory water. Inherent difficulties with measuring both the bioavailability of a contaminant in two systems as well as detecting the organisms response (e.g., mortality, loss of reproductive potential, growth or subtle physiological characteristics) are not as restrictive as the very fact that such single chemical criteria address effects in the absence of other pollutants in the water column. Most often a chemical of interest is usually one of many components in an effluent which may affect the chemical's biological availability and/or toxicity (Carlson and Roush 1985).

The most recent criteria (U.S.EPA 1985) for heavy metals has at least included empirical formulations considering hardness in establishing realistic estimates of "no effect" levels. This is a step forward in recognizing the important role water chemistry and toxicant concentration can play. However as Szumski and Barton (1983) note, the criteria conversion process for metals is far more complicated than the simple hardness analysis supporting these criteria. Their mechanistic model of the toxicity of metals would include the role that pH, temperature, alkalinity, hardness, and dissolved oxygen play in determining the apparent acute toxic concentration and then formalize those chemical and physiological processes responsible for variations in bioassay test results.

In the development of aquatic life criteria for any particular toxicant there is most often little agreement on the acute and chronic effect thresholds. Since the 1970's investigators have focused on those physiological and histological differences occurring in test mortalities that might elucidate factors affecting test variability. Even in the widely used Daphnia acute 48-h toxicity test that so many agencies employ because of its standardization, there is now growing concern regarding control survival and test repeatability (Lewis and Weber 1984). The current trend to replace Daphnia with the smaller, more ubiquitous Ceriodaphnia reflects increasing concern with the sensitivity of test

organisms to particular toxicants, test temperatures, and pH excursions (Cowgill et al. 1985).

These concerns are of fundamental importance for the validity of traditional acute and chronic test methods. Unfortunately the bulk of our present toxicological data base relies heavily upon acute responses, and the resulting median lethal concentrations that have skewed the water quality criteria do not always guarantee interpretation of thresholds for chronic effects. Although many materials might not exhibit thresholds, those that do at exposures that are subthreshold should not cause any "unacceptable effect" on aquatic organisms and their uses according to the recently revised Water Quality Criteria Guidelines (USEPA 1985). Interpretation of the Criterion Continuous Concentration (CCC) is intended to be a good estimate of this threshold of unacceptible effect that considers duration of exposure under fluctuating conditions. Further improvements on interpretation of existing numerical criteria will include a four-day averaging period which is intended to prevent increased adverse effects on sensitive life stages by limiting the durations and magnitudes of exceeding the CCC. For interpretation of averaging periods for Continuous Maximum Concentrations (CMC) (USEPA 1985), it is advised that concentrations above a CMC should not exist for as long as one hour. One hour represents an appropriate averaging period

based upon tests of high concentrations conducted in 48 to 96 hours.

Although existing criteria have significantly improved our approach to effluent regulation and demanded a more thorough treatment of acute toxicity testing with certain species, they have not resulted in a unified theory of toxic action for specific toxicants and consequently have not provided adequate data for criteria development. There now exists a growing demand for more reliable acute (Kimerle et al. 1985) and economically feasible chronic tests (Birge et al. 1985) as the EPA moves toward greater emphasis upon duration of exposure and gives more consideration to site-specific validation. Although the strategy of always placing single-species toxicity tests at the fore-front of sequential testing is under current scrutiny (Cairns 1983) single species testing will undoubtedly remain the most reliable source of data upon which numerical criteria will be based for years to come. Until the present data base can include results of such novel testing methods using analysis of multispecies, community or ecosystem reactions to perturbants, development of testing protocols must rely upon current test methods that exhibit low variability in measurements of survival rates, growth rates, and biochemical and physiological changes. Additional credibility from these improved existing methods can then be supportive for more realistic application to site-specific testing.

BIOCHEMICAL INDICATORS

Neff (1985) has critically evaluated the utility of biochemical tests for diagnosis of acute or chronic pollutant stress in fish. Those tests include analysis of toxification/detoxification systems, biochemical composition of blood and tissues, and blood and tissue enzyme activity. Many of these tests have been further examined for potential use with amphibians, aquatic birds, aquatic mammals, and invertebrates (Payne 1984). The detoxification systems examined thus far include the metallothioneins for detoxification of metals and the mixed-function oxygenase system for detoxifying certain hydrophobic organic chemicals. Chronic changes in biochemical composition of blood and other tissues in response to sublethal concentrations of toxic chemicals may result in one or more pathological conditions. Persistent pollutant stress has been found to exhaust certain endocrine pathways, deplete and redistribute tissue stores of catabolic substrates, such as glycogen and lipid, and of micronutrients involved in detoxification and resistance to stress, such as glutathione, vitamins A, C, E, and several B vitamins (Thomas 1982a,1982b; Neff 1985). Also, direct damage to cellular structure and or integrity of tissues and organs such as liver or kidney may result in release of tissue biochemicals into the blood or failure to remove waste products from the blood. Neff (1985) concludes that the ma-

jor difficulty in using biochemical parameters lies in the necessity to correlate changes in composition from stress in small groups of fish with impending adverse effects such as reduced growth or fecundity in the entire population. However, he does note that some enzyme activities and biochemical parameters do change in predictable ways in response to *in vivo* exposure of fish to certain pollutants. It is these responses, having been correlated with certain impairments of biological function and integrity, that show potential for diagnostic use in assessing fish populations.

Those natural exogenous and endogenous factors producing an influence on biochemical parameters must also be evaluated in order to classify those biochemical changes as either general or specific stress indices (Viarengo et al. 1982; Hodson 1983). General stress indices reveal a syndrome characteristic of an aquatic organism's response to a wide variety of environmental stressors, including pollutants and natural, physical and biological factors (e.g.temperature, salinity, season, age, sex, stage in reproductive cycle, and starvation). These generalized responses that are an integral part of more sensitive tests are also affected by procedural as well as biological and environmental factors, and their influence should be assessed and minimized by appropriate procedures and sampling designs (Hodson 1983). Specific stress indices are selected to reflect only responses to specific classes of contaminants, such as heavy metals or

organic compounds. As in Viarengo et al. (1982), increased concentrations of low molecular weight thionein-like metal-binding proteins in mussels was adopted as a specific stress index directly linked to presence of heavy metals in sea water. Variations in the rate of protein synthesis, amino acid uptake and RNA synthesis served as general stress indices. Supportive evidence from general stress indices has proven that specific stress indices alone are insufficient to evaluate confinement stress due to capture and handling in both field and laboratory studies (Neff 1985). This is shown with elevated levels of blood cortisol and glucose in fish that occurs within ten minutes of capture or handling (Thomas et al. 1980).

Those blood and tissue enzymatic changes examined thus far have resulted in attributing catalytic activity changes from pollutants to one of the following catagories as outlined by Neff (1985);

(a) direct interaction between enzyme and pollutant,

(b) a secondary response to a pollutant-mediated change in the concentration of an enzyme modulator (including oxygen),

(c) attempts by an organism to mitigate cellular damage resulting from exposure,

(d) leakage of cellular enzymes away from normal activity sites due to membrane damage, and

(e) pollutant-mediated depletion of micronutrient (enzyme cofactor) or buildup of a metabolic waste product.

ENZYME EFFECTS

The fact that many chemicals affect metabolism of aquatic organisms by altering normal enzyme activity, is now a well-known phenomenon (Brown 1976; Bitton 1982; Christensen 1982; Neff 1985). Information exists for both inorganic and organic inhibitory effects on selected enzymes (Hochster and Quastel 1963). Some interactions involve a high degree of inhibition of specific enzymes. This case has been presented for example with the inhibition of α-aminoleuvilinic acid dehydratase by lead salts (Goldstein 1972) and carbonic anhydrase by actazolamide (Wyeth and Prince 1977). Other interactions affect the activity of many enzymes thereby resulting in an ultimate debilitating effect from a variety of nonspecific biochemical malfunctions (Christensen 1982).

Due to this apparent variety of responses, Hodson (1984) has suggested there are no reliable enzyme assays to diagnose specific contaminant exposures to fish. He notes the reasons for this are that enzyme assays are not always sensible, sensitive and specific. There has been some success with enzyme assays when used to study the effects of toxicants on enzymatically controlled processes and these correlated with ecological-effect tests or biogeochemical tests involving nutrification, cellulose decomposition and sulfate reduction (Bitton 1981). But as the demand for correlation between *in vitro* systems for rapid screening and toxicogenesis *in vivo*

is stressed, confidence in enzymes as candidates for monitors waines. Since exposure to a range of stressors does affect enzyme activity, it has been thought that enzymes are logical candidates to use as biomonitors. However, overemphasis on rapid-screening *in vitro* enzyme assays to replace whole-animal test systems may be inappropriate because they remove any interaction involving nonspecific biochemical malfunctions, detoxification mechanisms and behavioral avoidance of the whole organism. For these reasons sensible enzyme assays must be tested to ensure that *in vitro* responses are detectable *in vivo* (Jackim et al. 1970; Gergart and Carlson 1978, Jackin et al. 1978) and measured activities should correspond to degree of contaminant exposure and toxicity. Hodson (1983) further emphasizes that sensitive enzymes should detect *in vivo* contaminant exposures at concentrations within the normal range of contaminated environments during chronic exposure. Also, responses should precede overt pathology and be statistically sound allowing assessment of a minimum sample size necessary to diagnose exposure or injury.

Greater emphasis upon sensitive enzyme assays that are correlated to *in vivo* responses demands greater consideration of fates of potentially hazardous chemicals and those exposure pathways whereby organisms will be affected. Such an example of specific enzyme selection is provided in much of Brown's (1976) examination of the action of heavy metals on

enzymes concerned with intermediary nitrogen metabolism. He states that the EC50 (effective concentration to yield 50% enzyme activity) value for Hg^{2+} is dependent upon the state of the purity of the enzyme tested. Mercury is known to form very strong complexes with sulfhydryl groups and therefore reducing its effective concentration attached to inhibitory sites on the enzyme studied while in the presense of other proteins. Investigators should for this reason take care in extrapolating from inhibition values with enzymes ,to some parameter such as LC50 values from intact animals. That the aquo-, hydroxo-, bicarbonate-,and organo-ligands can form and reform in a dynamic state and thereby affect the specific toxicity each has, further implies that analysis of enzyme inhibition from metal exposure must consider those chemical parameters existing not only in the water column or substrate but also at cell surfaces particularly gill, and gill and respiratory membranes (Christensen 1972). If more regard is given to those pathways of metal uptake involving contributions of food (Willis and Sunda 1984) factors such as food type and rate of consumption will also affect exposure levels once considered within the organism. Here the composition of the phytoplankton community at the base of the food chain may control levels of trace metals influencing metal accumulation within herbivorous communities.

CELLULOSE DEGRADATION

Cellulose is a major structural component of plants and is the most common microfibrillar component in the algae (Round 1975). In its native crystalline state, cellulose exists in hydrophobic molecules assembled into microfibrils. These cellulose microfibrils are then linked by hydrogen and covalent bonds to other structural components of plant cell walls (Preston 1979). Cellulose comprises about one-third of all the carbon dioxide fixed by plants and is the most abundant of all naturally occurring structural polysaccharides (Linkins et al. 1983). The fundamental importance of cellulose to the energy flow of streams may be the rate at which this refractory structural polymer can be hydrolyzed and assimilated by micro- and macroconsumers (Sinsabaugh et al. 1985). Studies concerning the assimilation of cellulose in freshwater macroconsumers have dealt largely with shredder utilization of detrital structural molecules (Bjarnov 1972; Martin et al. 1980; 1981a,1981b; Barlocher 1982; Sinsabaugh et al. 1985). While it has been demonstrated that stream invertebrates can derive nutritional benefit from plant cell wall polysaccharides, it is still unclear as to how efficiently cellulose is degraded and by which cellulose-degrading enzyme system this is accomplished. Sinsabaugh et al. (1985) outline three potential sources of cellulolytic enzymes associated with animals: tissue- level

synthesis, acquired microbial symbionts, and acquired (e.g. ingested fungal carbohydrases) enzymes from diet.

While it has been suggested that the presence of specific carbohydrase activity would be indicative of substrates most often consumed, this has been found not to be the case with consumers studied thus far. Comparative studies on carbohydrate degradation have been conducted on ecologically comparable species by a number of workers. Nielsen (1962) made qualitative comparisons of carbohydrases in 34 soil and litter invertebrates, B. O. Nielsen (1966) in 6 wrack invertebrates, Kristensen (1972) on 19 species of marine detritivores, and Yoko and Yasumasu (1964) investigated the presense of cellulase in seventy-four animal species. Monk (1976) concluded from all these surveys and from work by Bjarnov (1972) that there is a lack of correlation with diet and carbohydrase presence in aquatic invertebrates. Within certain taxa (e.g. the Crustacea, Mollusca and Annelida) the occurrence of cellulase appears to be universal, with sporadic occurrence found in Insecta. To date, there is wide acceptance of the presence of a cellulase system in many phyla of invertebrates. However, the physiological and ecological significance of the presence of weak cellulolytic activity in so many groups, remains to be further elucidated.

The degradation of crystalline cellulose is a complex process requiring the participation of many enzymes from the functional group known as cellulases. Reese et al. (1977)

first suggested a route for the conversion of native cellulose to soluble sugars based on a two-step sequential process (Fig. 1A). It was on the basis of observations made with actively growing fungi that the C_1 - C hypothesis was first formulated. Years later it is still work with cellulolytic microorganisms where the mechanisms of cellulose degradation are being determined. Most recent evidence supports the mechanism as described by Pettersson (1975) as follows.

(a) Regions of low crystallinity in the cellulose fibre are attacked by endo-glucanases and free chain ends are created.

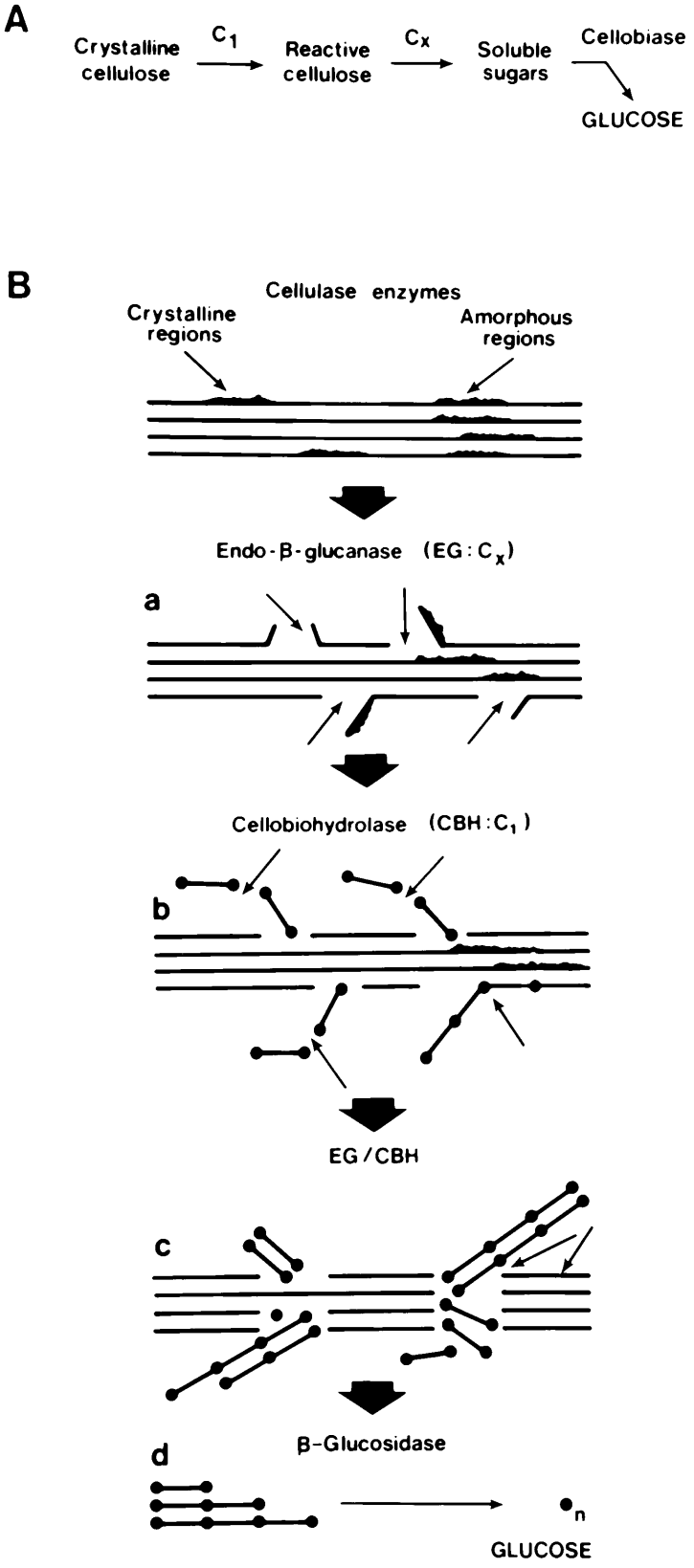
(b) Exo-glucanases start the degradation from the chain ends by hydrolytically removing cellobiose.

(c) Cellobiose is hydrolysed to glucose through the action of β-glucosidase.

As presented in Figure 1B, it is now understood that the endo-glucanases (endocellulases) act randomly over the cellulase chain and the exo-glucanases (exocellulases) act on exposed chain ends by splitting off cellobiose or glucose, with the endo- and exoglucanases having a strong synergistic action.

Agnisola (1981) has referred to this synergistic activity of the enzymes involved in the hydrolysis of cellulose to glucose as "cellulolytic activity" whereas cellulase as used in the literature indicates one of these enzymes in particular. While it is known that cellulose hydrolysis requires the synergistic activity of at least three destinct types of

Figure 1. The suggested route of cellulose conversion to glucose according to Reese's (1977) C_1-C_x hypothesis (A) and according to the synergistic action of enzymes in cellulolysis (Bisaria and Ghose 1981)(B).


A
Crystalline cellulose
C1
Reactive cellulose
Cx
Soluble sugars
Cellobiase
GLUCOSE
B
Cellulase enzymes
Crystalline regions
Amorphous regions
Endo-β-glucanase (EG:Cx)
a
Cellobiohydrolase (CBH:C1)
b
EG/CBH
c
β-Glucosidase
d
n
GLUCOSE

enzyme components (C_1, C , and cellobiase), the first or C_1 component which "activates" or deaggregates native cellulose to increase its suseptibility to hydrolysis by the other two, is the least understood. Consequently, much of the work as relates to cellulolytic activity in invertebrates has relyed on use of carboxymethylcellulose (CMC) as a soluble derivative of native or "crystalline" cellulose.

Several authors have noted that hydrolysis of CMC is not evidence that native cellulose is being hydrolyzed (Monk 1976; Martin et al. 1981). However, as Kesler (1982) has previously stated in his work with aquatic insects, the presence of CMCase indicates the ability to break β-1-4 glycosidic bonds. Snails with high cellulase activity have been found to efficiently assimilate Scenedesmus, a green alga which has a thick cellulose wall (Calow and Calow 1975; Kesler and Tulou 1980). Crosby and Reid (1971) also reported that cellulolytic activity in the Bivalvia corresponded with the level of cellulose in the food. Further support for the presence of an active cellulase system in mollusks is given by Agnisola (1981) who confirmed the existence of a complete cellulolytic system containing the C_1 component necessary for degrading native cellulose in mollusks already described as having β-1,4-glucanase activity on CMC. Even carnivorous gastropods and bivalves were found to have a "fossil" cellulase enzyme system following transition from herbivorous to carnivorous diet.

One cannot draw any general conclusions concerning cellulase distribution for all mollusks, however for those groups examined activity levels have been relatively high and often able to degrade native cellulose. Cellulose appears to be digested intracellularly and extracellularly in bivalves (Crosby and Reid 1971). Extracellular digestion is affected by cellulase secreted by digestive diverticula and the crystalline style in addition to exogenous enzymes from gut microflora.

STATEMENT OF HYPOTHESIS

The following hypothesis has been experimentally examined through the objectives listed for this research: A significant decline in cellulolytic activity in molluscs dosed with a range of sublethal heavy metal concentrations can be detected within 10 to 30 days.

OBJECTIVES

The objectives of this research were to evaluate cellulase enzyme activity in a benthic filter feeder, the Asiatic clam (*Corbicula fluminea*) and a grazer (*Mudalia dilatata*) as an indicator of long-term stress due to metal exposure. Two species of differing trophic levels were used to distinguish the effects of behavioral adaptation and food uptake upon

metal accumulation. These parameters are considered to determine a "no effects" concentration for long-term exposure to selected heavy metals.

EXPERIMENTAL DESIGN

Molluscs were chosen as test organisms due to presence of a complete cellulolytic system (Agnisola 1981) in addition to their usefulness as bioassay test organisms (Holcombe et al. 1984) and in situ field monitors for heavy metals (Graney et al. 1983). Both the Asiatic clam and the snail were resident species of the New River selected as test organisms in the field artificial stream studies. Both species are easily collected and maintained in laboratory and field artificial streams.

Adult clams (15-17mm) and snails (12-15mm) were exposed to levels of zinc sulfate (.025-1.0 mg/L) and cadmium sulfate (.012-0.10 mg/L) during 30-day long-term exposures in laboratory and field oriented artificial streams. Cellulolytic activity was determined on whole animal homogenates and compared to metal accumulation occurring in like individuals under the same exposure conditions. Use of the cellulase index as an indicator to heavy metal stress was analyzed with respect to duration (Days 0, 2, 5, 10 , 20, 30 inspection) of exposure, recovery from exposure and interaction of pH, and dual metal combinations. Exposures in laboratory and

field artificial streams were conducted to test the ability of these mollusks to integrate differences in the biological availability of metals as reflected in variations of the cellulase response.

RELEVANCE OF THIS RESEARCH

A statistically defensible enzymatic test that accounts for responses occurring as metals bioaccumulate can be used to examine recovery capabilities of molluscs. Molluscs have been shown to depurate metals by numerous modes (Bryan 1976). Since environmental exposures are rarely at fixed concentrations for defined periods as purported by the majority of current toxicity testing procedures (Buikema 1982), any test method sufficiently sensitive to intermittent or episodic exposure and concomitant dilution would allow a more realistic evaluation of perturbant effects. Demonstration of an *in situ* organismic response to metal exposure measured by a sufficiently sensitive cellulase index further supports enzymes as likely candidates for inclusion into biochemical parameters that may serve as indicators of stress (Brown 1976; Christensen et al. 1982; Simkiss 1982).

Examination of the inhibition of cellulases in mollusks is relevant to a more thorough examination of the degree of metal uptake that can be attributed to uptake routes via food and sediment. Routes of uptake involving particulate frac-

tion, food or sediment sources are dependent on the feeding strategies and behavioral adaptations of mollusks already found pertinent to the monitoring of heavy metal contaminants (Johnston and Hartley 1981; Simkiss and Mason 1982). There is even growing concern that existing data on the toxicity of metals to aquatic organisms fail to address those compartments where metals are complexed with organics and bound to sediments (Adams et al. 1985). Only a fraction of the total metal species present is "seen" by organisms (O'Donnel 1985). Criteria pertinent to this "seen" amount will depend upon a better understanding of those effects of metal speciation and the effects of various management practices (i.e. affecting temperature, pH, bioturbidity) on the speciation (Staples et al. 1985).

Evaluation of cellulolytic activity as a useful diagnostic tool is addressed. Hodson (1983) has defined sensible enzyme assays as inexpensive, simple, rapid, and should be chosen according to the known metabolic or enzymatic effects of contaminants. This method is evaluated as to its ability to detect *in vivo* responses to metal exposures at concentrations comparable to those found in contaminated environments and often at chronic exposure. These test conditions are fundamental to the development of data for estimating the Maximum Acceptable Toxic Concentration (MATC) or estimate of a no-effects concentration in revisions of the U.S. Numerical National Water Quality Criteria (1985). Although enzymatic

tests, in general, are not yet widely accepted methods for screening hazardous substances, they can add credibility to data generated for site-specific criteria development (USEPA 1983). Buikema et al. (1982) have warned of the dangers that exist in relying too heavily on overly standardized tests for every answer to regulatory decisions. Toxicity tests intended to answer questions about specific receiving systems may be permitted to deviate substantially from standardized methods if test conditions are dictated local water quality parameters.

This research will aid in further evaluation of a novel approach for the application of biochemical tests to the examination of long-term metal stress. Site-specific field validation using appropriate test organisms will lend additional credibility to those trends in criteria development that question standardized single species toxicity tests for testing all hazardous substances without regard to fate and effects.

CHAPTER TWO: CORBICULA CELLULASE AND GROWTH

INTRODUCTION

Use of bivalve molluscs as indicators of heavy metal pollution is well documented (Bayne et al. 1979; Johnston and Hartley 1981; Viarengo et al. 1982; Graney et al 1984). Some species are noted as being well suited as biomonitors of particular metals (Johnston and Hartley 1981), because they are known to have poor regulatory mechanisms affecting rates of metal uptake (Bryan 1976). Fundamental assumptions involving epithelial uptake systems as specific, energy dependent, saturable processes, deserve more critical testing (Simkiss and Mason 1982: Simkiss et al. 1982). Apart from those systems recognized in certain molluscs that enable metal loss by diffusion, excretion, and leucocyte storage and ejection (Bryan 1976), there are unanswered fundamental questions. These include mechanisms by which molluscs concentrate metals, the effects of certain environmental parameters (e.g., water hardness, pH, temperature) on metal uptake, and how these relationships influence toxicity in different species employed in monitoring.

One species that is currently being investigated for its suitability as an indicator of heavy metal pollution is the Asiatic clam, Corbicula fluminea (Phillips 1977: Graney et

al. 1983; Graney et al. 1984). Its tolerance to pollution, ability to bioaccumulate, resistance to desiccation, and hardiness in the laboratory makes it particularly appealing for use in monitoring studies as well as chronic bioassays. Graney (1983) noted that Corbicula possesses the necessary qualities to be an effective biological indicator as defined by Phillips (1977). While Graney et al.(1983) were able to predict environmental concentrations of Cd, Cu, and possibly Zn as demonstrated by the rate of metal uptake and BCFs, they do admit that more consideration must be given to fluctuation of environmental parameters (e.e., TSS, pH, temperature) before appropriate interpretation of BCFs can be included in data analysis. Two of Phillips (1977) ten requirements for choosing a biomonitor place emphasis on a simple correlation between metal content of an organism and average concentration of the surrounding water. However, the assumption that turnover and accumulation of trace metals is via water only has been refuted (Willis and Sunda 1984). It is now thought that metals are most likely acquired from both food and water. Uptake via food may account for avoidance behavioral adaptations associated with feeding, siphoning time, and metal content of food and substrate. While consideration of uptake routes other than water are not as restrictive to the qualification of Corbicula as an effective bioindicator of heavy metal stress, it does place greater emphasis on experimental designs incorporating more environmental realism

than has been included in many *in situ* or laboratory bioconcentration studies.

A growing body of literature dealing with the effects of substrate, temperature, pH, and diet on the responses of *Corbicula* to a range of toxicants further supports more intense investigations considering routes of metal uptake other than via solution alone.

Adding credibility to the use of *Corbicula* as a bioindicator of metals will depend upon the inclusion of better functional tests that can detect subtle physiological changes associated with accumulation and release of metals by tissues. Tests sufficiently sensitive to differences in environmental parameters, behavioral modifications and different metal uptake routes mentioned previously will provide greater environmental relevance.

Much of the information on inhibitory effects of inorganic and organic substances on enzymes suggests that enzyme systems of aquatic organisms are conducive to environmental monitoring (Brown 1976; Christensen et al. 1982). However, any attempt to simply associate changes in enzyme activity with an environmental pertubation, does not necessarily qualify the enzymatic reaction as an adequate measurement of stress. But by combining classical sublethal measurements of stress, (i.e., changes in growth, metal accumulation, and reproductive potential) with changes in enzyme activity, more reliable predictions can be made relating degree of enzyme

activation or inhibition with degree and duration of perturbant exposure.

The importance of the accumulation of metals through food vs water has been stressed by a number of authors (Pentreath 1973; Martincie et al. 1984). An enzyme directly involved with digestion in a suspension feeder like Corbicula could indicate subtle physiological changes resulting from metal accumulation. The cellulase enzyme complex responsible for producing soluble oligosaccharides, cellobiose, or glucose from cellulose is widely distributed among Mollusca (Yokoe and Yasumasu 1964; Elyakova 1972). Information is also available on the inhibition of cellulases by metals, although the bulk of the work deals with crude preparations from plant, animal, and microbial sources (Mandels and Reese 1965; Ferchak and Pye 1983).

Previous research by Graney et al. (1983) revealed that laboratory studies are insufficient to examine all environmental parameters that influence metal accumulation in Corbicula. Accumulation data from site-specific artificial streams has been more applicable to evaluation of Corbicula as a monitor. An improved artificial stream design was used in this study to examine cellulolytic activity and its relation to zinc accumulation and growth in Corbicula. Parallel 30-d tests were conducted in artificial streams under controlled laboratory conditions and at a field site receiving river water. Possible stress factors associated with

behavioral avoidance and laboratory maintenance of Corbicula during chronic testing are examined as they relate to evaluation of biomonitoring with bivalves.

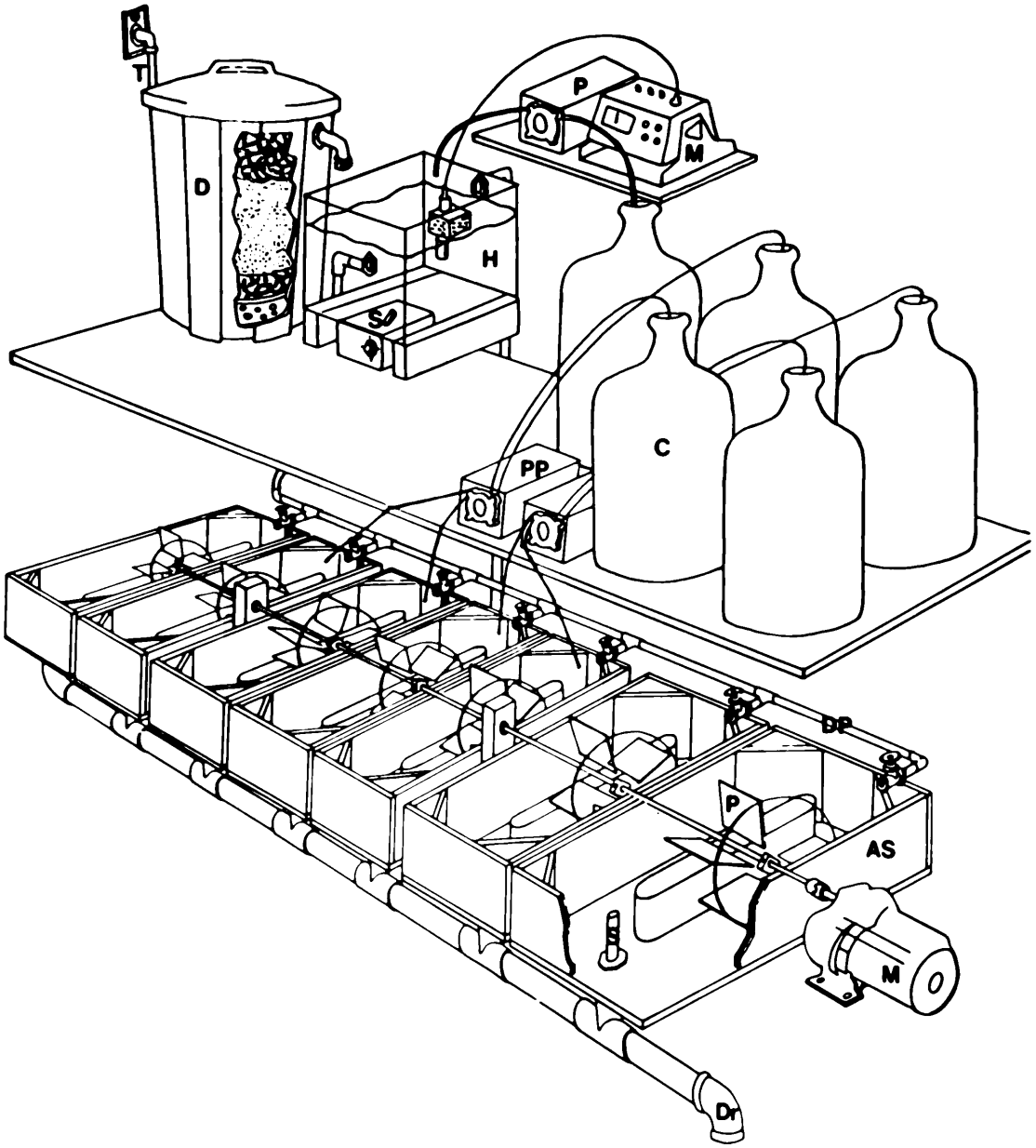
MATERIALS AND METHODS

DESCRIPTION OF ARTIFICIAL STREAMS

Thirty-day chronic exposures were carried out at an outdoor site-specific field laboratory at Glen Lyn, Virginia with untreated New River river water (pH approximately 8.3) and at the Virginia Polytechnic Institute and State University (Va Tech) Ecosystem Simulation Laboratory (ESL) with dechlorinated tap water (pH approximately 7.8). A series of oval, paddle driven streams, suitable for either field or laboratory applications, was designed (Fig. 2). Surber and Thatcher (1963) describe a similar system constructed of stainless steel tanks and paddle wheels. Our design utilizes 16 mm exterior grade plywood and 25 x 150 mm shelving for simplifying construction and reducing total costs. Streams were 76 x 33 x 15 cm with a capacity of 13 L. Joints were sealed with silicon sealant and surfaces treated with three coats of chemical and moisture resistant enamel paint.

Dechlorinated, charcoal-filtered tap water or natural stream water from an on-site submersible pump in the New River entered each system via a headbox. Gravity provided

Figure 2. Schematic representation of artificial stream and metal dosing system showing dechlorination (D), headbox (H) on stirrer (S), peristaltic pump (P) with pH meter controller (M), carboys (C) containing zinc pumped (PP) to streams (AS) with paddle wheels (P), motor (M), standpipe (S), diluent pipe (DP) and drain (Dr).


T
D
P
M
H
S
C
PP
DP
P
AS
M
Dr

constant pressure through a headbox drain pipe that led to each of the streams. Drain pipes, constructed of 19-mm schedule 40 PVC pipe, were fitted with 19-mm straight valves that allowed regulation of water flow to each stream. Inflow rates were checked daily at the ESL and every other day at the Glen Lyn site. Experience showed that only minor adjustments in flow rates to the streams were necessary each monitoring period. At an inflow rate of 0.5 L/min, retention time was ca. 26 min.

A plexiglass headbox was used to adjust pH at the ESL before the water entered the artificial streams. Regulation of influent pH at the ESL was regulated by a Fisher Model 650 pH meter/controller and a Cole Parmer peristaltic pump that delivered a 1% sulfuric acid solution into the headbox. Mixing was ensured by a magnetic stirrer positioned beneath the base of the headbox. Stream water depth was regulated by 19-mm diameter PVC standpipes mounted in bulkhead and male CPVC adapters. Current was provided by a series of plexiglass paddle wheels attached to a 10-mm steel rod powered by a 1/4 h.p. continuous-use motor.

In order to increase the environmental realism of the outdoor Glen Lyn streams, coarse sand sediment (2 cm in depth) was added (83% of the sediment was 2.5-9.0 mm in diameter); whereas, no sediment was added to the ESL streams. At Glen Lyn natural photoperiod was used and a 14L:10D light regime was established for ESL streams.

Artificial streams at both the ESL and at Glen Lyn were treated with $ZnSO_4$ at nominal concentrations of 0.05 and 1.0 mg/L at a pump flow rate of 0.5mL/min from Cole Parmer variable speed peristaltic pumps. Stock solutions held in 25-L carboys were changed every other day. Inflow dilution rate was maintained at 1.2 L/min by adjustments of valves to each stream. Current was maintained at 25 cm/sec. In the ESL, green algae (Chlamydomonas rheinhardti) cultured in Bold's Basic Medium was added to each stream to achieve a final density of approximately 625 cells/L each day. At Glen Lyn, clams fed on algae brought in via New River water and endogenous periphytic growth in the artificial streams. The laboratory feeding strategy was based upon research by Graney et al.(1984).

EXPERIMENTAL DESIGN

Adult Corbicula (13-16 mm) were collected from the New River in 1984 for ESL (27 May-26 June) and Glen Lyn (23 June-23 July) studies. Sixty clams were placed in each artificial stream. A 2-week acclimation period was provided. Analyses (enzyme activity, growth, water chemistry) were performed on days 0, 5, 10, 20, and 30.

Water samples were collected from the ESL and Glen Lyn and returned to Virginia Tech on ice for chemical analysis (Table 1). Total zinc was determined using a Perkin-Elmer 640 Atomic

Table 1. Water chemistry parameters for summer, 1984, at Glen Lyn (above) and ESL (below) studies. Mean ± SD are cited.

Parameter	n	Zinc Concentration (mg/L) Control	0.05	1.0
Temp (°C)	9	25.11 ± 1.76	-	-
	3	16.5 ± 1.5	-	-
pH	18	8.39 ± 0.30	8.31 ± 0.38	8.06 ± 0.26
	6	7.88 ± 0.08	7.87 ± 0.08	7.82 ± 0.09
Alkalinity (mg/L)	18	49.8 ± 7.1	49.5 ± 5.9	49.8 ± 6.1
	6	27.6 ± 2.1	28.7 ± 1.9	29.1 ± 2.5
Hardness (mg/L)	18	71.1 ± 13.5	70.7 ± 14.6	72.3 ± 10.6
	6	57.5 ± 2.7	55.0 ± 4.5	55.8 ± 5.8
Conductivity (uV)	18	122.8 ± 25.5	124.5 ± 22.9	128.45 ± 20.6
	6	133.3 ± 16.6	127.7 ± 15.5	128.38 ± 12.1
NO_3 (mg/l)	12	5.92 ± 1.83[a]	4.77 ± 1.33	4.95 ± 1.30
	2	4.44 ± 1.68	3.39 ± 0.39	3.39 ± 0.18
PO_4^{-3} (mg/L)	12	0.31 ± 0.31[a]	0.0 ± 0.0	0.0 ± 0.002
	2	0.11 ± 0.01	0.11 ± 0.01	0.03 ± 0.04
SO_4^{-2} (mg/L)	12	13.28 ± 4.16[a]	11.99 ± 2.36	14.63 ± 3.00
	2	12.69 ± 1.54	12.26 ± 0.94	13.67 ± 2.58
Cl^- (mg/L)	12	3.43 ± 0.78[a]	2.66 ± 0.32	2.58 ± 0.32
	2	12.77 ± 0.14	12.28 ± 0.60	12.36 ± 0.71

[a]Sample size = 9.

Abosorption Spectrophotometer (APHA et al. 1981). Alkalinity and hardness were determined titrimetrically, and pH was determined using a Model 650 Fisher Scientific pH meter. Anions (NO_3^-, PO_4^{-3}, SO_4^{-2},Cl&supmin.) were determined by column ion chromotography using a Dionex Model 10 Ion Chromatograph (U.S. EPA 1983).

ENZYME ANALYSIS

Six clams from each treatment were randomly chosen and transferred to the laboratory for dissection and weighing. Enzyme extracts from individual clams were prepared from whole body homogenates. Samples were homogenized in 0.15M phosphate buffer at pH 6.0 at a wet mass to buffer ratio of 0.2g/mL. Homogenates were centrifuged for 15 min at 15,000 x g. Supernatants (extracts) were decanted and the final extract volume recorded. Pellets were recovered for dry mass measurments. Two cellulase assays were used. The first was a viscometric assay using 1% (wt/vol) carboxymethylcellulose (CMC; Hercules type 7H3SF) solution (Almin and Eriksson 1967). The second assay was a colorimetric reducing sugar determination, which also used CMC as the substrate and dinitrosalicylic acid (DNS) as a reagent (Miller 1959). Details of these assays are reported by Sinsabaugh 1980 and Sinsabaugh et al. 1985. The viscometric assay measured endocellulase (β-1,4-endoglucanase) activity in units pro-

portional to absolute activity. The reducing sugar assay estimated exocellulase activity. The production of reducing sugars is the result of the synergistic interaction of endo- and exocellulase, but only the products of exocellulase and beta-glucosidase activity were determined by the exocellulase assay. Activity was expressed as mg of glucose equivalents evolved per hour. Soluble protein in extracts was measured by a colorimetric procedure (Bradford 1976; Kley and Hale 1977 using Coomassie blue dye (Bio-Rad Laboratories Technical Bulletins 1051, 1977). All assays were performed at 20 C. Cellulase activity is expressed in two ways: first, both as absolute activity and, secondly as a relativized product index that incorporated both endo- and exocellulase estimates (and their synergistic interaction) to compare units of activity of clams exposed to zinc in relation to control activities. One unit of the enzyme is defined in this context as the amount of enzyme required to liberate one mg of reducing sugar equivalent to that of glucose per hour using CMC as a substrate.

GROWTH ANALYSIS

At the beginning of the Glen Lyn study, 90 clams were measured for shell length with Vernier calipers (precision ± 0.025 mm) and weighed on a digital Metler PC 440 microanalytical balance (precision ± 0.0005 g). Groups of 10 clams

were placed in each artificial stream and held in nylon mesh 2-mm^2 baskets filled with 2 cm of coarse sand sediment.

The length and weight of control, 0.05, and 1.0 mg/L treatment groups were 15.65 ± 0.15 mm and 2.332 ± 0.053 g, 15.85 ± 0.15 mm and 2.332 ± 0.053 g, and 15.93 ± 0.16 and 2.425 ± 0.075 gm, respectively. Individual clams could be identified in each basket by their unique combination of length and weight and frequent measuring. On sampling days each clam was measured, weighed, and returned to the artificial stream. Dead clams were removed and shell length recorded.

Growth was analyzed by comparing length and weight measurements for each individual through time in a cumulative manner. Cumulative growth was considered as the addition (or loss) of shell length and whole clam weight from the condition of clams on day 0. Preliminary analyses indicated that the clam did not grow well during 30-d artificial stream tests at the ESL; therefore, growth data were not collected for laboratory studies.

ZINC ACCUMULATION ANALYSIS

Three *Corbicula* and precolonized glass rods covered with periphyton were removed from streams at each exposure concentration on days 0, 5, 10, 20, and 30. *Corbicula* were dissected and algae scraped from the glass rods. Periphyton was

then filtered onto 0.45 um Metricel membrane filters. Clams and algae were dried at 100 C for 24 h. Dry weights were recorded, and acid digestion for bioaccumulation was accomplished according to the methods of Valdes et al. (1982).

STATISTICAL ANALYSIS

Enzyme activty, growth, and zinc chemistry data were analyzed statisitcally using the Kruskal-Wallis Test, a nonparametric one-way analysis of variance rank analogue (Hollander and Wolfe 1973). In analyses of total zinc data, a concentration of 0.012 mg/L was assumed for purposes of calculations when samples read below the lowest standard solution analyzed (0.025 mg/L). Significantly different means were determined by a rank-like Least Significant Differences procedure (α=0.05) (Hollander and Wolfe 1973). Product-moment correlation calculations were made of enzyme activity, growth, and bioaccumulation data for the purpose of comparing measurements.

RESULTS

ARTIFICIAL STREAM DOSING

Target zinc concentrations were met for control, 0.05, and 1.0 mg/L in both field and laboratory studies. At Glen Lyn,

control and 0.05 mg/L realized concentrations were not significantly different, although Zn measurements of the 0.05 mg/L target were within 70% of the calculated concentration (i.e., 0.035±0.012 and 0.038±0.013 mg/L for Glen Lyn vs ESL streams, respectively)(Table 1). The effect of presuming that a 0.012 mg/L baseline concentration was always present in controls altered the outcome of the Kruskal-Wallis test. The high dose (1.0 mg/L) target had an average concentration of 1.101±0.955 mg/L. The realized Zn concentrations at the ESL (1.130±0.478 mg/L) were more consistent than those at Glen Lyn. All ESL treatments (0, 0.05, 1.0 mg/L) were significantly different from each other.

Dosing reproducibility for each target concentration at Glen Lyn was established according to the Kruskal-Wallis test (Table 2). Treatment replicates were not significantly different (control, 0.05, and 1.0 mg/L).

CELLULASE DYNAMICS

Total activity, as represented by the exo- and endocellulase product index declined with increasing Zn exposure concentration and time during both 30-d chronic studies (Table 3; Fig. 3). Enzyme activities in clams exposed to 0.05 mg/L Zn at Glen Lyn were significantly different from control activities by day 10 and continued so for the remainder of the study. Clams exposed to 1.0 mg/L Zn did

Table 2. Analyses of variance for total zinc content for the three target concentrations and replicates in the Glen Lyn artificial stream study. This analysis substantiates the reliability of dosing replicate. Means (±SE) with the same symbol are not significantly different ($\alpha = 0.05$).

Replicate	n	Control	Kruskal-Wallis (p-value)	0.05 mg/L Target	Kruskal-Wallis (p-value)	1.0 mg/L Target	Kruskal-Wallis (p-value)
A	6	0.026 ± 0.010		0.042 ± 0.005		0.642 ± 0.136	
B	6	0.022 ± 0.005		0.033 ± 0.004		1.622 ± 0.589	
C	6	0.024 ± 0.008	0.790 (p>0.95)	0.028 ± 0.004	4.60 (p>0.10)	1.040 ± 0.239	2.92 (p>0.250)

not possess significantly different activity levels until day 20 which were depressed thereafter through day 30. No significant differences in the product index were seen in clams exposed in ESL streams until day 30 (832.1, 384.1, and 274.1 for control, 0.05 mg/L, and 1.0 mg/L, respectively.

Cellulase activities of control clams at Glen Lyn consistently increased throughout the 30-day study (Table 3). Control clams held in ESL streams and fed an artificial diet also responded in the first 10 days with increasing product indices. These clams however exhibited a decline in cellulase activity by day 20 that continued until product indices of exposed and controls were more similar.

The ratio of

Table 3. Analysis of variance by Kruskal-Wallis for growth and enzyme activities (parameter mean ± SE) of adult Corbicula exposed to zinc sulfate at Glen Lyn and ESL in artificial streams. Means with the same symbol are not significantly different ($\alpha = 0.05$).

Parameter	Zn Target Concentration (mg/L)	Length of Exposure (Days)				
		0	5	10	20	30
Length gain[a]	0	-	0.062±0.048	0.129±0.032*	0.188±0.040*	0.387±0.045*
Glen Lyn	0.05	-	0.091±0.029	0.139±0.029*	0.203±0.030*	0.172±0.030+
(mm)	1.0	-	0.015±0.021	-0.014±0.026+	0.010±0.022+	-0.028±0.030+
Weight gain[b]	0	-	31.9±13.4	50.0±15.9	103.4±16.23*	184.5±27.9*
Glen Lyn	0.05	-	107.3±35.3	118.3±38.6	137.2±42.5*	85.5±51.65*
(mg)	1.0	-	52.0±24.6	44.7±33.3	-1.3±18.8+	14.7±29.4+
Exo X Endo X 10^{5}[c]	0	823.8±288.1	606.9±104.7	634.9±105.4	1062.4±305.4*	1822.7±421.8*
Glen Lyn	0.05		793.9±510.2	252.3±107.3	123.4±29.9+	176.6±45.1+
(units/g dry weight)^{2}	1.0		427.7±89.9	572.4±114.9	85.6±28.9+	41.5±13.0+
Exo/Endo X 10^{-4}[d]	0	88.3±16.7	129.1±20.3	154.8±5.9	176.4±5.4	153.5±14.1
	0.05		139.4±64.9	158.6±13.7	165.8±27.5	320.5±56.7
	1.0		152.5±81.5	143.6±18.5	134.1±19.4	217.7±65.7
Exo X Endo X 10^{5}[e]	0	2587.6±244.5	4190.2±109.9	5588.8±236.4	3571.8±1994.5	832.1±169.9
ESL	0.05		3049.8±873.9	2489.0±353.2	1465.1±322.4	384.1±107.2
(units/g dry weight)^{2}	1.0		3661.1±224.7	1491.4±363.5	1151.3±299.2	274.1±110.7
Exo/Endo X 10^{-4}[f]	0	43.9±12.5	65.9±11.7	56.8±6.0	77.4±8.8	106.2±11.0
ESL	0.05		65.8±14.2	69.2±3.6	69.8±2.6	105.1±11.9
	1.0		43.0±19.7	75.0±8.0	65.8±6.6	85.2±15.2

[a]The statistic (and in parentheses, p-values) were 4.725 ($p>0.10$); 14.620 ($p<0.05$); 16.410 ($p<0.05$); and 21.053 ($p<0.05$) for days 5 to 30, respectively.

[b]The statistic (and in parentheses, p-values) were 6.785 ($p>0.10$); 5.760 ($p>0.10$); 14.382 ($p<0.05$); and 9.530 ($p<0.05$) for days 5 to 30, respectively.

Table 3. Continued.

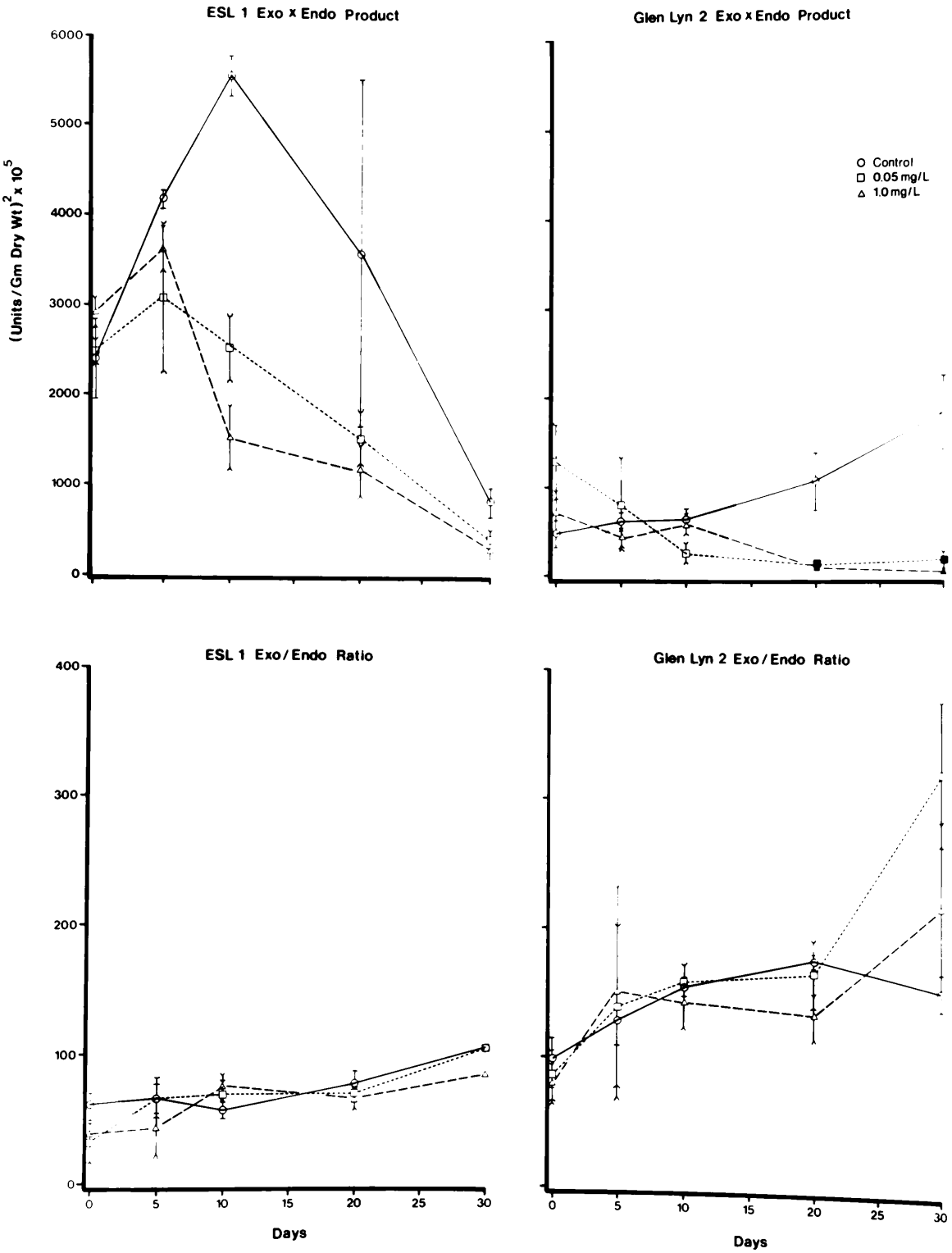
[c]The statistic (and in parentheses, p-values) were 0.327 ($p>0.75$); 3.239 ($p>0.10$); 4.643 ($p>0.05$); 5.888 ($p<0.05$); 7.240 ($p<0.05$) for days 0 to 30, respectively.

[d]The statistic (and in parentheses, p-values) were 5.632 ($p>0.05$); 6.538 ($p<0.05$); 0.783 ($p>0.75$); 0.225 ($p>0.75$); 0.327 ($p>0.75$) for days 0 to 30, respectively.

[e]The statistic (and in parentheses, p-values) were 0.153 ($p>0.75$); 3.561 ($p>0.10$); 0.363 ($p>0.75$); 0.539 ($p>0.75$); 0.433 ($p>0.75$) for days 0 to 30, respectively.

[f]The statistic (and in parentheses, p-values) were 2.844 ($p>0.75$); 4.897 ($p>0.05$); 0.784 ($p>0.75$); 0.080 ($p>0.75$); 25.75 ($p>0.25$) for days 0 to 30, respectively.

Figure 3. Results of Ecosystem Simulation Laboratory (ESL) and Glen Lyn stream cellulase products and ratios of Corbicula during 30-d exposures to 0.05 and 1.0 mg/L zinc sulfate. One standard error of the mean was used for all indications of variability about the mean.


ESL 1 Exo x Endo Product
Glen Lyn 2 Exo x Endo Product
(Units/Gm Dry Wt)^2 x 10^5
6000
5000
4000
3000
2000
1000
0
O Control
□ 0.05 mg/L
△ 1.0 mg/L
ESL 1 Exo/Endo Ratio
Glen Lyn 2 Exo/Endo Ratio
400
300
200
100
0
0
5
10
15
20
25
30
Days

exo- to endocellulase activities varied between field and laboratory experiments, but not necessarily between functional cellulase components (Table 3; Fig. 3). The general trend was for the cellulase ratio index to increase throughout both 30-d studies. However, neither endo- or exocellulase activity was declining with respect to the other; therefore, both cellulase complex components were affected equally during zinc exposure (Table 3).

GROWTH OF CORBICULA

By day 20 at Glen Lyn, significant growth inhibition ($p < 0.05$) was evident in clams exposed to 1.0 mg/L $ZnSO_4$ (Table 3). By day 30, the 0.05 mg/L dosed clams were growing at only one-half the rate of controls, and 50% of Corbicula exposed to 1.0 mg/L were dead. No mortality was observed in control and 0.05 mg/L dosed clams.

Growth was not consistently related to the cellulase product index. The strongest correlation was for product index versus shell growth (0.497; p-value =.06). When the 0.05 mg/L dosed Corbicula stopped growing by day 20, with negative growth between days 20 and 30, the cellulase index dropped (Table 3; Fig. 4). The 1.0 mg/L dosed animals did not grow after day 5; in fact, clams decreased in shell length and the cellulase index dropped rapidly (Fig. 5).

Figure 4. Response of Corbicula cellulase complex to zinc in (A) Ecosystem Simulation Laboratory (ESL) and (B) Glen Lyn exposures. Data are relativized to the control product index on each day. Means ±1 SE are given on each day. Means which are significantly different from the control are indicated by an asterisk (*).

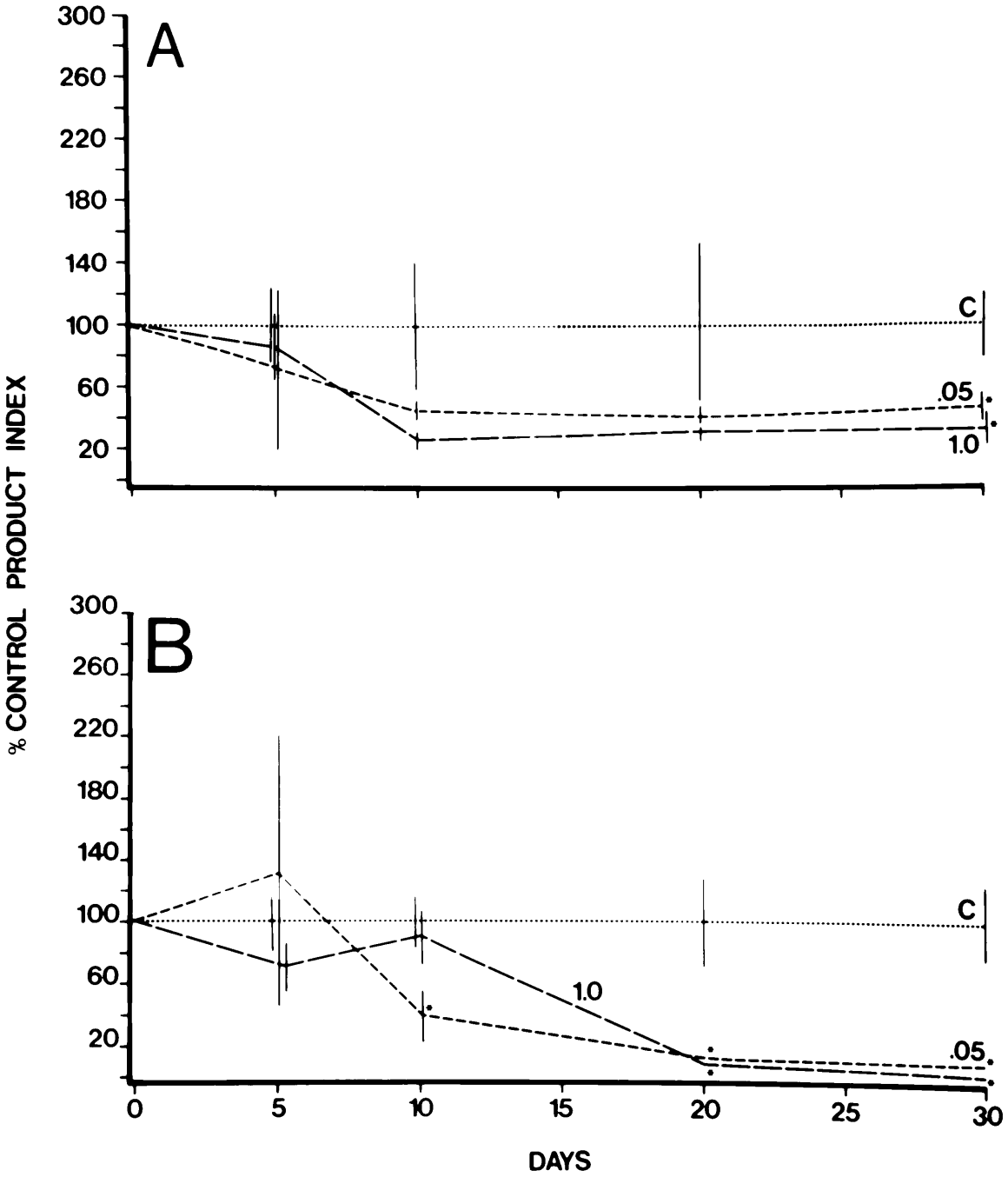
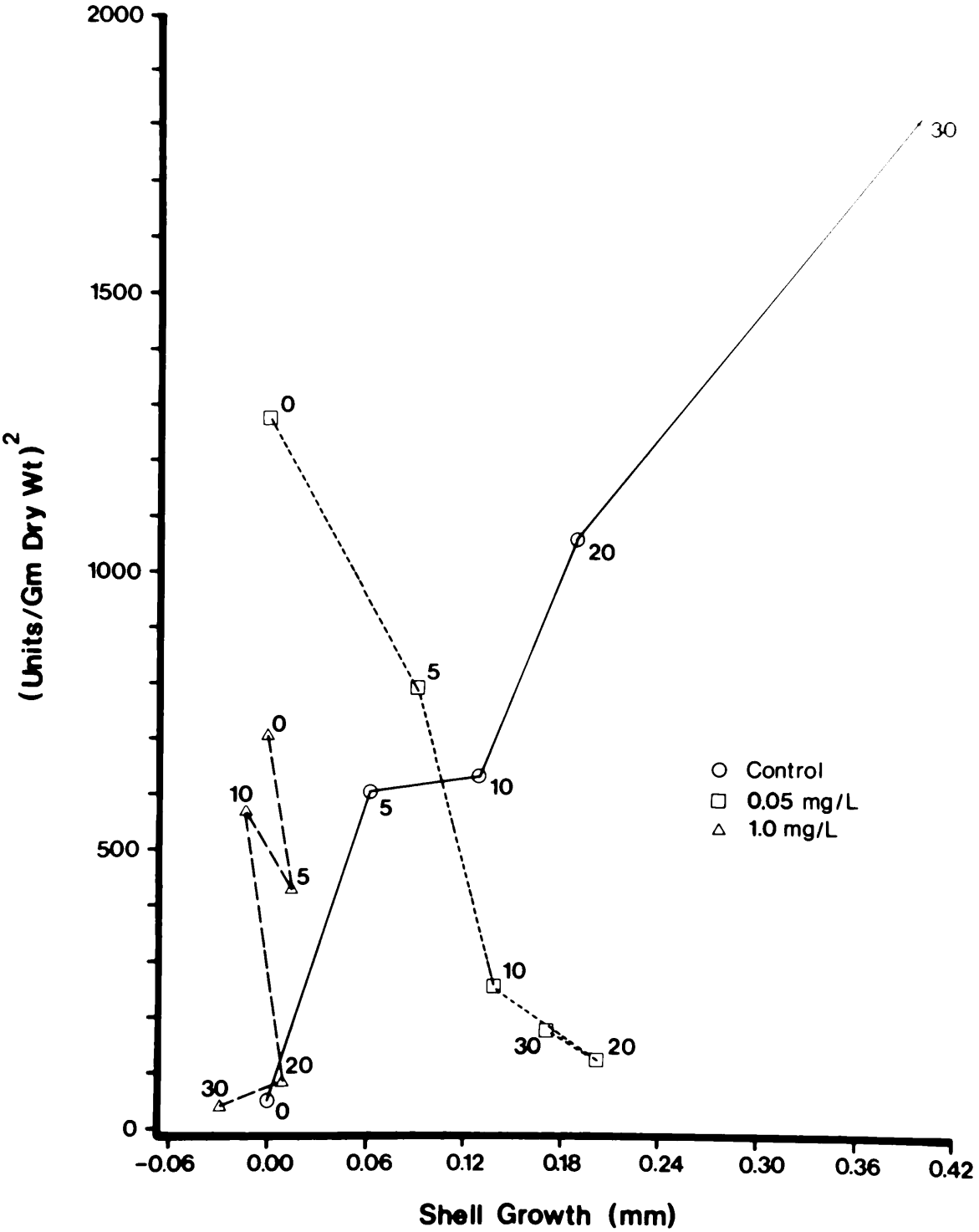

A
B
300
260
220
180
140
100
60
20
C
.05
1.0
0
5
10
15
20
25
30
% CONTROL PRODUCT INDEX
DAYS

Figure 5. Comparison of Corbicula cellulase activity (units/g dry wt)[2] and shell growth (mm) through 0, 5, 10, 20, and 30 d exposures in control, 0.05, and 1.0 mg/L zinc streams. One standard error of the mean was used for all indications of variability about the mean.


2000
1500
1000
500
0
(Units/Gm Dry Wt)^2
-0.06
0.00
0.06
0.12
0.18
0.24
0.30
0.36
0.42
Shell Growth (mm)
Control
0.05 mg/L
1.0 mg/L
0
5
10
20
30

ZINC ACCUMULATION

Accumulation of Zn was influenced by concentration and length of exposure (Table 4). A steady state condition was attained in both the periphyton and *Corbicula* in the 0.05 mg/L Zn streams by day 10. Periphyton from the 1.0 mg/L Zn streams displayed a linear relationship between length of exposure and Zn uptake up to day 20, followed by a leveling off of uptake rate by day 30. However, a sustained equilibrium between uptake and excretion of Zn in *Corbicula* did not occur during the 30-d exposure.

High correlations were found for zinc in algae versus clams ($r = 0.695$, $p < 0.008$). Also, cellulase product indices were correlated with zinc uptake in *Corbicula*. The cellulase product index - zinc in *Corbicula* correlation coefficient was -0.762 ($p < 0.001$) and for relativized data the coefficient was -0.739 ($p < 0.002$). Growth measurements were most closely associated with zinc accumulation in algae (for length gain, $r = -0.559$, $p < 0.05$; for weight gain, $r = -0.541$, $p < 0.06$).

DISCUSSION

Response of the cellulase enzyme complex to zinc exposure in this study indicates that this functional measurement can reflect sublethal stress occurring in chronic tests with

Table 4. Metal accumulation (ug Zn/g dry wt) of Corbicula and periphyton in 30-d exposure in artificial streams at Glen Lyn, Virginia. Means ± SD are cited (n=3).

		Periphyton	Corbicula
Day 0 (6-h dose for periphyton)	0	393 ± 39	181 ± 21
	0.05	856 ± 60	180 ± 3
	1.0	2562 ± 26	183 ± 6
Day 5	0	795 ± 80	171 ± 13
	0.05	2047 ± 568	361 ± 57
	1.0	14414 ± 4471	568 ± 26
Day 10	0	1327 ± 417	201 ± 7
	0.05	2608 ± 309	540 ± 127
	1.0	35901 ± 14355	491 ± 43
Day 20	0	686 ± 88	206 ± 12
	0.05	4369 ± 409	453 ± 41
	1.0	56247 ± 7460	615 ± 36
Day 30	0	585 ± 55	169 ± 5
	0.05	3917 ± 211	433 ± 29
	1.0	32411 ± 1804	827 ± 120

Corbicula. A number of studies emphasizing enzyme inhibition within aquatic organisms from heavy metal cations have presented possible applications to monitoring (Brown 1976). Yet no single reliable combination of measurements involving all pathways of exposure has proven to validate dose-effect responses. Brown (1976) found that some heavy metals may activate cellular enzymes or enhance their activity at low concentrations but may be inhibiting at higher concentrations.

This trend was supported by exposures to 0.05 mg/L zinc in that day 5 activities were increased above controls before declining to significantly lower levels by day 10 at Glen Lyn. The influence of avoidance behavior in clams exposed to 1.0 mg/L was reflected in delayed effects on cellulolytic activity, i.e., no significant differences occurring until day 20. This behavior modified response tends to contradict the majority of the work supporting enzymes as candidates for biomonitoring using in vitro quick screening tests to replace whole-animal test systems (Christensen et al. 1982; Bitton 1982). If the purpose of a chronic bioassay is to elucidate biochemical pathways involved with detoxification, response variability, or recovery following exposure, then in vivo combinations of one or more relevant enzyme systems coupled with parameters such as growth and metal uptake is a more effective approach. Combinations involving classical measurements that are responsive to metal uptake mechanisms more

adequately address the relative contributions of food and water to trace metal contamination. The importance of an organism's feeding response and ability to detect Zn at low levels further supports consideration of pathways where metal accumulation is affected.

It has been shown that metal uptake from the diet is more important than direct uptake of dissolved metals from water (Phillips 1977; Graney et al. 1984). Willis and Sunda (1984), using ^{65}Zn-labelled Chlamydomonas, set up a model food chain and found that 78 to 82% of total metal accumulation by fish was attributable to dietary accumulation. Martinicie et al. (1984) found marine bivalves exposed to particulate suspended material containing zinc, copper and lead (about 100 to 150 ng/kg sea water) and to cadmium at only 3.5 ng/kg accumulated zinc, cadmium, and copper predominantly from the dissolved state. Accumulation in their studies showed metals to be above levels required for catalytic activity of enzymes. However, when compared with other findings on tissue accumulation, they note that accumulation may be metal, tissue, and species specific with respect to particulate or dissolved metal-state.

In our studies, clams were able to detect acutely toxic levels of zinc and remain closed, thus avoiding zinc exposure and zinc-laden algae. This avoidance response has been corroborated by Belanger et al. (1986). At low zinc concentrations (e.g., 0.025-0.050 mg/L) clams siphoned and took up

zinc and algae. Zinc laden algae may have contributed a major portion of the observed total body burden. But at higher concentrations (0.5 -1.0 mg/L) clams avoided zinc or zinc-laden algae and only maintained total body burdens comparable among all treatments. As in our studies, by day 20, clams in high zinc waters continued to accumulate due to Corbicula's estimated avoidance response of only 10-15 days (McMahon 1979). Graney et al. (1983) also reported highest BCF's to occur at the lowest metal exposure. Corbicula exposed to 0.218, 0.433, and 0.835 mg/L Zn had BCF's of 631, 358, and 511 respectively, during 28-day field artificial stream exposures.

Algae accumulated up to 5,000 ug Zn/g dry weight at 0.05 mg/L and 60,000 ug Zn/g dry weight at 1.0 mg/L in our artificial streams at Glen Lyn. Apparently this accumulation not only accounted for the observed total body burdens in Corbicula, but also affected the total zinc in water, enzyme activities, growth and calculated nominal zinc concentrations. This was even more evident in exposures using lower zinc concentrations in chronic field tests where target exposure concentrations were difficult to maintain as algae served as a sink for the water-borne metal (Belanger et al. 1986). As algal biomass increased throughout the exposure period, zinc often dropped below detection limits in these studies.

Cellulase indices for activity in Corbicula apparently reflect either direct enzyme inhibition, or indirect inhibition upon the crystalline style by zinc. Several authors have noted that hydrolysis of CMC does not prove that native cellulose is being degraded (Hylleberg 1976; Martin et al. 1981; Monk 1976). However, Kesler (1982) points out in his work with aquatic insects that the presence of CMCase indicates the ability to break β-1-4 glycosidic bonds. Even if it is believed that structural carbohydrates may not be completely degraded during passage through guts of bivalves during optimal siphoning, it is possible that CMCase may be important during periods of starvation or stress when gut residence times are extended. Sinsabaugh et al. (1985) have also found significant crystalline cellulose digestion in aquatic insects that have variable levels of CMCase activity. However, the origin or exact action of cellulase was not an objective in this study with Corbicula. The fluctuations in activity of this enzyme group in relation to stream conditions, food variability and growth suggest that Corbicula does utilize CMCase in its obtaining digestible materials.

Those changes in growth seen in 30-day Zn exposures were supportive of the measured cellulase indices for the same clams. Correlations between zinc uptake in algae and growth made apparent the trends seen for reduced enzymatic activity compounded by behavioral changes. Since dietary needs were not being met as Corbicula failed to siphon at acutely lethal

Zn levels, the observed degrowth could at first be attributed to the behavioral modification. Belanger et al. (1986) has not only shown this to be typical in Corbicula growth reductions to Zn exposures, but also upon exposure to chrysotile asbestos (Belanger et al. 1986). The observed period of degrowth occurring in the first 20-days in clams exposed to 1.0 mg/L Zn combined with the observed cellulase indices and zinc accumulation would lead one to believe that clams were undergoing an avoidance-starvation response. This hypothesis is supported by similar declining cellulolytic activity seen in not only dosed laboratory clams but even in starved control clams. Decreasing visceral biomass of Corbicula in these instances reflects a change in metabolic budgeting. These shifts, resulting in degrowth, are seen in populations of mollusks subjected to seasonal periods of starvation (Russel-Hunter et al. 1984) as well as in dissolution of Corbicula shell when subjected to acidic waters (Kat 1982). The difficulty of maintaining an adequate food supply to insure growth of Corbicula in the laboratory is known by many (Dauble et al. 1985; Foe and Knight 1985; Foe and Knight 1986). Those enzymatic and growth responses occurring in all clams being stressed in laboratory conditions did not differ from those in starving individuals avoiding zinc exposure in the field. Corbicula dietary needs were met in field exposures where phytoplankton was present in sufficient amounts in river water. In field exposures it was possible to meas-

ure responses of Corbicula's with respect to uptake mechanisms and avoidance of a toxicant and not responses of an organism already undergoing metabolic shifts from inadequate feeding.

The observed interaction between zinc accumulation and growth at Glen Lyn was evident where clams continue to siphon in 0.05 mg/L Zn exposures. This was further substantiated by a rapidly declining cellulase index. In laboratory exposures where siphoning was not induced by an adequate food supplies, changes in cellulolytic activity were not significant until day 30. Even then, high and low level exposure responses were not significantly different from one another.

Relationships between cellulase activity and zinc accumulation, and growth and zinc accumulation were evidence for two functional measurements that are responsive to chronic metal exposure. However, zinc availability through water alone is an inadequate approach to assess Corbicula's ability to avoid or actively accumulate a metal. Corbicula requires dietary management in toxicity testing if food is a route of exposure and if more sensitive functional methods are of use in assessing chronic exposure to metals.

CHAPTER THREE: SNAIL AND CLAM COMPARISONS

INTRODUCTION

Efforts to monitor the impacts of heavy metals upon aquatic environments have included the examination of partitioning of metals in aqueous and suspended particulate phases, sediment phases, and the availability of these phases to organisms qualifying as biomonitors. The present data base dealing with effects levels of metal toxicity exhibits large variability in organismic response. This variability is influenced by differences in species selection, methodology in testing, water quality parameters, and various factors known to affect the range of effects levels (O'Donnel 1985). This apparent lack of cohesion between toxicity estimates and what is now known about the bioavailability of metals demands more consideration be given to those tests which can address effects occurring as stress is encountered prior to the onset of mortality. The use of indigenous biota (specifically molluscs) to monitor metal contamination in water and sediments has gained recognition due to the ability of sedentary or sessile organisms to estimate actual concentrations "seen" by organisms (Phillips 1977; Goldberg et al. 1978). The use of a biomonitor whose body burden of metal may be correlated with ambient water concentrations, does not re-

quire information on all changes occurring in metal complexation or speciation, rather it examines those metal forms accumulated within the organism through specific uptake routes.

Although molluscs have been used extensively as monitoring organisms for heavy metal pollution (Phillips 1980; Goldberg et al. 1978; Graney et al. 1983), there is a paucity of information on the effects of metal accumulation on metabolism and physiology (Bayne et al. 1976; Viarengo et al. 1980). Recent efforts to quantify stress to molluscs that accumulate metals above storage and depuration capacities, have led to the development of general and specific stress indices (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 which renders the individual or the population more vulnerable to further environmental changes. Stress indices may clarify nonspecific processes (including mucus binding, endocytosis, and diffusion [Simkiss 1983]) involved in metal uptake by molluscs. 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 which may affect indicator ability (Phillips 1980).

This study was undertaken to investigate cellulolytic responses in two molluscs (a filter-feeding bivalve and a

phytophagous gastropod that are ecologically and physiologically distinct) to long term exposure and recovery to zinc. The Asiatic clam, Corbicula fluminea, has been adequately qualified as a bioindicator of pollutants (Johnston and Hartley 1981; Graney et al. 1983, 1984) and has recently been given more attention for use in biomonitoring and toxicity testing (Harrison et al. 1984; Belanger et al. 1986a). In addition to examining zinc accumulation and cellulolytic activity in clams, comparisons were made with a freshwater gastropod (Mudalia dilatata) to evaluate the ability of gastropods to accumulate metals through different uptake routes and as affected by behavioral alterations. Stress indices from these two potential monitors were examined seasonally in both laboratory and field-oriented artificial stream systems. Data reported in this manuscript represent a portion of a larger study designed to determine the feasibility of using cellulolytic activity as a general stress index.

MATERIALS AND METHODS

Seven, 30-day chronic exposures of the Asiatic clam, (Corbicula fluminea) and a snail (Mudalia dilatata) to zinc were carried out at the Glen Lyn field laboratory (GL) and VPI&SU Ecosystem Simulation Laboratory (ESL) (Table 5). The molluscs were analyzed for variations in cellulolytic activ-

Table 5. 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	28 April - 28 May, 1984	30-day
GL 2	Summer, 1984	23 June - 23 July, 1984	30-day
GL 3	Fall, 1984	7 Sept. - 7 Oct., 1984	30-day + clam recovery
GL 4	Spring, 1985	26 April - 26 May, 1985	30-day + clam and snail recovery
ESL 1	Spring, 1984	27 May - 26 June, 1984	30-day
ESL 2	Winter, 1984	26 Nov. - 26 Dec., 1984	30-day
ESL 3	Spring, 1985	9 April - 9 May, 1985	30-day

ity and zinc accumulation in response to duration and degree of exposure.

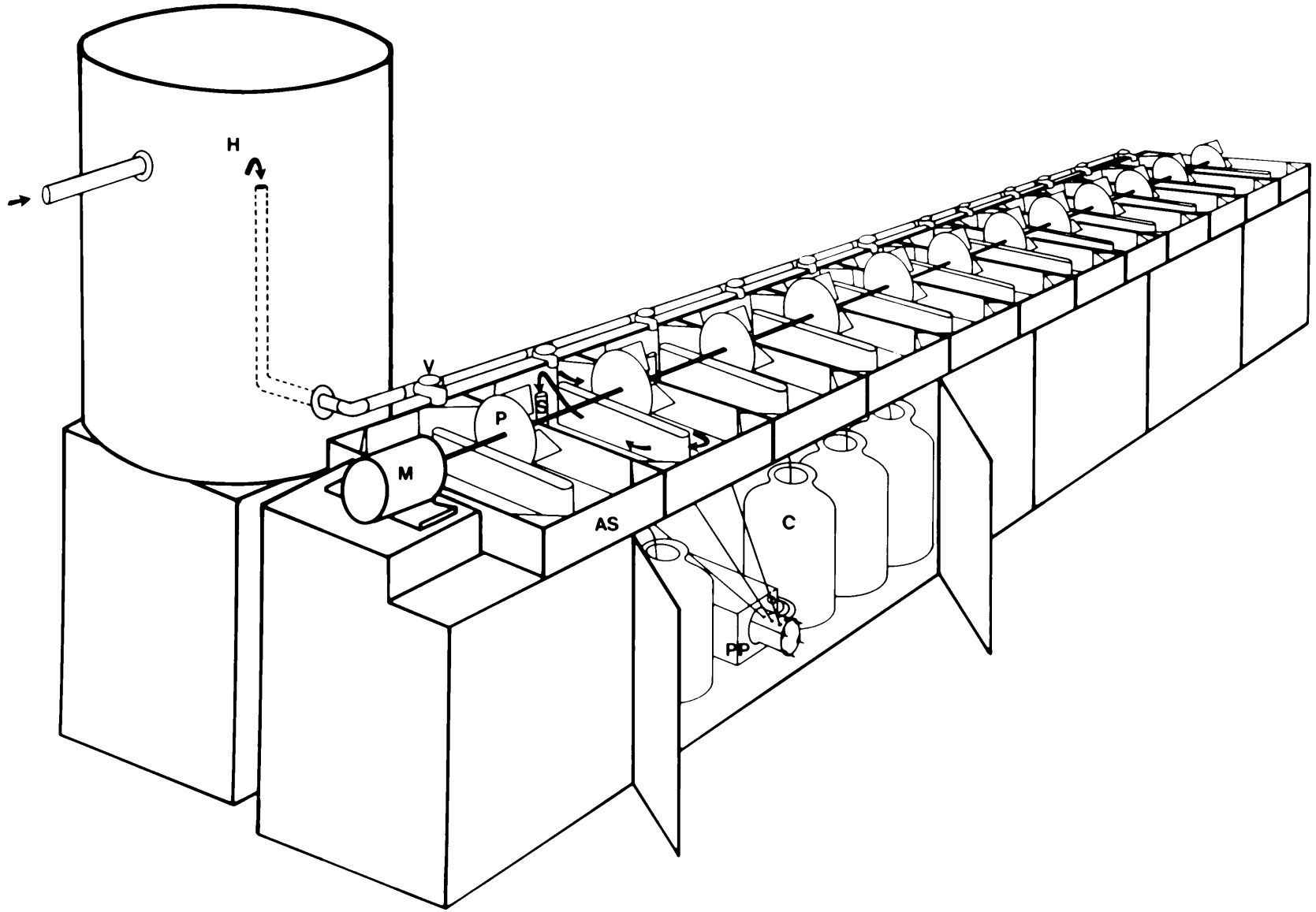
DESCRIPTION OF ARTIFICIAL STREAMS

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). Experimental units consisted of a series of oval, paddle-driven streams adapted for both field and laboratory applications (Fig. 6). This design utilizes 16 mm plywood and 25 x 150-mm shelving. Streams were 76 x 33 x 15 cm, with a capacity of 20 L. Joints were sealed with silicon sealant and surfaces with three coats of chemical and moisture resistant enamel paint.

Dechlorinated, charcoal-filtered tap water or natural stream water entered each system via a headbox. Gravity provided constant pressure through a headbox drain pipe that led to each of the oval streams. The drain pipes, constructed of 19-mm schedule 40 PVC pipe, were fitted with 19-mm straight valves that allowed precise regulation of water flow to each individual stream. Inflow rates for Glen Lyn and ESL streams was 1.2 L/min and 0.5 L/min respectively.

A plexiglass headbox was used to adjust various water quality parameters before the water entered the ESL artificial streams. Influent pH at the ESL was regulated by a

Figure 6. Schematic representaion of artificial stream and metal dosing system showing headbox (H), peristaltic pump (PP), carboys (C), containing zinc 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.


H
V
P
M
AS
C
PP

Fisher Model 650 pH meter/controller and a Cole-Parmer peristaltic pump that fed a 10 % sulfuric acid solution into the headbox. Mixing was ensured by a magnetic stirrer posi- beneath the headbox. Water depth was regulated by 19-mm di- ameter PVC standpipes mounted in bulkhead and male CPVC adapters. Current was provided by a series of plexiglass paddle wheels attached to a 10-mm steel rod powered by a 1/4 h.p. continuous-use gearmotor.

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 at Glen Lyn were dosed with $ZnSO_4$ at concentrations ranging from 0.025 to 1.0 mg/L at a pump flow rate of 0.5 ml/min from Cole-Parmer var- iable speed peristaltic pumps. Stock solutions held in 25-L carboys were changed every other day. To provide food for Corbicula, 250 ml of Chlamydomonas rheinhardti cultured in Bold's Basic medium (625 cells/L) were added to ESL streams every day; Glen Lyn streams were colonized by resident algae from the New River, so no food supplements were needed. During acclimation , precolonized rocks transferred from the New River to ESL streams provided substrate for Mudalia grazing.

Water samples were collected from ESL and Glen Lyn and returned to Va Tech on ice for chemical analysis on each

sample day (Tables 2 and 3). Total zinc was determined (APHA et al. 1984) using a Perkin-Elmer 640 Atomic Absorption Spectrophotometer. Alkalinity and hardness were determined titrimetrically (APHA et al. 1984) and pH was determined using a Model 650 Fisher Scientific pH meter. Anions were determined by column ion chromatography using a Dionex Model 10 Ion Chromatograph as previously described in chapter three.

Exposures at Glen Lyn were conducted by using three replicate streams at three to four concentrations (Tables 2 and 3). In previous studies we have documented that replicate streams were not significantly different in actual Zn concentrations (Farris et al., in review); consequently, the spring, 1985 Glen Lyn study and all formal laboratory studies were conducted with single streams per target concentration.

In the fall 1984, and spring 1985 studies, the recovery of clams exposed to Zn for 30 days was determined. This recovery analysis also included snails in the spring 1985. After the fall 1984 exposure period, clams were removed to fish hatchery troughs previously colonized by algae for use as a food source fed with New River water (Clark et al. 1980). The response parameters were analyzed after 10, 20 and 30 days of recovery in clean water. After the spring 1985 exposure period, both snails and clams were allowed to recover in the same artificial streams previously dosed with zinc for

30 days. Response parameters were analyzed as in the previous study.

ENZYME ANALYSIS

Corbicula were collected from a population in the New River, VA (River mile 100) from fine sand sediment. Mudalia were removed from rocks upstream from Glen Lyn Power Plant. Both clams and snails were immediately transferred to artificial stream systems following collection. Following an acclimation period of 10-14 days six clams and snails from each dosage 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 eliminated and recovery days 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 of 0.2 g/ml. Homogenates were centrifuged for 15 min at 15,000 x 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 and 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 Sinsabaugh et al.

(1985). The viscometric assay measured endocellulase (β-1,4-endoglucanase) activity in units proportional to absolute activity. Reducing sugar production (mg glucose equivalents/h) reflects the synergistic action of all the enzymes responsible for cellulolysis. Soluble protein in extracts was measured by a colorimetric procedure (Bradford 1976, Kley and Hale 1977) using Coomassie blue dye (Bio-Rad Laboratories Technical Bulletins 1051, 1977). All assays were performed at 20 C. All activity measurements for endo- and exo-products were reported as units/dry mass. 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 relativized to control activity levels on each day of examination.

Enzyme activity data (the cellulase product index) was analyzed by the Kruskal-Wallis Test, a one-way analysis of variance rank analogue (Hollander and Wolfe 1973), to determine the effect of zinc 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 control, 0.05, 0.1, 0.5, and 1.0 mg/L in both field (Table 6) and laboratory (Table 7) studies. The 0.025 mg/L target was met in laboratory streams but fell below detection limits in field studies at Glen Lyn. At Glen Lyn, control and 0.05 mg/L realized concentrations were not significantly different, although zinc measurements of the 0.05 mg/L target were within 70% of the calculated concentration (i.e., 0.035 ± 0.012 (SE) and 0.038 ± 0.013 mg/L for Glen Lyn vs ESL streams, respectively). The effect of presuming a 0.012 mg/L baseline concentration always present in controls altered the outcome of the Kruskal-Wallis test. The high dose (1.0 mg/L) target had an average concentration of 1.101 ± 0.955 mg/L. The realized zinc concentrations at the ESL (1.130 ± 0.478 mg/L) were more consistent than those at Glen Lyn. All ESL treatments (0, 0.05, 1.0 mg/L) were significantly different from each other.

Dosing reproducibility for each zinc 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) existed. The pattern was consistent for all experiments, therefore, for the purpose of

Table 6. Means (± 1 SE) of selected water chemistry parameters analyzed during the Glen Lyn studies (n=15).

Season	Target Concentration (mg/L)	Actual Zinc Concentration (mg/L)	Temp (°C)	pH	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
GL 1 (Spring, 1984)	0	0.020 (±0)	25.1 (±1.8)	8.39 (±0.30)	71.1 (±13.5)	49.8 (±7.1)
	0.05	0.043 (±0.060)	-	8.31 (±0.38)	70.7 (±14.6)	49.5 (±5.9)
	1.0	0.819 (±1.014)	-	8.06 (±0.26)	72.3 (±10.6)	49.8 (±6.1)
GL 2 (Summer, 1984)	0	0.028 (±0.016)	25.5 (±0.8)	8.44 (±0.10)	70.2 (±3.65)	47.6 (±1.5)
	0.05	0.035 (±0.012)	-	8.42 (±0.09)	70.5 (±4.2)	48.5 (±1.3)
	1.0	1.101 (±0.955)	-	8.08 (±0.08)	70.8 (±2.7)	48.4 (±1.4)
GL 3 (Fall, 1984)	0	0.094 (±0.228)	20.6 (±1.9)	8.31 (±0.12)	88.8 (±2.3)	56.2 (±0.7)
	0.05	0.087 (±0.109)	-	8.27 (±0.09)	88.3 (±2.6)	55.5 (±0.8)
	0.50	0.504 (±0.286)	-	8.20 (±0.05)	87.1 (±2.6)	59.7 (±1.1)
	1.0	0.975 (±0.299	-	8.14 (±0.02)	88.3 (±2.3)	56.1 (±0.7)

Table 6. Continued.

Season	Target Concentration (mg/L)	Actual Zinc Concentration (mg/L)	Temp (°C)	pH	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
GL 4 (Spring, 1985)	0	.020 (±0)	17.2 (±0.4)	8.17 (±0.26)	68.0 (±5.8)	44.4 (±1.9)
	0.025	.020 (±0)	-	8.12 (±0.05)	66.0 (±5.1)	38.6 (±3.2)
	0.10	0.12 (±0.056)	-	8.39 (±0.20)	69.0 (±6.7)	41.2 (±2.3)

Table 7. Mean (± 1 SE) of selected water chemistry parameters analyzed during the ESL studies (n=4).

Season	Target Zinc Concentration (mg/L)	Actual Total Zinc Concentration (mg/L)	Temp (°C)	pH	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L 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 ± .03	7.7 ± 0.03	56 ± 1.8	27.0 ± .3
	0.05	0.090 (±.02)	-	7.7 ± 0.02	62 ± 2.4	28.8 ± .12
	0.50	0.531 (±.05)	-	8.05 ± 0.0	60 ± 1.2	28.2 ± .2
	1.0	0.980 (±.12)	-	7.3 ± 0.03	58 ± 2.4	30 ± .02
ESL 3 (Spring, 1985)	0	0.021 (±0)	16.25 ± .23	8.05 ± 0.01	60 ± 1.2	23.1 ± 1.7
	0.025	0.020 (±01)	-	8.1 ± 0	65 ± 2.4	32.0 ± .02
	0.10	0.070 (±.02)	-	8.06 ± .34	65 ± 0.0	33.3 ± .44

citing realized zinc concentrations all replicates were pooled for analysis.

CELLULOLYTIC ACTIVITY IN CORBICULA

Total activities as represented by the relativized exo- and endocellulase product indices declined both with high zinc doses and time over the course of most 30-day exposures (Fig. 7 and 8). Declining cellulolytic activity in Corbicula 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 zinc only on day 20 of exposure (Fig. 7). A decline in cellulolytic activity for exposure periods during all seasons was most often evident after 20 days of exposure and especially during fall, 1984 for all days of exposure at 1.0 mg/L. Only in the fall, 1984 did high zinc exposures (0.5 and 1.0 mg/L) significantly reduce cellulolytic activity of clams as early as day 5, and their activity failed to recover to levels displayed for controls following 30 days in control water. Clams exposed to zinc levels ranging from 0.025 to 0.1 mg/L, however, did respond with increased cellulolytic activities following 10 to 30 days of recovery (Fig. 7).

Patterns of decline in cellulolytic activity were inconsistent in most zinc exposures for Corbicula that were con-

Figure 7. Response of Corbicula cellulase enzyme complex to zinc in (A) spring, 1985; (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 which are significantly different from the control are indicated by an asterisk (*).

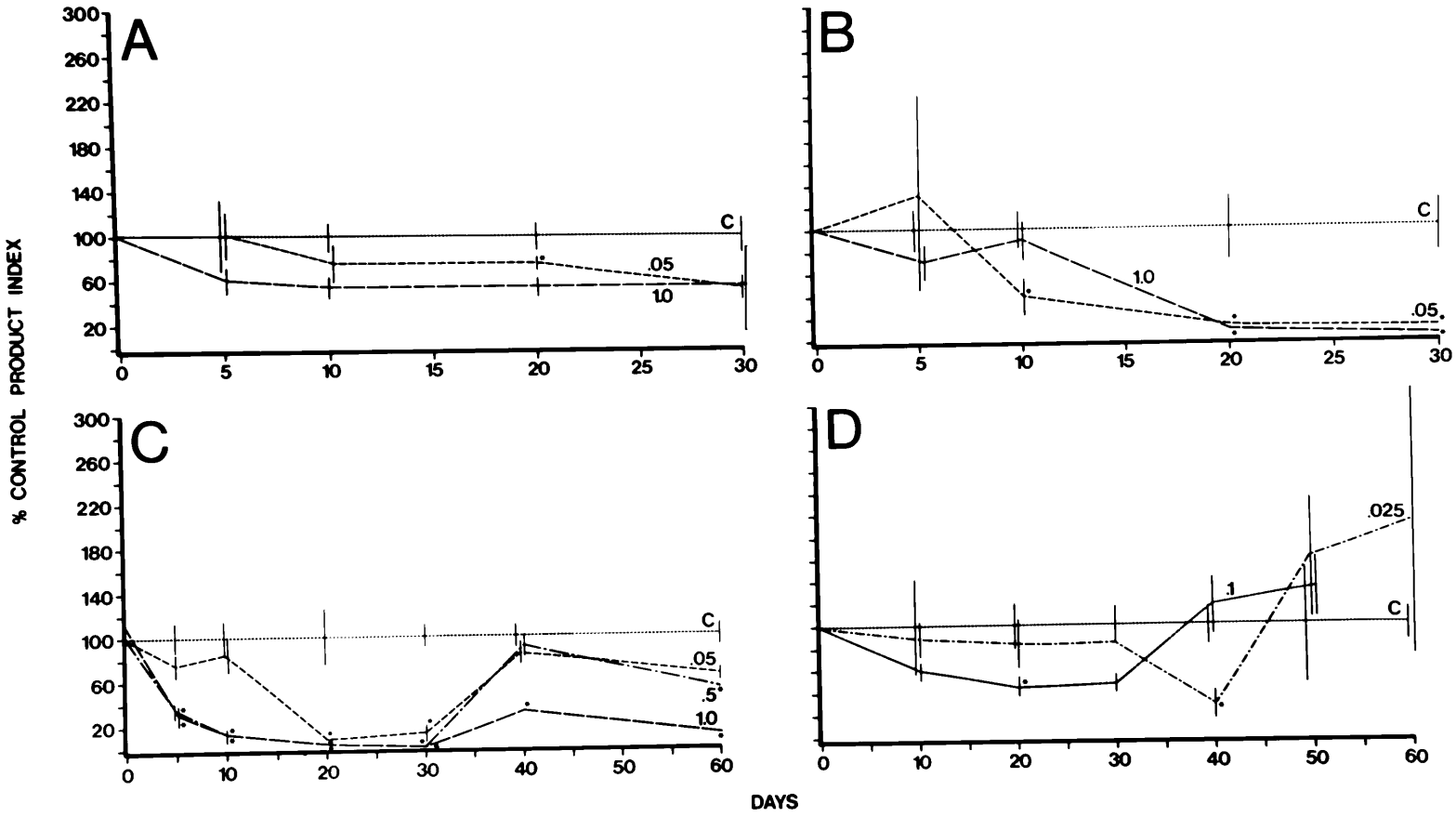

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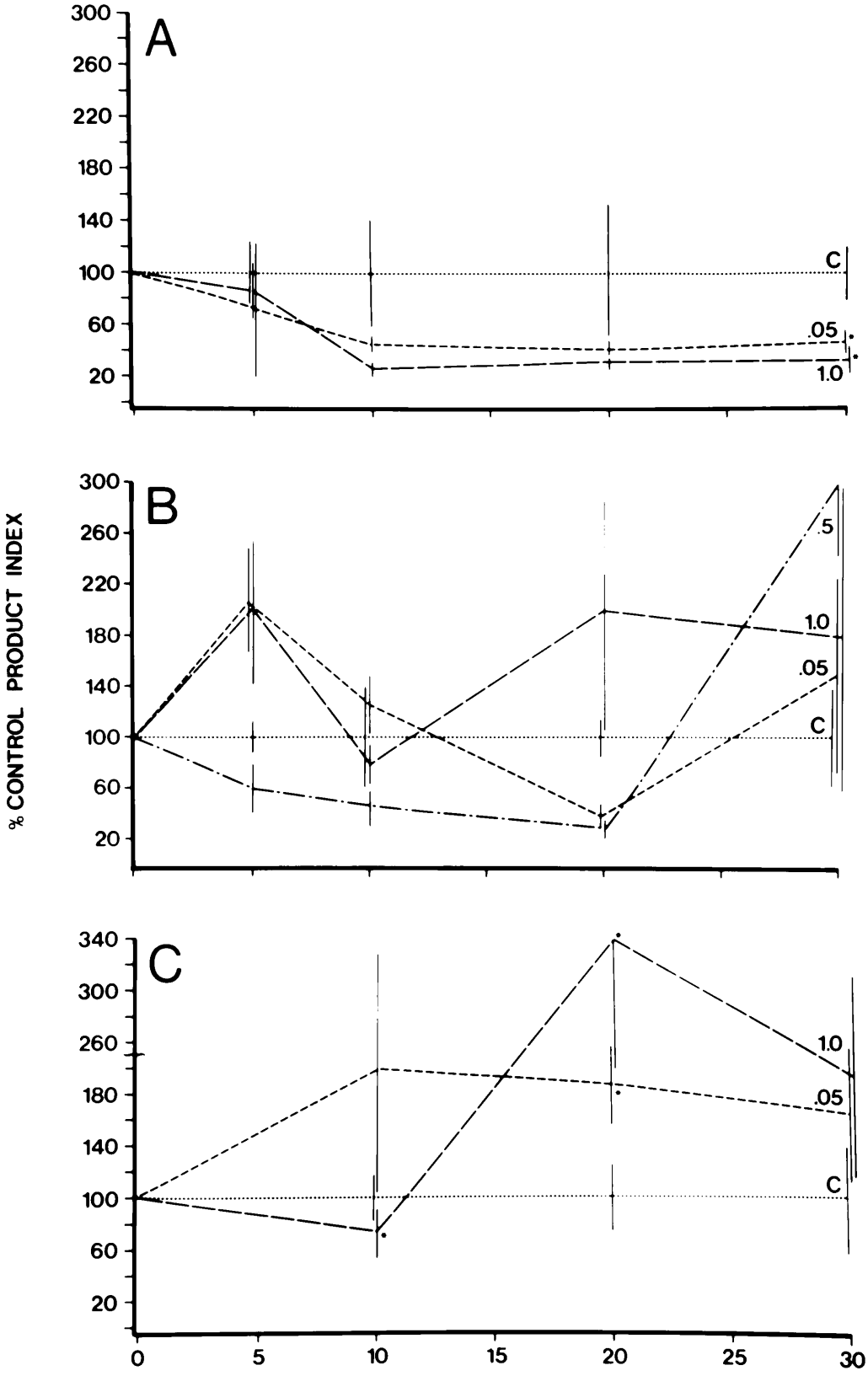
Figure 8. Response of Corbicula cellulase enzyme complex to zinc in (A) spring, 1984; (B) winter, 1984; and (C) spring, 1985 Ecosystem Simulation Laboratory studies. Data are relativized to the control product index on each day. Means ± 1 SE are given on each day. Means which are significantly different from the control are indicated by an asterisk (*).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ducted in the Ecosystem Simulation Laboratory studies compared to Glen Lyn (Fig. 8). Only in exposures conducted during spring, 1984 did activities show any dose dependent response and only after 30 days of exposure. Product indices measured during the winter, 1984 study had no discernible patterns of significant increase or decline to zinc. 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 zinc.

CELLULOLYTIC ACTIVITY IN MUDALIA

Product indices for cellulolytic responses in Mudalia showed consistent patterns of reduced enzyme activity in all exposures conducted at Glen Lyn (Fig. 9). All zinc exposures in the four seasonal tests except 0.025 mg/L caused cellulolytic activity to decline significantly below controls by day 10 at 0.05-1.0 mg/L. Enzyme activity levels of snails exposed to 1.0 mg/L zinc in spring and summer, 1984 studies had abruptly 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. 10). Enzyme activity was difficult to describe in the winter, 1984 study due to the

Figure 9. Response of Mudalia cellulase enzyme complex to zinc 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 which are significantly different from the control are indicated by an asterisk (*).

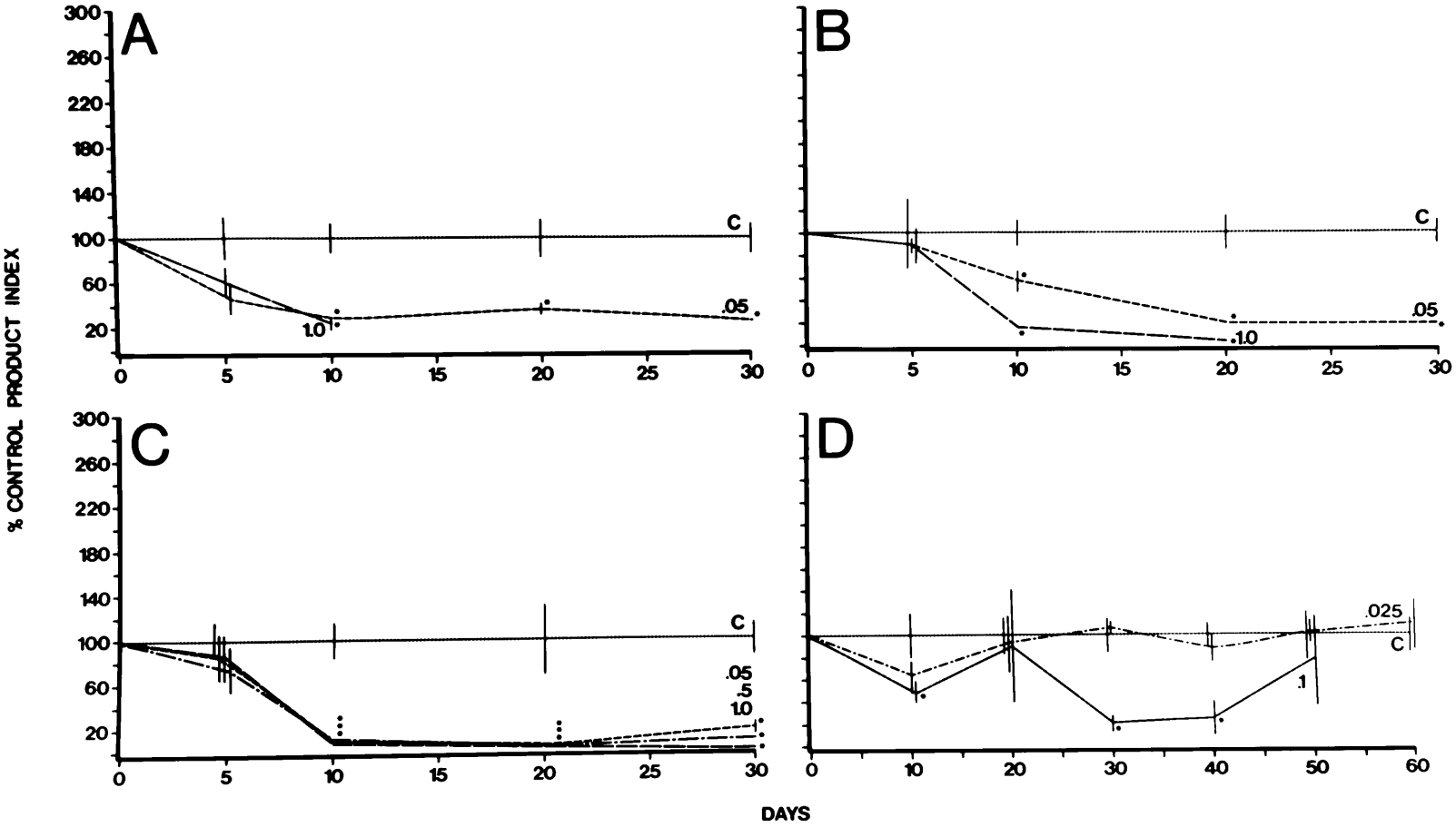
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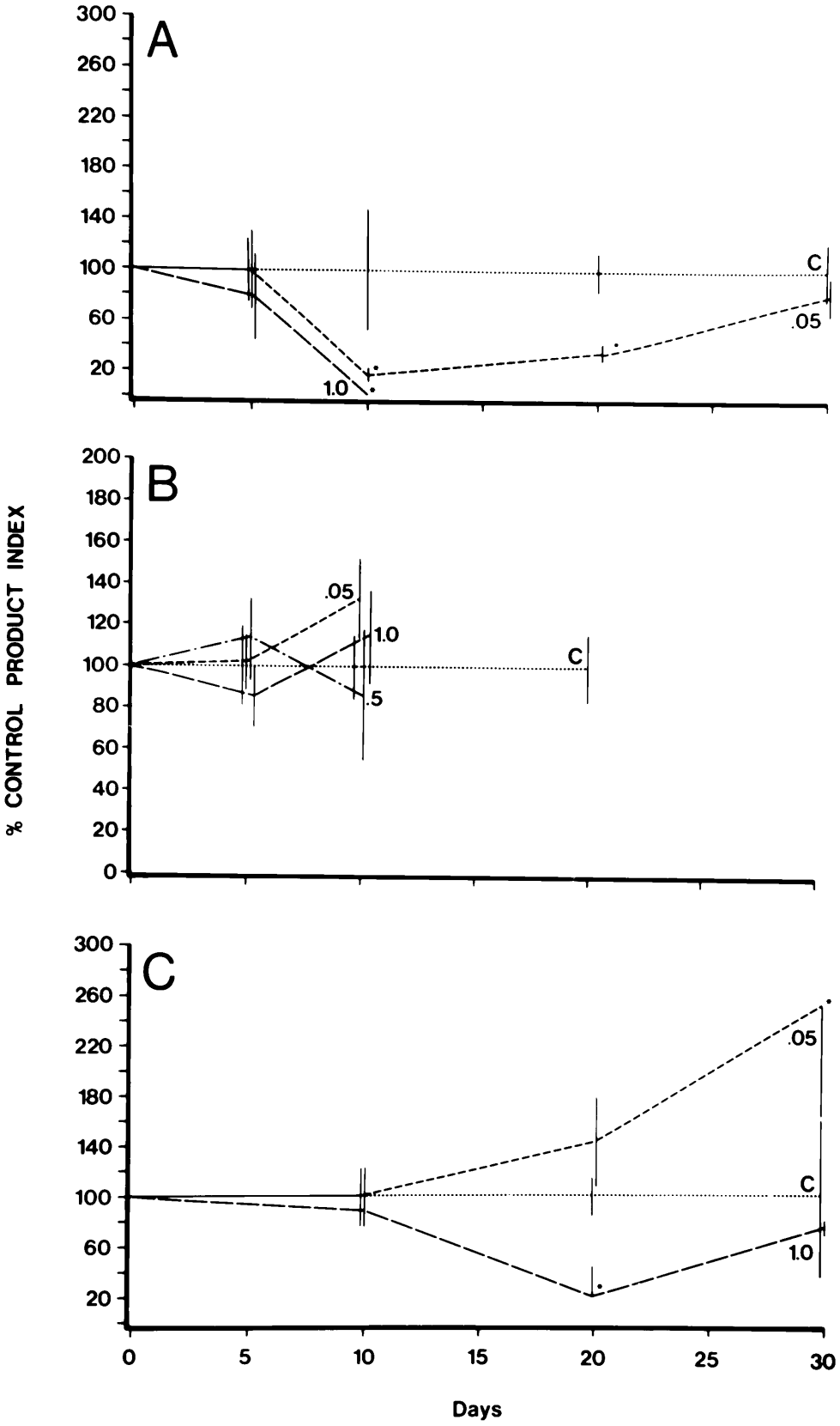
Figure 10. Response of Mudalia cellulase enzyme complex to zinc in (A) spring, 1984; (B) winter, 1984; and (C) spring, 1985 exposures at the Ecosystem Simulation Laboratory. Data are relativized to the control product index on each day. Means ± 1 SE are given on each day. Means which are significantly different from the control are indicated by an asterisk (*).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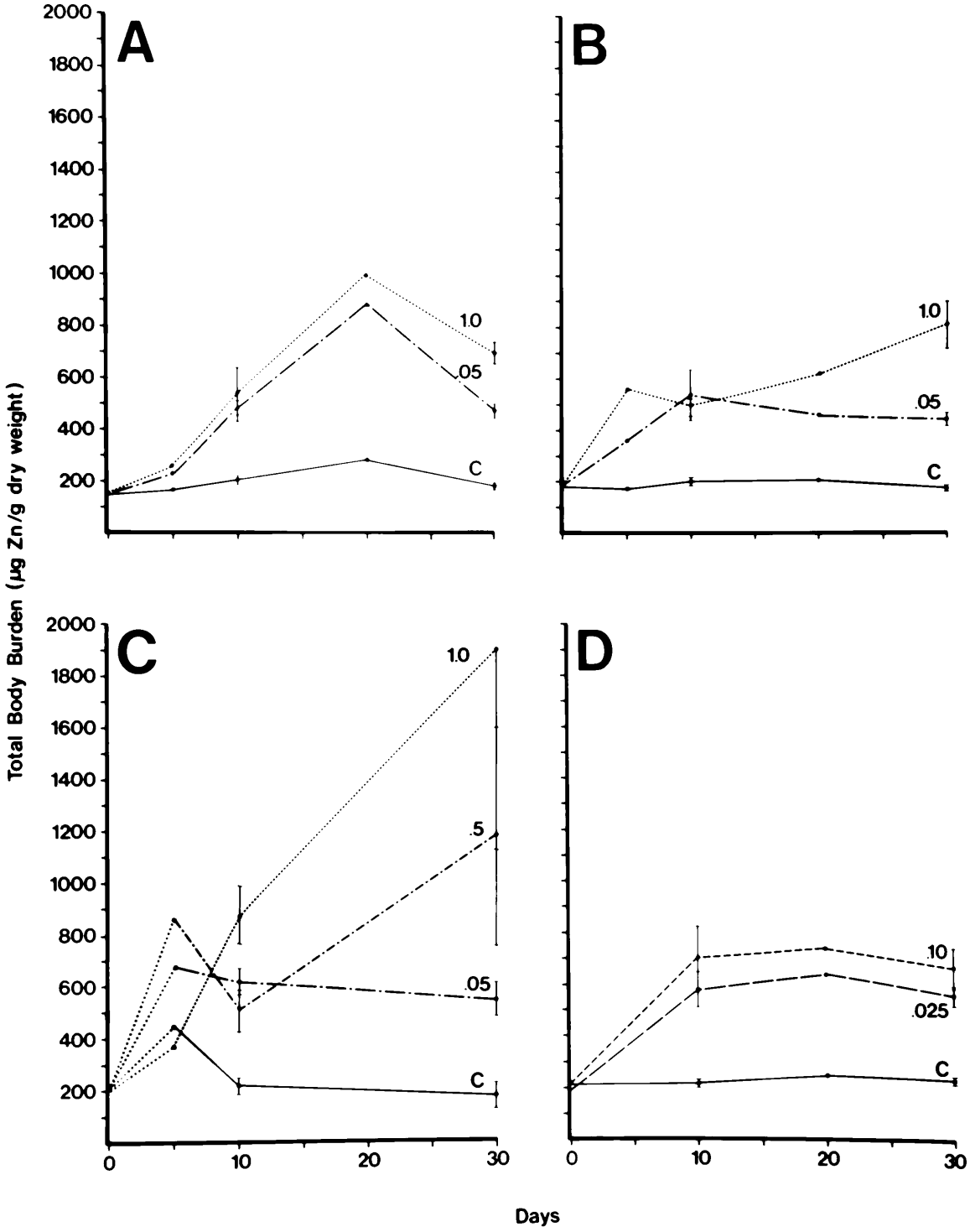
high mortality that occurred at all levels tested by day 20 with no surviving controls by day 30. Variations in cellulolytic activity during the spring , 1985 study in Mudalia were similar to responses of Corbicula for that same study where no consistent patterns of increase or decline were evident.

ZINC ACCUMULATION

Accumulation of zinc in clams exposed to low levels of zinc (0.025 and 0.05 mg/L) was similar to that occurring in clams at higher exposures during the first ten days of any study at Glen Lyn (Fig. 11). By days 20-30, high dosed clams (1.0 and 0.5 mg/L) continued to accumulate zinc when low-dosed clams (0.025 and 0.5 mg/L) failed to display continuing accumulation. This pattern was especially evident in the fall, 1984 study for both 1.0 and 0.5 mg/L exposures (Fig. 11). Background body burdens of controls were generally between 150-250 ug Zn/g. The highest accumulations observed were 1940 ug/g at 1.0 mg/L on day 30 in fall, 1984. Maximum accumulations for clams exposed to 0.05 mg/L concentrations ranged from 440 to 890 ug/g (summer and spring, 1984, respectively).

Accumulation patterns in snails at Glen Lyn showed that zinc uptake was more rapid than in Corbicula, with maximum total body burdens at higher exposures most often occurring

Figure 11. Total body burden of zinc 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 zinc exposures.


A
B
C
D
Total Body Burden (µg Zn/g dry weight)
Days
2000
1800
1600
1400
1200
1000
800
600
400
200
0
10
20
30
1.0
.05
.5
.025
C

by day 10 (Fig. 12). Snails failed to accumulate the metal at high zinc doses throughout the 30-day exposures while at lower concentrations (0.025-0.1 mg/L) they most often had uptake patterns similar to that for Corbicula. Background body burdens for controls ranged from 108 to 391 ug/g zinc. The highest accumulations observed were 1403 ug/g at 1.0 mg/L on day 10 in the fall, 1984. Snails exposed to lower (0.05 mg/L zinc) concentrations accumulated zinc at moderately low levels, 401 to 516 ug/g (spring and fall, 1984, respectively).

In the fall, 1984 study at Glen Lyn, Corbicula quickly depurated accumulated zinc body burdens in 17 days at all levels of exposure after being placed in streams not contaminated with zinc (Fig. 13). This depuration pattern was again evident at lower level zinc exposures (0.025 and 0.1 mg/L) as early as 10 days following addition of uncontaminated river water into the artificial streams in the spring, 1985. Mudalia also depurated zinc accumulation within 10 days following cessation of zinc exposure and subsequent addition of clean water. Depuration was considered complete after 20 days of recovery in the 0.025 mg/L concentration exposed snails.

Figure 12. Total body burden of zinc 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 zinc exposures.

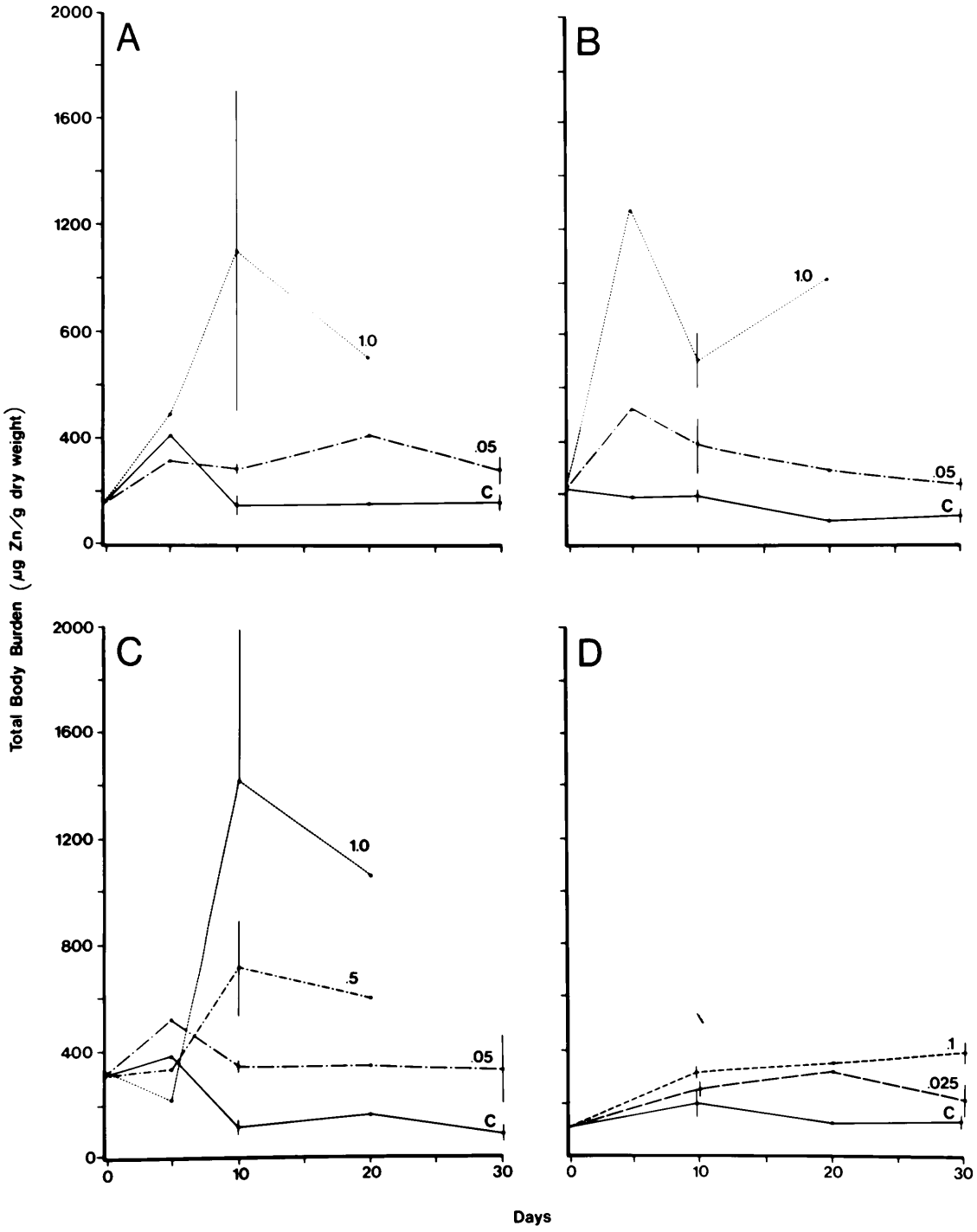
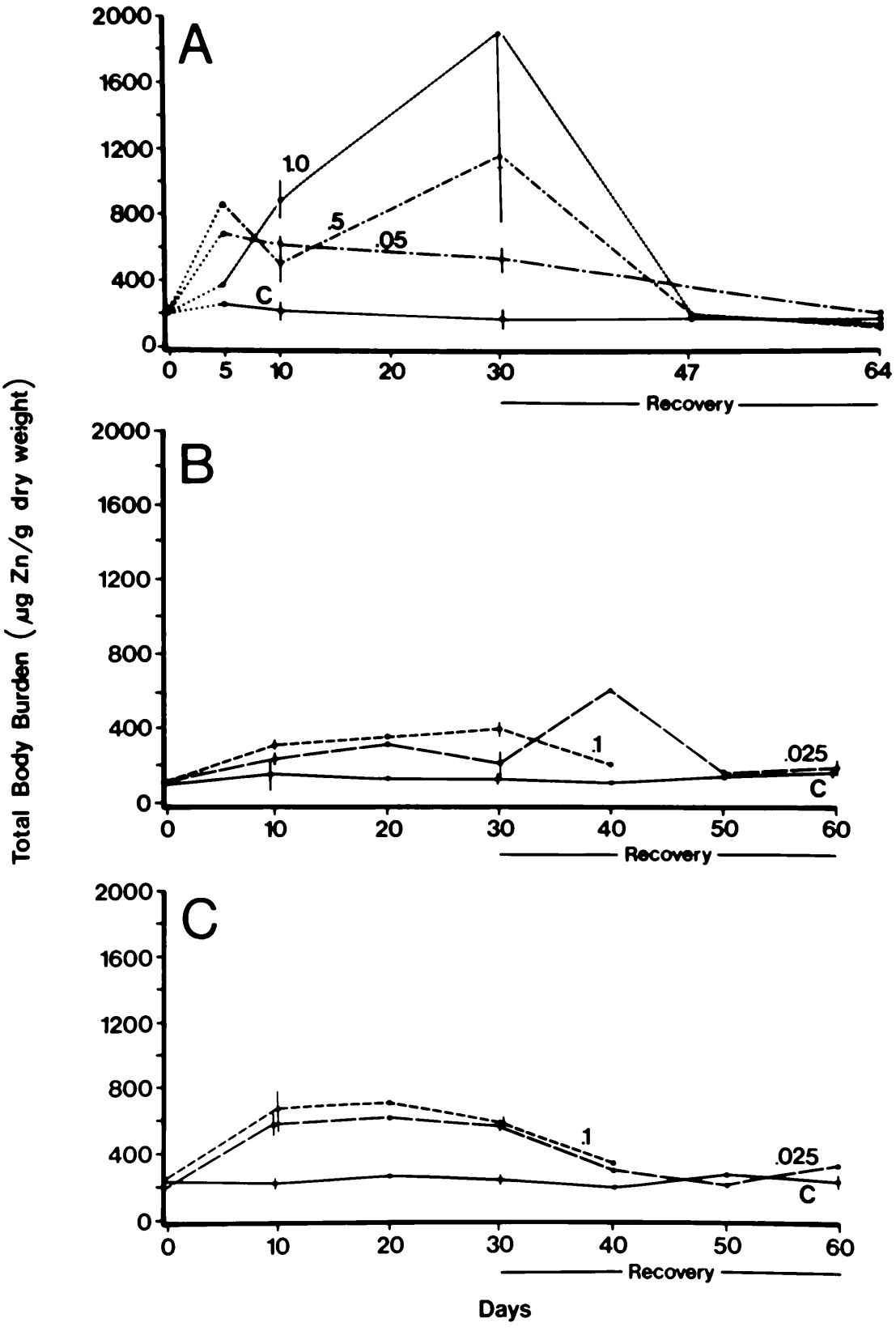

A
B
C
D
2000
1600
1200
800
600
400
0
1.0
.05
C
.5
.025
1
0
10
20
30
Days
Total Body Burden (µg Zn/g dry weight)

Figure 13. Total body burden of zinc for *Corbicula* during recovery following exposures in (A) fall, 1984 and (B) spring, 1985 at Glen Lyn; total body burden of zinc for *Mudalia* during recovery following exposure in (C) spring, 1985 at Glen Lyn. Means ± 1 SE are given on days 10, 30, and 60.


A
B
C
1.0
.5
.05
C
.1
.025
C
.1
.025
C
2000
1600
1200
800
400
0
0
5
10
20
30
47
64
40
50
60
Recovery
Total Body Burden (µg Zn/g dry weight)
Days

DISCUSSION

The utility of the cellulolytic product index as a general stress indicator was substantiated by the significantly reduced activity found in both Corbicula and Mudalia in long-term exposures to zinc in both laboratory and field-oriented artificial streams (Figures 7-10). Laboratory artificial stream exposures generally were inconclusive for indicating any direct correlations with zinc induced stress except to show that even control snail and clam populations undergo stress attributed to starvation by day 30 during long-term holding. 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 (Figs. 8 and 10). However, as in all ESL experiments, unrelativized activity of both endo- and exocellulase activity of control organisms were inconsistent (Farris et al. in review). 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 were obviously met in field-laboratory experiments where suspended periphyton were transported to streams via river water. This assumption was not only supported by more consistent trends in cellulolytic

activity in both snail and clam control organisms, but also agrees with simultaneous growth analysis performed upon Corbicula. Belanger et al. (1986) found tissue and shell degrowth in control populations for the spring 1984 ESL study. Their work was able to show that algal growth in artificial streams together with transported suspended phytoplankton was able to support growth in Corbicula held in artificial streams at Glen Lyn. Dauble et al. (1984) has reported that algal densities of 1,000 cells/ml 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. 1984; Foe and Knight 1985, 1986; Belanger et al. 1986a) have shown that dietary requirements of Corbicula are fairly specific. Studies using Corbicula to examine toxicity (Hartley and Johnston 1983; Harrison 1984; Belanger et al. 1986a) or bioindicator potential (Graney et al. 1984, 1984) may have failed to adequately assess clam health during long-term exposure. Effects on cellulolytic activity due to starvation, which complicated interpretation of zinc effects, were not apparent until days 20 to 30 in our laboratory exposures. This complication related to duration of testing may be even more important when considering the initial state of Corbicula with respect to testing at different periods of the year. Seasonal differences in energy allocations in

Corbicula has been shown to affect asbestos uptake as reflected in growth rates (Belanger et al. 1986b).

Metal uptake and variations in cellulolytic activity in our studies was clearly more pronounced during certain seasons at comparable zinc exposures (0.05 and 1.0 mg/L). 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. and the Seasonal fluctuation in bioavailability is 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 (Poe and Knight 1985).

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 zinc 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 suffi-

ciently sensitive to detect this difference in indicator species of differing trophic strategies, and supports the selection of these organisms and an enzymatic test specific to feeding responses.

Body loads of metals among molluscan herbivores, suspension feeders, carnivores and detritivores are obtained through various routes (Pentreath 1973; Young 1977). Forstner and Whittmann (1979) proposed filter-feeding bivalves as the most suitable indicator among molluscs; however, they stressed that the importance of gastropods is related to the uptake of heavy metals within a food chain in which the species can be at any trophic level (phytophagous, deposit-feeding, and carnivorous). In comparing the uptake of zinc 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 studies. In outdoor streams, algae accumulated up to 5000 µg Zn/g dry weight at 0.05 mg/L and 60,000 µg Zn/g dry weight at 1.0 mg/L (Farris et al. in review). Graney et al. (1983) found no relationship between accumulation in Corbicula and zinc exposure at concentrations from 0.2-0.8 mg/L for thirty days. However, they failed to consider alternative exposure routes where algae, particulates or sediment may have affected exposure levels.

Belanger et al. (1986a) demonstrated that growth rates of Corbicula feeding on zinc laden algae were reduced even though zinc was not detectable in test water.

Accumulations of zinc by algae at Glen Lyn most likely contributed to the maximum zinc accumulations that occurred by day 10 in snails exposed to high concentrations (0.5-1.0 mg/L). Accompanying significant declines in cellulolytic activity always accompanied those periods of maximum accumulation (Fig. 9). 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 zinc exposures (0.05 mg/L) 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 zinc exposure concentrations tested (0.025 and 0.05 mg/L).

Graney et al. (1983) found no significant differences in the body burdens of clams exposed to 0.2 and 0.4 mg/L zinc and proposed that Corbicula was able to regulate zinc uptake and/or excretion. Clams and snails in our studies also effectively regulated zinc body burdens at lower exposure levels (0.025 and 0.05 mg/L). Regulation of zinc accumulation and its relavance to snail condition was confounded by depuration and/or excretion which 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 zinc in clams on day 20 in spring, 1984 and spring 1985 studies corresponded with nonsignificant differences in cellulolytic activity occurring on day 30 following earlier periods of significant decline.

Snails were unable to effectively depurate zinc in the first 10 days of exposure. However, clams had adelayed response of uptake and declining cellulolytic activity which occurred by day 20. Ths suggests 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. Bivalve molluscs can adduct the valves tightly in unfavorable conditions and some gastropods withdraw into the shell and tightly close the operculum. This behavioral avoidance mechanism can be affected by a number of natural parameters (Bayne 1976). Bivalves also react to toxicants by incorporating changes in valve adduction with adjustments in filtration rates, as well as depressing normal burrowing behavior (Phillips 1980). 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 absense of sufficient available food in laboratory experiments not only removed an uptake route but in addition may have 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 served as a general stress indicator for zinc exposure in both Corbicula and Mudalia at all zinc levels and seasons tested. Uptake routes, behavioral modifications, and species selection are factors of critical importance to the consideration of effective monitors of heavy metal discharges. Our study further supports the use of site-specific artificial stream systems together with in situ or laboratory studies for evaluating more sensitive functional tests to qualify indicator potential of selected organisms.

CHAPTER FOUR: VALIDATIONS AND COMPARISONS

INTRODUCTION

Assessment of the extent and severity of damages caused by power plant effluents most often involves either documenting qualitative and quantitative shifts in resident populations (structural measurements of organisms present) or documenting the presence of principal toxicants in both biotic and abiotic compartments within the receiving system. Major constituents within these complex effluents known to adversely affect aquatic ecosystems include thermal and pH excursions, suspended particles associated with fly ash, heavy metals and other metals such as arsenic and selenium, and chlorine (Cherry et al. 1977; Cherry et al. 1979). These components are known to cause ash siltation of benthic habitat (Specht et al. 1984), heavy metal bioaccumulation in invertebrates and fish, and alter osmoregulatory capacities of fish and macroinvertebrates (Peters et al. 1985). The effects of these perturbations include simplification of food chains, reduction in number and diversity of heterotrophic bacteria populations, reductions in diversity and density of benthic macroinvertebrate communities, and reductions in fish populations (Specht et al. 1984). However, measurements of shifts in resident populations fail to provide information

on recovery potential of surviving individuals , or on potential affects that may occur in response to persistent toxicants. Similarly, determination of metal body burdens may help assess exposure level or persistence, but does not necessarily relate to the potential of toxicity or relative health of the surviving organisms (Mehrle and Meyer 1980).

A number of biochemical tests for diagnosing acute or chronic pollutant stress have been successfully developed (Bitton 1982; Payne 1984; Neff 1985). Although these tests are sensitive at sublethal exposures, their usefulness in monitoring complex effluents is complicated by the need to correlate sensitive internal changes in individuals with adverse effects occurring in entire populations in receiving systems. The current use of laboratory bioassays to predict effects on aquatic ecosystems is being challenged as not providing an accurate representation of the responses of resident aquatic biological communities (Kimball and Levin 1985). Attempts to overcome limitations have included direct instream measurement of biological response (Weber 1981), integrated field- laboratory approaches to toxicity testing (Cherry et al. 1986) and simultaneous toxicity testing at several levels of biological organization (Cairns, 1983).

In addition to identifying biological measures best suited for evaluating effects of power plant effluents, there is inherent difficulty in relating biological responses to various components of complex effluents. Fetterolf et al.

(1986) noted that consideration of changes in effluents through time must include variability in composition and discharge rates. It is clearly more difficult to assess complex effluents than single chemicals. For this reason alone, careful consideration must not only be given to the distribution and concentration of the effluent as it interacts with the receiving system, but also to the distribution of aquatic habitats in relationship to the distribution of the effluent (Dickson and Rodgers 1986).

The purpose of this investigation was to incorporate functional measurements (rates of change in cellulase indices and metal accumulation in two molluscs, Corbicula fluminea and Mudalia dilatata, from field artificial stream exposures with information from laboratory and field bioassays to provide essential information on the interaction of chosen components of power plant effluents (cadmium and zinc; alkaline and acidic pH and zinc). Cellulolytic indices from these component studies were compared with cellulolytic responses in Clinch River caged molluscs. Cellulolytic responses were then compared to conventional instream biological responses (e.g. macroinvertebrate community structure) and water quality monitoring. Laboratory generated toxicity estimates were compared to more rigorous testing schemes in field-laboratory artificial streams and in-stream monitoring to provide evidence for incorporating an integrated field-laboratory assessment of complex power plant effluents with

standard bioassay protocols. Data reported in this manuscript represent further application of the use of cellulolytic activity as a general stress index in bioindicator species. Utilization of the index in previous field artificial stream exposures to zinc (Chapters 2 and 3) have shown that its application to field exposures of higher complexity deserves further examination.

MATERIALS AND METHODS

Both short (96-hr) and long-term (14 to 30-day) bioassays were conducted in the Ecosystem Simulation Laboratory (ESL) and at Glen Lyn under conditions ranging from static to artificial stream flow-through from July 17, 1984 to September 16, 1985.

Two, 30-day exposures using the Asiatic clam (*Corbicula fluminea*) and a snail (*Mudalia dilatata*) were conducted in artificial streams at the Glen Lyn field laboratory. Clams and snails were exposed to zinc and cadmium alone and in combination from April 26 to May 26, 1985. This exposure period was followed by a 30-day recovery study involving both species. Clams and snails in the second study were exposed to a low level of zinc (0.05 mg/L) under pH controlled conditions from July 9 to August 8, 1985. Molluscs were analyzed for cellulolytic activity and metal accumulation in response to duration and degree of exposure.

Cellulolytic activity in caged clams and snails transplanted within the confines of the Clinch River Power Plant Carbo, Virginia, during the period from September 12 to September 25, 1985, was compared with results from in-stream monitoring of water quality parameters and macroinvertebrate sampling for that same period.

ORGANISM COLLECTION AND HANDLING

Adult Corbicula, 15 to 17 mm in shell length, were collected from the New River, Virginia (River mile 100). Adult Mudalia, 12.0- to 15.0-mm shell length, were removed from rocks upstream from the Glen Lyn Power Plant in the New River, Virginia. Both clams and snails were immediately transferred to artificial streams either in the ESL or at Glen Lyn following collection. Clams in ESL streams were fed 500 ml of Chlamydomonas rheinhardti cultured in Bold's Basic medium (625 cells/L) every day; Glen Lyn streams were colonized by resident algae from the New River, so no food supplements were needed. During acclimation, precolonized rocks transferred from the New River to ESL streams provided food for Mudalia.

SHORT AND LONG-TERM BIOASSAYS

Corbicula and Mudalia were exposed to zinc as $ZnSO^4$, at concentrations ranging from 0.5 to 40 mg/L by placing 10 clams and 10 snails on a raised plexiglass platform in 15-L polycarbonate containers after Belanger et al. (1986a). All ESL exposures had dechlorinated tap water as diluent. A second test was conducted using the same target concentrations but containers were stirred.

Identical test conditions were used in exposures to alkaline pH using 6M sodium hydroxide to adjust pH to 8.5, 9.5, 10.0, 10.5, 11.0, and 12.0. Also, identical test conditions were incorporated using 6M HCl to adjust pH to acidic exposures of pH 4.0 5.0 6.0 and 7.0. Ten clams were used in each replicate test container (twenty clams total) and pH was monitored and manually adjusted daily.

Semi-static (only toxicant added but with current) artificial stream bioassays were conducted in the ESL using paddle driven streams with 20-L capacities (Farris et al.,in review). Regulation of pH to 9.0, 9.5, 10.0, 10.5, and 11.0 was accomplished using separate Fisher Model 650 pH meter controllers and Cole-Parmer peristaltic pumps to feed 6M sodium hydroxide solutions into mixing zones of each stream.

Flow-through artificial stream toxicity tests using clams and snails at the Glen Lyn field laboratory, were conducted using the aforementioned artificial streams continually re-

newed with New River water at 1.2 L/min. Peristaltic pumps were used in 96-hr tests to continuously dose $ZnSO_4$ at concentrations ranging from 0.1 to 5.0 mg/L at a pump flow rate of 0.5 ml/min. Stock solutions were held in 25-L carboys and were changed every other day. Thirty clams and thirty snails were randomly distributed in artificial streams filled to a depth of 2 cm with coarse sand.

The same experimental design as that used in zinc exposures at Glen Lyn was used for the acid pH bioassay. Regulation of pH to targeted concentrations (5.0, 5.5, 6.0, 6.5, 7.0, and 7.5) in each stream was achieved using pH meter/controllers and peristaltic pumps that delivered a 10% sulfuric acid solution to each stream.

CADMIUM AND ZINC INTERACTION TEST

Adult clams and snails were exposed in the manner as previously described in precolonized artificial streams at Glen Lyn to targeted cadmium concentrations of 0.012, 0.025 and 0.1 mg/L; zinc concentrations of 0.025 and 0.1 mg/L; and to cadmium/zinc combinations of 0.012/0.025 and 0.025/0.025 mg/L. Cadmium and zinc stock solutions were maintained separately in 25-L carboys which were changed every other day during the 30-day exposure. Metal and diluent delivery was accomplished as previously described for all other Glen Lyn experiments. Following 30 days of exposure, metal addition

to the streams was discontinued and streams were allowed to recover for 30 days with inflowing New River water.

ZINC AND PH INTERACTION TEST

Clams and snails were exposed for 30-days to zinc sulfate at 0.05 mg/L at ambient New River pH (8.39 ± 0.35) and at targeted, controlled pH concentrations of 6 and 9. Clams and snails were also exposed to controlled pH conditions of 6 and 9 without zinc addition. Each concentration tested was replicated using two streams per concentration. All metal dosing and stream experimental design was as described for Glen Lyn cadmium and zinc experiments. Streams controlled for pH were adjusted using pH meter/controllers and peristaltic pumps which delivered a 10% sulfuric acid solution for pH 6 and 6M sodium hydroxide solution for pH 9.

ENZYME ANALYSIS

Following an acclimation period of 10-14 days, six clams and snails from each exposure treatment were randomly chosen and transferred to the laboratory for dissection and weighing on days 0, 10, 20, and 30. Days 40, 50 and 60 were included for analysis of recovery during the cadmium and zinc spring, 1985 exposure. Enzyme extracts from individuals were prepared from whole body homogenates as previously described in

chapters 2 and 3. All cellulase and protein assays were described in these chapters. Activities for clams and snails were expressed as cellulase product indices which were relativized to control activities on each day of examination. Enzyme activity was statistically analyzed by the Kruskal-Wallis Test (Hollander and Wolfe 1973).

ZINC AND CADMIUM ACCUMULATION ANALYSIS

Four Corbicula and four Mudalia were removed on each sampling day from streams at each exposure, dissected and dried at 60 C for 24 h. Dry weights were recorded, and acid digestion for bioaccumulation was accomplished according to the methods of Valdes et al. (1982).

FIELD IN SITU EXPOSURE

Thirty adult Corbicula and thirty adult Fluminicola were placed in nylon mesh cages (2-mm^2 mesh size) containing precolonized cobble from the Clinch River, Virginia. Cages were tied to iron stakes at predetermined stations which coincided with macroinvertebrate sampling stations 1, 8, 11, and 15 on September 12, 1985. Six specimens of each species on day 1 were transferred to Virginia Tech for enzyme analysis as previously described. Following 14 days in the field,

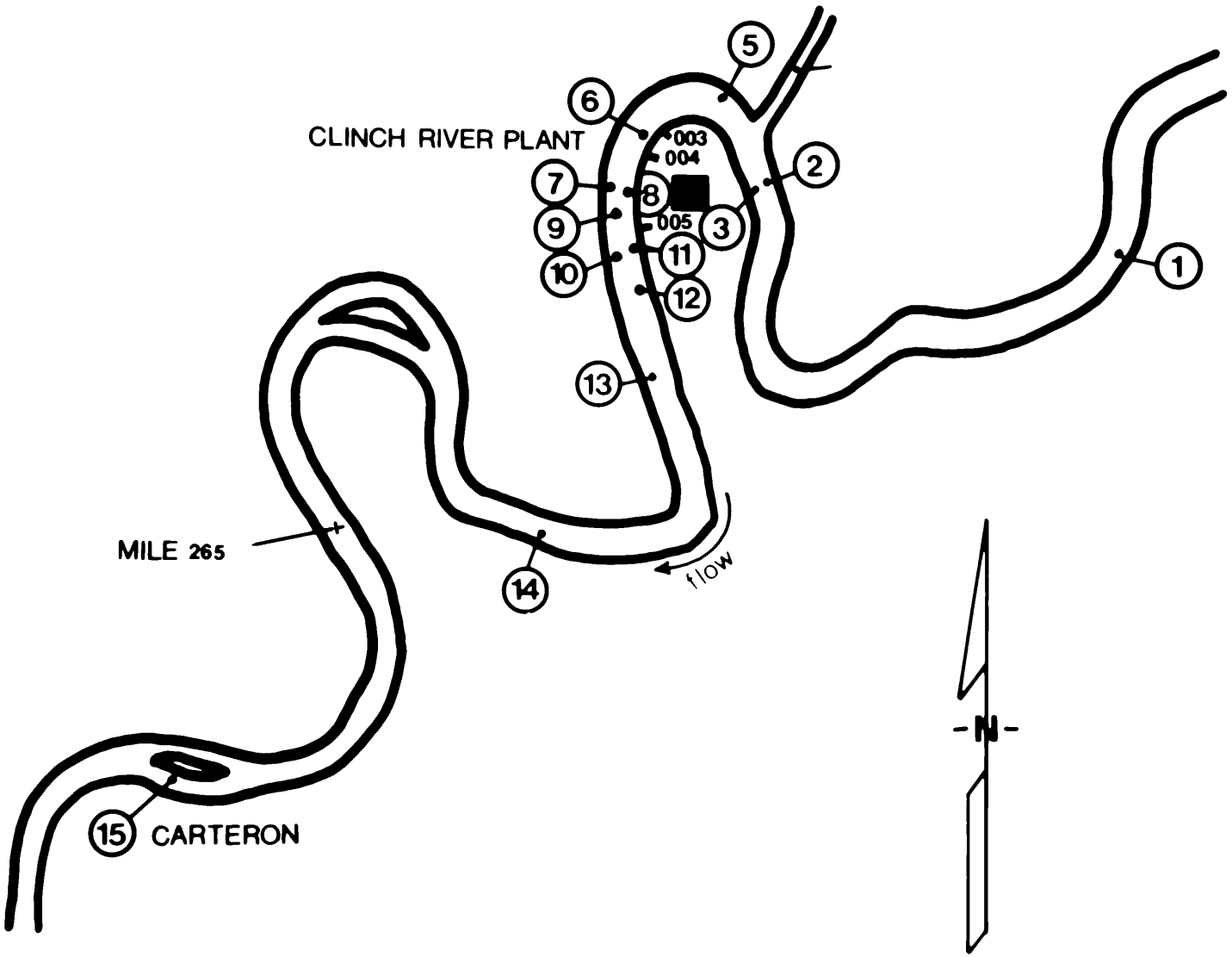
clams were retrieved from all stations and returned to Virginia Tech for enzyme analysis.

HESTER-DENDY SAMPLING/CLINCH RIVER

Benthic invertebrate monitoring using Hester-Dendy samplers was carried out at 15 sampling stations above, within, and below the confines of the Clinch River Plant (Fig. 14). Samplers were placed at each location on August 15, 1985 by securing each sampler to a rebar into the river sediment. These samplers were approximately 5 cm above the substrate and were collected September 12, 1985, for a total of 4 weeks of invertebrate colonization. Three replicates were set at each station whereby each station had all replicates set 0.3 m behind each other (replicate A being most upstream followed by B, then by C).

Stations 1, 2, 5, 7, 10, 14, and 15 were located on the right side of the river facing downstream; stations 3, 6, 8, and 11 on the left side; and stations 9, 12, and 13 in the middle (Figure 14). To determine potential plant or other industrial impacts, stations 6, 8, and 11 (left side of the river) were located downstream from discharges 003, 004, and 005, respectively. The coal washing facility was represented by station 4 (within Dumps Creek), 5 (Clinch River just below Dumps Creek), and 7 (Clinch River below station 5 on the same side). Stations 14 and 15, furthest from the plant, repres-

Figure 14. Schematic drawing of the Clinch River, Dumps Creek, and associated discharges from the Clinch River Plant showing sampling stations for in-stream monitoring.


CLINCH RIVER PLANT
003
004
005
MILE 265
flow
CARTERON
N

ented areas that were either pool-like or potentially stressed by siltation from road-side repairs, respectively.

Samples were retrieved, washed, sieved in the field with a 500 µm sieve, and preserved with 10% formalin. Samples were randomly selected, sorted to taxonomic orders and placed in 70% ethanol. Chironomids were identified to tribe by characteristics given in Merritt and Cummins (1984). This key allowed identification of remaining aquatic insects to genus, while species identification was made possible by Brigham et al. (1982). Mollusc identification was based on family characteristics given in Burch (1982). Summaries of trophic relationships was compiled from Merritt and Cummins (1984).

Invertebrate data were analyzed by analysis of variance (ANOVA) by the Statistical Analysis System (SAS Institute Inc. 1982). The effects of sample location on total abundance, diversity, richness, and functional group classifications were tested with significance inferred at $\alpha=0.05$. Groups were classified using Duncan's Multiple Range Test.

RESULTS

BIOASSAYS

The results of all zinc and pH bioassays are presented in Table 8. The 96-hr LC50 value for zinc (as $ZnSO_4$) was lowest for Mudalia exposed in precolonized flow-through artificial

Table 8. LC-50 determinations for Corbicula and Mudalia exposed to zinc sulfate, acid and alkaline pH. FT indicates flow-through bioassays; S indicates static bioassays; SS indicates static-stirred; SAS indicates static bioassays in artificial streams receiving no diluent. ESL indicates bioassays conducted in the Ecosystem Simulation Laboratory and GL indicates bioassays conducted in artificial streams at the Glen Lyn field laboratory.

Toxicant	Assay conditions	Time interval	LC-50	95% Confidence Limits (Lower, Upper)	Range
Zinc	FT - GL	96-hr	snail	1.58 (1.58, 1.58)	0.00
Zinc	FT - Gl	96-hr	clam	no mortality at highest concentration	
Zinc	S - ESL	96-hr	snail	2.07 (1.43, 2.99)	1.56
Zinc	S - ESL	96-hr	clam	10% mortality at 40 mg/L	
Zinc	SS - ESL	96-hr	snail	2.43 (2.06, 2.86)	0.80
Zinc	SS - ESL	96-hr	clam	no mortality at highest concentration	
Acid	FT - GL	96-hr	snail	6.52 (6.38, 6.63)	0.25
Acid	FT - GL	96-hr	clam	no mortality at lowest pH	
Base	SAS - ESL	96-hr	snail	9.94 (9.79, 10.10)	0.31
Base	SAS - ESL	96-hr	clam	no mortality at highest pH	
Base	S - ESL	14-day	clam	10.90 (10.71, 11.09)	0.38
Base	S - ESL	21-day	clam	9.82 (9.65, 10.00)	0.35
Base	S - ESL	30-day	clam	9.39 (9.19, 9.59)	0.40

streams (1.58 mg/L) when compared with LC50 values from static tests conducted in the (ESL) (Table 8). Zinc was less toxic to clams in all bioassays conducted in both Glen Lyn and ESL studies with only 10% mortality occurring at the highest zinc concentration tested (40 mg/L) and in one single static test. Corbicula was resistent to all exposures of zinc and pH with 100% mortality occurring only after 14-day static alkaline exposures. Longer exposures to alkaline pH did result in increased toxicity in clams as LC50 values declined 1.51 standard pH units for 30-day exposures. The 96-hr LC50 value for alkaline pH for Mudalia (9.94) in ESL artificial stream exposures, was comparable to that value resulting from 21 days of static exposure for Corbicula (9.82). The 96-hr acidic exposures in Glen Lyn artificial streams resulted in no clam mortalities. The 96-hr LC50 value for snails was pH 6.52.

ZINC AND CADMIUM INTERACTION TEST

Mudalia exposed to cadmium and zinc had significantly reduced cellulolytic activity at all cadmium concentrations tested (Fig. 15 and 16), even though cadmium was measured above detection only in the 0.10 targeted concentration (Table 9). Measured zinc concentrations in cadmium and zinc combinations were closest to targeted metal levels. All other low level metal addition was lost to absorption in test

Figure 15. Response of Mudalia cellulase enzyme complex to cadmium and zinc exposures (A) separately and (B) in combination in 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 which are significantly different from the control are indicated by an asterisk (*).

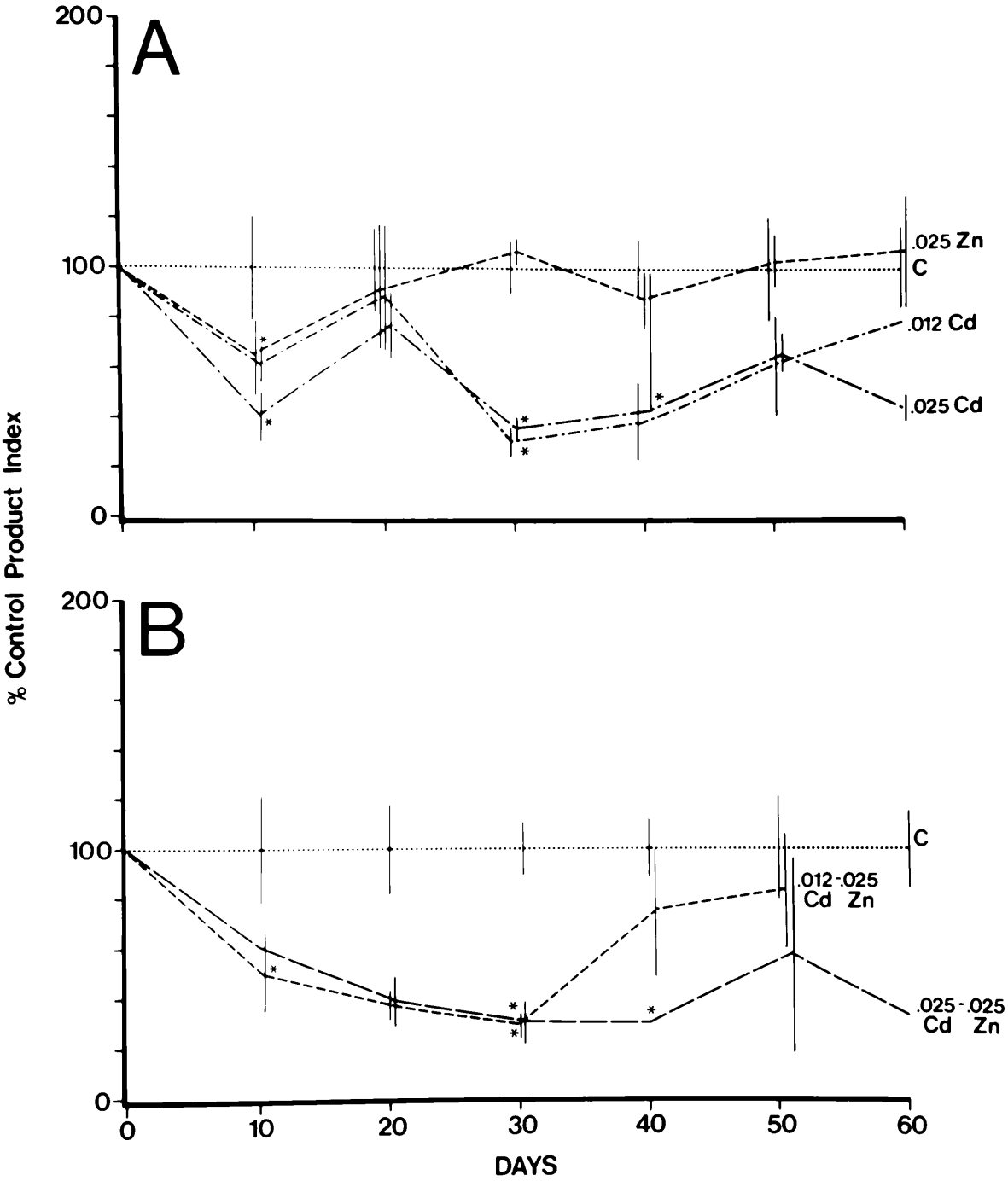

A
B
200
100
0
% Control Product Index
.025 Zn
C
.012 Cd
.025 Cd
.012-.025
Cd Zn
.025-.025
Cd Zn
0
10
20
30
40
50
60
DAYS

Figure 16. Response of (A) Mudalia and (B) Corbicula to cadmium exposures in spring, 1985, 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 which are significantly different from the control are indicated by an asterisk (*).

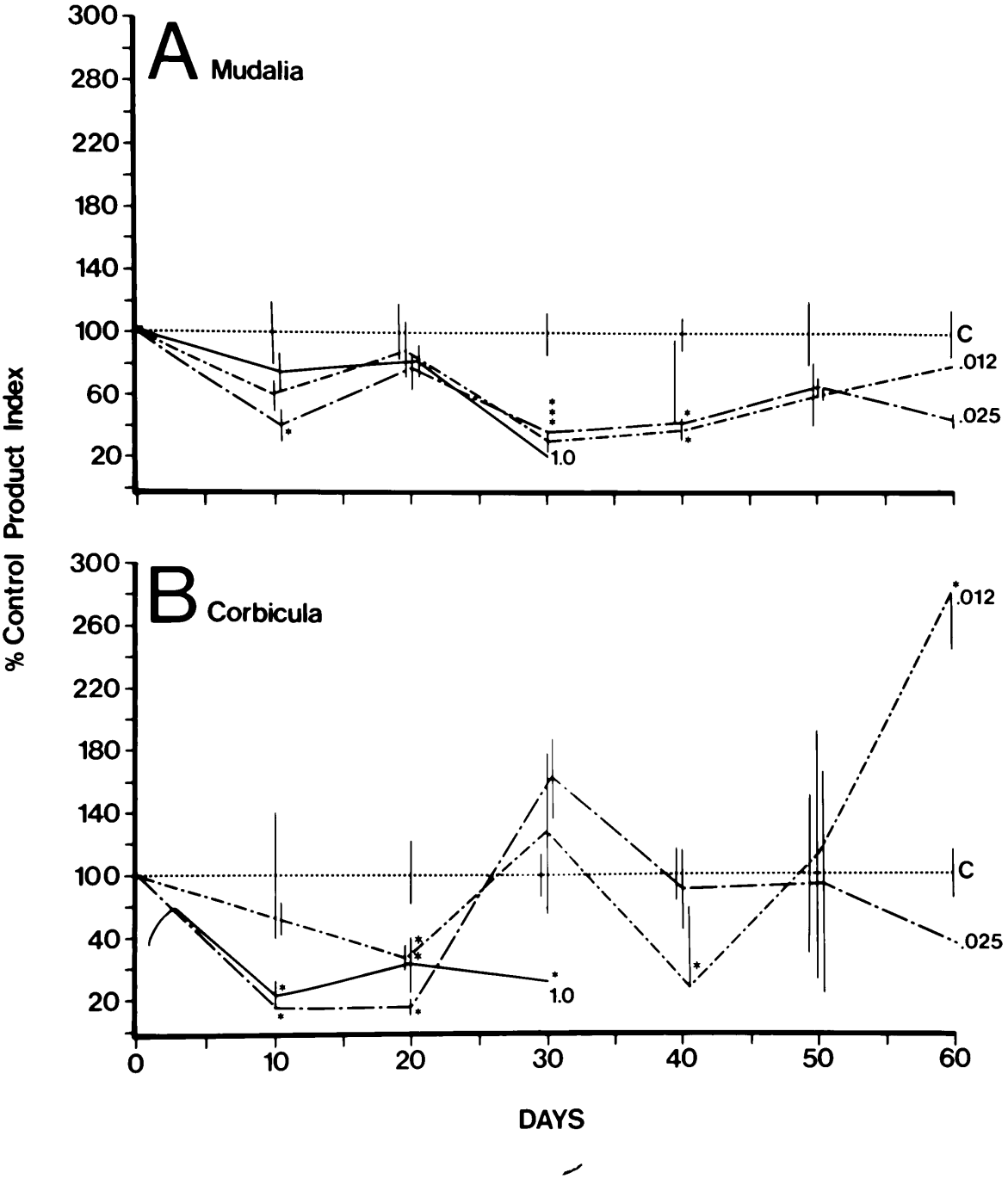

A Mudalia
B Corbicula
% Control Product Index
DAYS
C
.012
.025
1.0
300
280
260
220
180
140
100
60
40
20
0
10
20
30
40
50
60

Table 9. Means (± 1 SE) of selected water chemistry parameters analyzed during the Glen Lyn studies involving cadmium and zinc during spring, 1985 and zinc and pH control during summer, 1985 (n = 14). When toxicant is a metal, measurement is in mg/L and in standard units for pH.

Toxicant		Measured Total Metal Concentrations (mg/L)		Temp (°C)	pH	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)
A	B						
Cadmium	Zinc						
0	0	0.012 (±0)		17.2 (±0.4)	8.19 (±0.04)	69.9 (±1.6)	67.7 (±3.1)
0.025	0	0.012 (±0)		-	8.22 (±0.22)	68.0 (±5.6)	39.3 (±2.0)
0.025	0	0.012 (±0)		-	8.20 (±0.10)	66.0 (±5.1)	41.3 (±2.0)
0.10	0	0.027 (±0.01)		-	8.15 (±0.02)	67.0 (±1.4)	41.8 (±2.2)
0	0.025	0.020 (±0)		-	8.12 (±0.05)	66.0 (±5.1)	38.6 (±3.2)
0	0.10	0.12 (±0.056)		-	8.39 (±0.20)	69.0 (±6.7)	41.2 (±2.3)
0.012	0.025	0.012 (±0.0)	0.019 (±.003)	-	8.16 (±.08)	67.0 (±5.4)	41.3 (±2.5)
0.025	0.025	0.015 (±.003)	0.021 (±.005)	-	8.22 (±.25)	66.0 (±5.1)	38.4 (±2.9)

Table 9. Continued.

Toxicant A	Toxicant B	Measured Total Metal Concentrations (mg/L)	Temp (°C)	pH	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)
pH	Zinc					
Ambient	0	0.012 (±0.0)	24.8 (±0.8)	8.39 (±.35)	65 (±6.35)	41.2 (±1.4)
6	0	0.017 (±.005)	-	6.30 (±.57)	71 (±3.35)	13.1 (±8.3)
9	0	0.011 (±.001)	-	8.70 (±.25)	69 (±2.2)	42.9 (±2.7)
Ambient	0.05	0.011 (±.001)	-	8.19 (±.52)	68.5 (±6.35)	38.8 (±3.5)
6	0.05	0.026 (±.005)	-	6.41 (±.59)	69 (±1.65)	17.6 (±7.1)
9	0.05	0.014 (±.002)	-	9.10 (±.25)	65 (±2.2)	45.0 (±7.2)

systems resulting in no detectable metal in water. Exposure to 0.025 mg/L zinc alone caused no significant decrease in enzyme activity throughout 30 days of exposure and subsequent recovery. Snails exposed to both zinc and cadmium combinations had reduced cellulolytic activity that had no accentuated effects different from those variations seen in snails exposed to cadmium alone. Activity levels in snails exposed to cadmium (0.012 mg/L) both alone and in combination with zinc, returned to levels not significant from controls after 10 days of recovery. The cellulolytic index was sensitive to low levels of cadmium by day 10 of exposure.

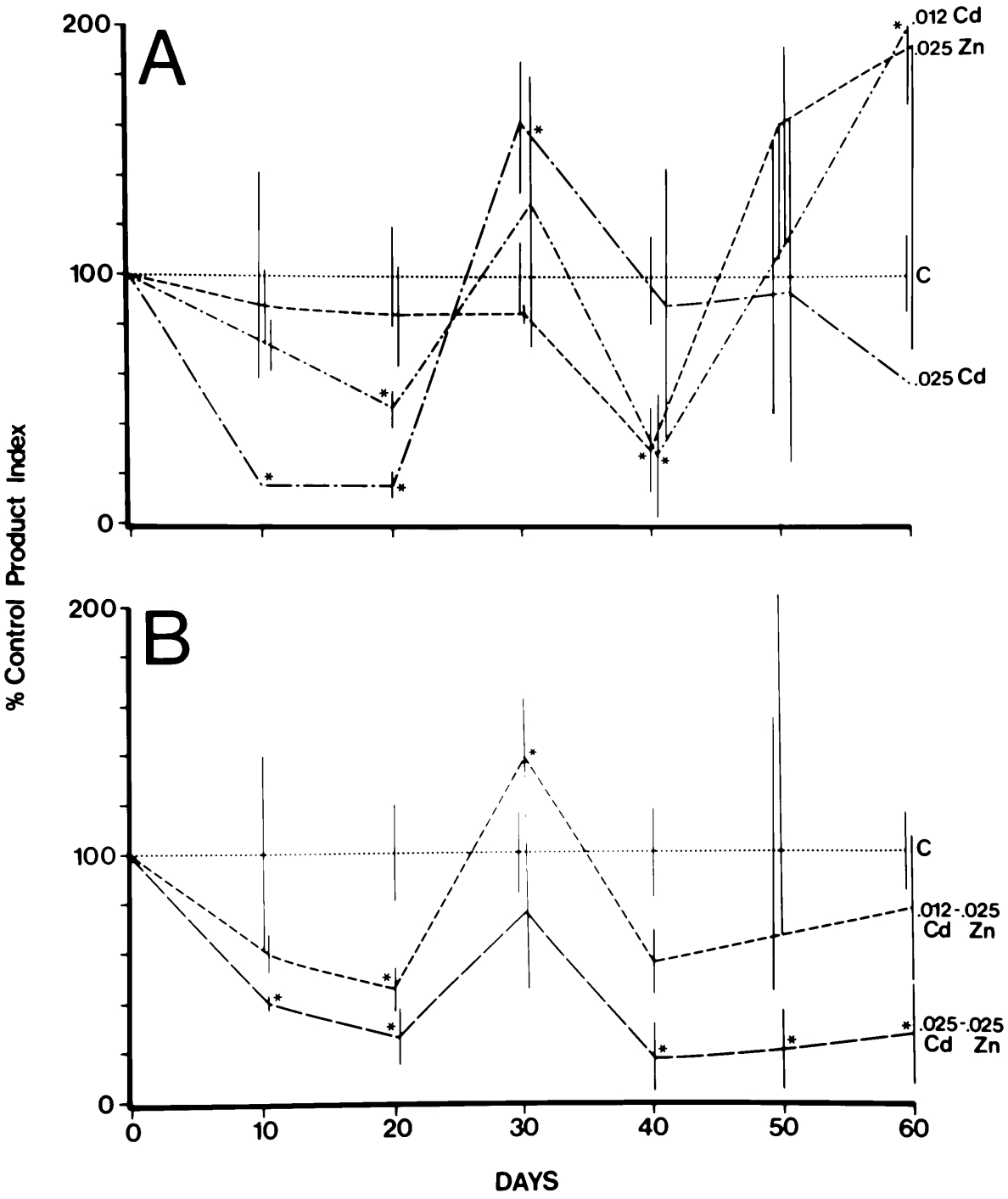
Accumulation of zinc in snails followed a general pattern of snails at low zinc exposure alone (0.025 mg/L) having body burdens that approximated that of snails exposed to the same zinc exposures plus cadmium at 0.012 mg/L (Table 10). Highest zinc accumulations occurred in snails exposed to 0.10 mg/L alone. Snails continued to accumulate both cadmium and zinc during 30 days of exposure to the 0.025 mg/L cadmium and 0.025 mg/L zinc combination to body burdens greater than those of snails exposed to either metal concentration alone. Background body burdens of controls were generally between 135-173 µg/g zinc and were not detectable for cadmium. All snails died after day 30 at 1.0 mg/L exposure zinc.

Cellulolytic activity in clams exposed to 0.025 mg/L cadmium both with and without zinc addition was significantly reduced by day 10 (Fig. 16 and 17). Activity in clams exposed

Table 10. Total body burden (µg/g) of cadmium and zinc for Mudalia during exposures and recovery in spring 1985 at Glen Lyn. Means ± 1 SE are given on all sample days (n=3).

Days Exposed	Cd	Zn	Cd			Zn		Cd, Zn			
	0	0	0.012	0.025	0.1	0.025	0.10	0.012 / 0.025		0.025 / 0.025	
0	<0.012	108.67 (±24.38)	<0.012	<0.012	<0.012	122.58 (±3.84)	112.34	<0.012	118.88 (±2.61)	<0.012	142.70 (±9.99)
10	<0.012	163.16 (±92.83)	43.40 (±0.41)	60.27 (±4.61)	94.85 (±13.41)	243.42 (±26.85)	318.31 (±23.77)	38.12 (±4.51)	213.80 (±12.79)	50.86 (±13.05)	250.08 (±3.77)
20	<0.012	135.35 (±8.60)	62.10 (±5.93)	50.09 (±8.72)	77.30 (±13.62)	316.14 (±31.52)	349.26 (±101.08)	50.62 (±8.30)	224.25 (±5.89)	66.38 (±6.91)	248.48 (±15.88)
30	<0.012	136.80 (±29.80)	72.32 (±2.58)	111.44 (±57.15)	203.39 (±0.0)	212.54 (±60.58)	400.03 (±35.01)	57.84 (±4.99)	232.04 (±23.02)	170.85 (±17.35)	330.39 (±64.21)
Days Recovered											
10	<0.012	119.77 (±10.96)	43.21 (±12.13)	63.65 (±12.56)	- -	611.00 (±443.81)	211.67 (±44.33)	33.72 (±1.17)	185.53 (±34.85)	59.17 (±19.10)	220.67 (±32.56)
20	<0.012	141.58 (±13.73)	38.97 (±3.06)	80.38 (±0.0)	- -	165.81 (±27.77)	- -	50.10 (±21.94)	258.29 (±84.53)	- -	- -
30	<0.012	173.06 (±16.89)	- -	- -	- -	184.35 (± 28.63)	- -	- -	- -	- -	- -

Figure 17. Response of Corbicula cellulase enzyme complex to cadmium and zinc exposures (A) separately and (B) in combination in 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 which are significantly different from the control are indicated by an asterisk (*).


A
200
100
0
.012 Cd
.025 Zn
C
.025 Cd
B
200
100
0
C
.012-.025
Cd Zn
.025-.025
Cd Zn
0
10
20
30
40
50
60
DAYS
% Control Product Index

to 0.012 mg/L cadmium was significantly decreased by day 20. Clams exposed to 0.012 and 0.025 mg/L cadmium, with and without zinc addition had a substantial increase in activity upon day 30, followed by a decrease in activity by day 10 of recovery. Activity levels in clams exposed to 0.1 mg/L cadmium without zinc addition did not show this pattern of inconsistent activity, but instead had significantly reduced activity throughout exposure (Fig. 16). Cellulolytic activity in clams exposed to all levels of zinc and cadmium, except the 0.025 mg/L zinc and cadmium combination and the 0.1 mg/L cadmium exposure, recovered to levels nonsignificant from control levels in 20 days of recovery. Cellulolytic activity in clams exposed to the highest cadmium and zinc combination remained reduced throughout recovery for 30 days. No recovery analysis was performed on clams exposed to 0.1 mg/L cadmium since 100% mortality by day 10 of recovery precluded any further analysis.

Accumulation of zinc and cadmium was greater in clams than in snails (Tables 10 and 11). Clams had the highest rate of zinc accumulation occurring within the first 10 days of all exposures while cadmium accumulation was additive in higher exposure concentrations. Two notable exceptions to this pattern occurred in clams exposed to 0.025 mg/L cadmium and to the 0.012 cadmium and 0.025 zinc combination exposures, where cadmium accumulations were depurated by day 20 with a subsequent return to higher body burdens of cadmium by day

Table 11. Total body burden of (µg/g) of cadmium and zinc for Corbicula during exposures and recovery in spring 1985 at Glen Lyn. Means ± 1 SE are given on all sample days (n=3).

Days Exposed	Cd 0	Zn 0	Cd 0.012	Cd 0.025	Cd 0.1	Zn 0.025	Zn 0.1	Cd, Zn 0.012 / 0.025	Cd, Zn 0.025 / 0.025
0	<0.012	216.54 (±125.02)	<0.012	<0.012	<0.012	192.82 (±3.50)	218.85 (±18.23)	<0.012 / 189.21 (±1.01)	<0.012 / 201.04 (±2.27)
10	<0.012	213.98 (±11.79)	375.87 (±126.85)	293.88 (±19.06)	223.54 (±26.00)	570.16 (±70.94)	699.00 (±123.95)	369.92 (±62.19) / 352.65 (±31.68)	165.87 (±28.70) / 346.18 (±69.07)
20	<0.012	244.92 (±25.82)	429.34 (±34.19)	68.93 (±22.76)	174.83 (±11.30)	640.95 (±57.98)	734.63 (±35.08)	71.31 (±0.0) / 617.57 (±226.78)	302.77 (±97.15) / 331.50 (±27.42)
30	<0.012	229.75 (±15.68)	366.08 (±36.66)	748.58 (±14.33)	303.83 (±72.75)	554.22 (±30.57)	560.84 (±52.91)	537.82 (±29.04) / 345.25 (±45.07)	651.04 (±131.25) / 339.45 (±14.54)
Days Recovered									
10	<0.012	191.20 (±7.27)	356.33 (±104.25)	590.96 (±0.0)	- -	270.60 (±43.49)	272.05 (±41.77)	279.20 (±21.58) / 206.37 (±7.84)	399.40 (±5.92) / 220.55 (±6.25)
20	<0.012	255.35 (±22.64)	-	-	-	188.09 (±26.00)	-	184.78 (±0.0) / 236.73 (±0.0)	- -
30	<0.012	220.12 (±11.45)	355.05 (±183.39)	265.17 (±12.61)	-	304.07 (±16.99)	-	154.95 (±33.58) / 244.44 (±15.82)	- -

30. Higher maximum cadmium accumulations occurred in low level exposures (748.58 µg/g at 0.025 mg/L cadmium) than at higher cadmium exposure (303.83 µg/g at 0.1 mg/L cadmium). All cadmium and zinc exposed clams depurated body burdens of zinc and cadmium within 10 days of recovery, with the exception of clams exposed to 0.012 mg/L cadmium alone which maintained a consistent body burden throughout the exposure and recovery period (374.03 ± 33.13 µg/g cadmium). Background body burdens of controls were between 108-173 µg/g zinc and were below detection limits for cadmium. Clams exposed to 0.10 mg/L cadmium had 50% mortality after 30 days of exposure (Table 12), while those exposed to 0.025 mg/L cadmium and 0.025 mg/L zinc combination had 35% mortality.

PH/ZINC INTERACTION TEST

Mudalia exposed to 0.05 mg/L at ambient pH and at alkaline controlled pH had the only significant declines in cellulolytic activity (Fig. 18). Significantly reduced activity occurred in snails by day 10 and continued so for the remaining 20 days in alkaline exposure. The only other significant variation in activity occurred in snails exposed to acidic pH without zinc addition having a significant increase in cellulolytic activity on day 20. This pattern was also apparent for Corbicula on days 10 and 20 of exposure to acidic pH. In both snails and clams the significant increase

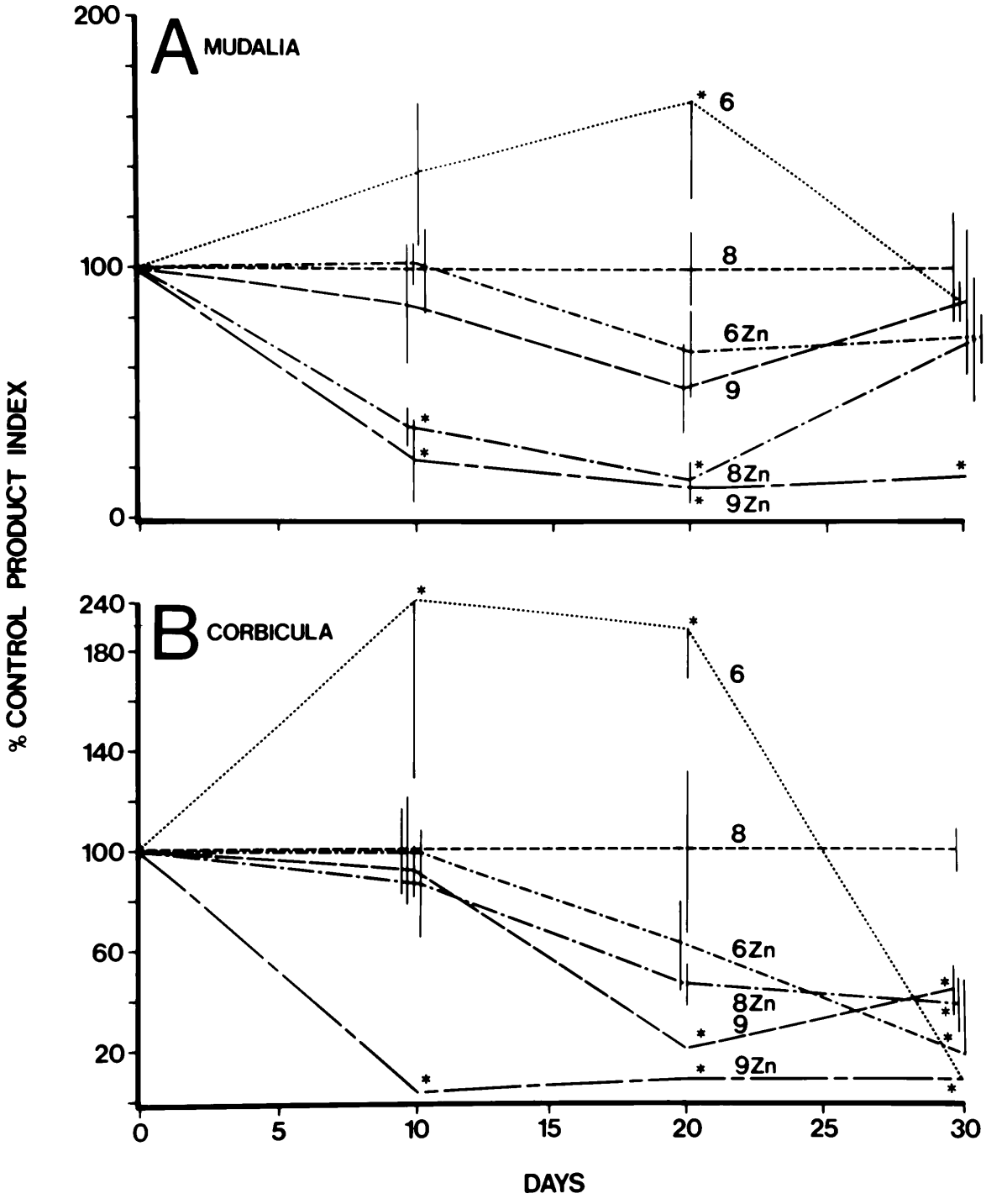
Table 12. Mortality of Corbicula after 30-days of Glen Lyn exposures to (A) cadmium and zinc exposures in spring, 1985; and (B) zinc and pH controlled conditions in summer 1985.

Target Toxicant Concentration			Percent Mortality at Each Concentration
A.	Cd	Zn	
	0	0	0
	0	0.025	5.0
	0	0.10	5.0
	0.012	0	10.0
	0.025	0	15.0
	0.10	0	50.0
	0.012 +	0.025[1]	0
	0.025 +	0.025[1]	35.0
B.	pH	Zn	
	Ambient	0	3.3
	6	0	50.0
	9	0	0
	Ambient	0.05	3.3
	6 +	0.05[2]	13.3
	9 +	0.05[2]	40.0

[1]Cadmium and zinc applied simultaneously in streams.

[2]Zinc and acidic or alkaline pH applied simultaneously in streams.

Figure 18. Response of (A) Mudalia and (B) Corbicula cellulolytic enzyme complex to 0.05 mg/L and alkaline and acidic pH exposures at Glen Lyn field-laboratory in summer, 1985.


A MUDALIA
B CORBICULA
% CONTROL PRODUCT INDEX
DAYS
200
100
0
240
180
140
100
60
20
0
5
10
15
20
25
30
6
8
6Zn
9
8Zn
9Zn

in cellulolytic activity on day 20 was followed by a rapid decline below that of controls on day 30 (Fig. 18). This was supported by 30-day mortality estimates for snails and clams exposed to acidic pH alone of 100% and 50%, respectively (Table 12).

Accumulation patterns in snails exposed to zinc and acidic pH generally followed those of controls until day 30 when maximum body burdens in snails occurred (926.94 ± 84.12 µg/g zinc) (Table 13). Snails exposed to pH control conditions without zinc addition had accumulation patterns similar to controls. Snails exposed to 0.05 mg/L zinc and alkaline pH consistently accumulated zinc to a maximum of 680 ± 195.97 on day 20. Snails from this level of exposure then depurated to 188.35 ± 30.39 µg/g by day 30.

Cellulolytic activity in clams was significantly depressed in all exposures by day 30 except for acidic pH without zinc addition. Significantly reduced activity for clams exposed to 0.05 mg/L zinc at alkaline pH was evident by day 10, and remained so for the remaining exposure period. Clam cellulolytic activity was more sensitive to pH controlled exposures than snails (Fig. 18).

Zinc accumulation was greatest in clams exposed to zinc and alkaline pH (827.20 ± 89.02 µg/g zinc) (Table 13). Clams exposed to 0.05 mg/L zinc and acidic pH had zinc accumulation patterns approximating that of controls. Accumulation pat-

Table 13. Total body burdens (μg/g) of zinc for (A) Mudalia and (B) Corbicula during artificial stream exposures in summer, 1985 at Glen Lyn. Means ± 1 SE are given on all sample days (n=4).

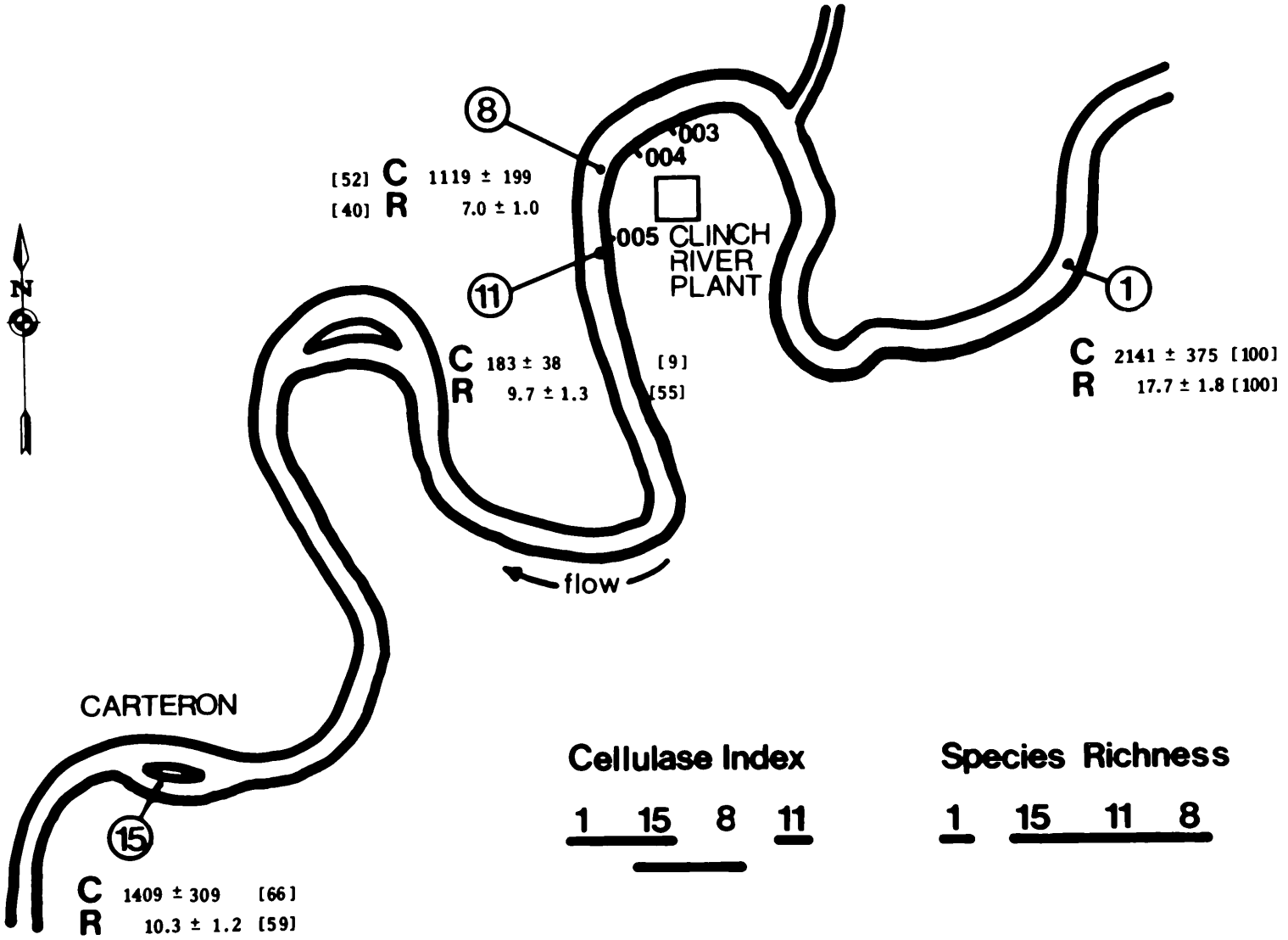
Days Exposed	Streams Without Metal Addition			0.05 mg/L Zinc Addition		
	Ambient	Controlled pH 6	Controlled pH 9	Ambient	Controlled pH 6	Controlled pH 9
A. 0	236.95 (±21.70)	227.06 (±33.98)	199.17 (±13.79)	170.76 (±17.21)	198.92 (±9.92)	231.12 (±21.91)
10	157.20 (±24.10)	130.72 (±8.31)	103.42 (±32.14)	509.69 (±101.34)	214.20 (±20.99)	427.13 (±1.14)
20	150.09 (±16.88)	162.20 (±22.12)	169.40 (±31.69)	398.45 (±37.53)	215.66 (±20.93)	680.72 (±193.97)
30	198.00 (±5.36)	254.96 (±28.44)	172.54 (±8.86)	565.74 (±49.81)	926.94 (±84.12)	188.35 (±30.39)
B. 0	229.40 (±23.72)	206.66 (±25.61)	214.96 (±19.23)	271.26 (±33.31)	206.69 (±25.48)	228.81 (±45.58)
10	209.79 (±7.30)	213.99 (±17.31)	191.03 (±35.32)	622.12 (±78.21)	291.38 (±38.18)	547.19 (±60.79)
20	217.02 (±21.30)	173.61 (±0.08)	218.26 (±34.11)	510.59 (±35.53)	261.65 (±32.87)	827.2 (±89.02)
30	273.35 (±44.25)	366.28 (±96.58)	370.51 (±17.28)	187.77 (±29.46)	159.15 (±9.50)	171.09 (±36.17)

terns for snails under pH controlled conditions without zinc addition was also similar to controls.

FIELD IN SITU EXPOSURE

Snails failed to survive 14-day exposures at stations 8 and 11 in the Clinch River, precluding any analysis of cellulolytic activity. Clams suffered no mortality at any location and were available for cellulolytic activity measurements. Clams at station 1 had the highest activity levels (2141 ± 375 [units/g dry wt]^2) (Fig. 19). Clam activity levels from stations 8 and 11 were 52 and 9 % respectively of levels measured in clams from station 1. Cellulolytic activity in clams from station 15 also had reduced activity (1409 ± 309 [units/g dry wt]^2) which was 66% of that observed in clams from station one. Activity levels measured from clams at stations 8 and 11 were significantly different from levels at station 1. Clam cellulolytic activity at station 15 however, was not significantly different from levels of clams at either station 1 or 8. Reasons for reduced cellulolytic activity at stations 8, 11, and 15 were possibly due to levels of zinc in plant effluent (e.g., 17, 28, 145, and 68 mg/L at stations 1, 15, 8, and 11, respectively).

Figure 19. Schematic drawing of the Clinch River and associated discharges from the Clinch River Power Plant. Stations 1, 8, 11, and 15 are shown and their associated cellulase indices and species richness estimates. Percentages of upstream indices are marked in parenthesis adjacent to absolute measurements. Results of Duncan's Multiple Range test are shown for measurements from these stations.


8
003
004
[52] C 1119 ± 199
[40] R 7.0 ± 1.0
005 CLINCH RIVER PLANT
11
1
C 2141 ± 375 [100]
R 17.7 ± 1.8 [100]
N
C 183 ± 38 [9]
R 9.7 ± 1.3 [55]
flow
CARTERON
15
C 1409 ± 309 [66]
R 10.3 ± 1.2 [59]
Cellulase Index
1 15 8 11
Species Richness
1 15 11 8

HESTER-DENDY SAMPLING/CLINCH RIVER

Diversity of aquatic invertebrates was lowest in Station 15 (1.98) followed by stations 8, 5, 7, 4, and 11 (2.25-2.54; Table 14, Fig. 20). Richness (the number of taxa) was lowest at station 8 followed by 4, 11, 15, and 5 (7-10.7 taxa) while the number of individuals was lowest at stations 8, 11, and 4 (29.2-49.7). Those stations with the potentially highest environmental impact (4, 5, 8, 11, and 15) had the highest percentage of gatherers (primarily chrionomids) followed by filterers with predators comprising the lowest to second lowest groups (Fig. 21). Predators were most abundant at impacted stations 4, 5, 8, and 11. Stations with the highest diversity (2.9) included 2, 3, 6, 9, and 12. Station 8 (25 m downstream of Outfall 004) had the fewest number of organisms (mean = 29.7), while Station 13 (mid-river, 0.6 km downstream of outfall 005) had the most (mean = 573). Dipterans (primarily chironomids) were the most abundant organisms at all sampling stations representing 43 to 86% of all organisms collected in a sample (Table 15). The other major insect orders collected, in decreasing order of abundance, were Ephemeroptera (0-47%), Trichoptera (1-33%), Coleoptera (0-9%), and Odonata, Plecoptera, and Megaloptera (0-7% each). Non-insect taxa comprised a low percentage of collections at all sampling stations (0-6%).

Table 14. Mean numbers of invertebrate individuals, taxa, diversity, and functional groups by station from Hester-Dendy (3 replicates) samples in the Clinch River and Dumps Creek, Virginia.

Parameter	Stations															Statistics	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	ANOVA F-value	p-value
Structural Analysis																	
Number of Individuals	294.0	116.3	94.3	49.7	131.7	98.0	196.7	29.7	192.3	160.7	34.3	180.7	573.0	75.7	93.3	5.75	.0001
Number of Taxa	17.7	15.7	15.7	9.0	10.7	14.3	14.3	7.0	17.0	15.0	9.7	16.0	20.0	12.0	10.3	7.64	.0001
Diversity	2.69	2.93	3.08	2.52	2.35	2.92	2.46	2.25	3.10	2.68	2.54	3.12	2.79	2.60	1.98	3.34	.0025
Functional Groups (%)																	
Gatherers	23	55	53	59	70	70	25	51	50	34	62	39	26	59	74	6.53	.0001
Filterers	70	34	33	17	17	23	69	34	45	51	15	54	70	24	23	8.02	.0001
Scrapers	6	9	11	12	3	3	5	1	3	12	9	4	3	5	2	1.37	.2239
Predators	2	3	2	12	9	4	1	12	2	2	14	3	1	11	1	6.08	.0001

Figure 20. Taxon diversity, total taxon abundance, and taxon richness at the 15 collection stations in the Clinch River and Dumps Creek. The mean ± 1 SE is indicated for each parameter.

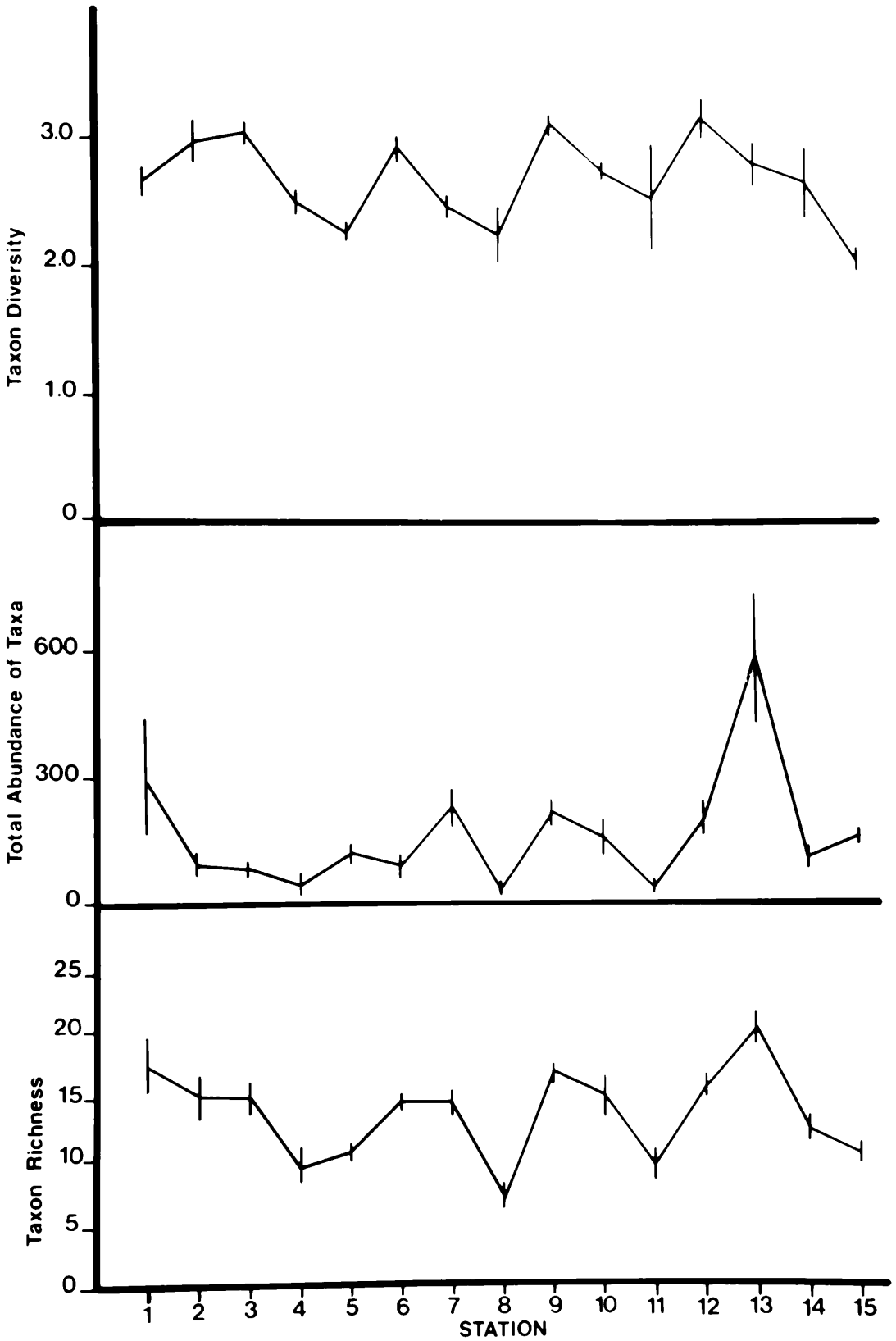

Taxon Diversity
3.0
2.0
1.0
0
Total Abundance of Taxa
600
300
0
Taxon Richness
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
STATION

Figure 21. The mean percent composition of invertebrate functional groups by station.

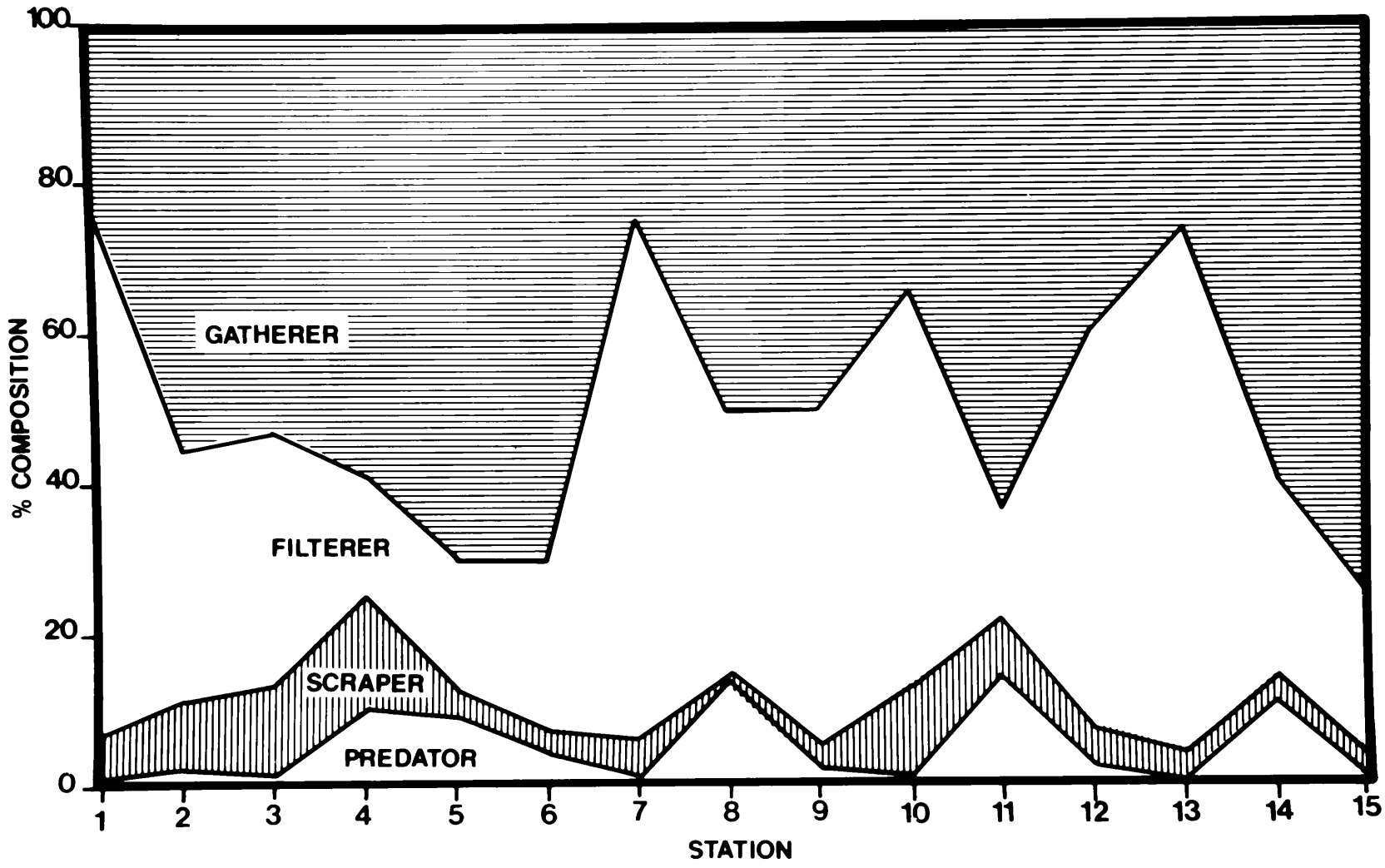

100
80
60
40
20
0
% COMPOSITION
GATHERER
FILTERER
SCRAPER
PREDATOR
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
STATION

Table 15. Percent composition of individuals in major insect orders comprising each sampling station in the Clinch River drainage system.

Station	Diptera	Ephemeroptera	Trichoptera	Coleoptera	Odonata	Other[1]
1	66.7	11.1	14.5	1.9	0	5.6
2	42.7	46.9	4.0	2.0	0	4.3
3	48.4	42.4	2.8	2.1	0.3	3.9
4	77.8	2.0	0.7	0	3.4	16.7
5	78.5	17.2	1.0	0.5	0.7	2.0
6	63.3	26.9	5.1	1.0	0.3	3.4
7	66.7	15.9	11.3	0.5	0	5.6
8	53.9	0	25.8	0	0	21.3
9	45.7	25.0	27.6	0.6	0.2	0.8
10	62.2	25.3	5.6	3.1	0	3.7
11	68.0	2.9	14.6	8.7	1.0	1.9
12	48.7	15.5	32.6	0.7	0	2.3
13	67.3	9.3	20.9	0.2	0	2.2
14	65.2	19.8	12.3	2.6	0	0
15	85.8	3.9	8.6	1.1	0.7	0
$\bar{X}$	62.7	17.6	12.5	1.7	0.4	4.9

[1]Comprised of Gastropoda, Nematoda, Oligochaeta, Plecoptera, and Megaloptera.

Mayfly proportions in the collections ranged from none collected at Station 8 to 47.2% of all organisms collected at Station 2 (0.33 km upstream of the Clinch River Plant river water intake).

In analyzing diversity, abundance, and taxon richness for selected station groups, several significant differences were noted (Table 16). In grouping stations 2, 3, 7, 10, 8, and 11 (selected pairs of left and right bank stations above the plant, and below the 004 and 005 discharges) taxon richness was significantly lower in stations 8 and 11. When analyzing differences between selected stations 7, 8, 9, 10, and 11, which were in or adjacent to the 004 and 005 discharges of the plant, taxon abundance and richness were significantly lower in those stations directly receiving the two effluents (stations 8 and 11).

DISCUSSION

IMPLICATION FROM LABORATORY TOXICITY TESTS

The effect of either static or flow-through conditions on resistence of Corbicula to zinc, alkaline, or acidic exposures were not significant in all 96-hr tests. The duration of exposure to alkaline pH before mortality was consistent with Corbicula's ability to detect and avoid toxic substances by closing the valves for 14-21 days (personal observations).

Table 16. Results of Duncan's Multiple Range Test for taxon diversity, abundance, and richness for selected groups of sampling stations. Stations connected by the same line are not significantly different.

Parameter	ANOVA Statistics	Sampling Stations	Station Group
Diversity	F=2.11; p 0.1250	2 3 7 10 11 8	Selected left and right bank
Abundance	F=5.21; p 0.0070	7 10 2 3 11 8	
Richness	F=11.9; p 0.0002	2 3 7 10 11 8	
Diversity	F=2.50; p 0.1335	6 3 11 8	All left bank
Abundance	F=15.0; p 0.0012	6 3 11 8	
Richness	F=19.2; p 0.0005	3 6 11 8	
Diversity	F=12.7; p 0.0039	2 10 7 5	Selected right bank
Abundance	F=10.2; p 0.0067	2 10 7 5	
Richness	F=1.90; p 0.2280	2 10 7 5	
Diversity	F=31.3; p 0.0037	2 1 10 14 7 5 15	All right bank
Abundance	F=3.49; p 0.0256	1 2 10 7 14 5 15	
Richness	F=1.16; p 0.3962	1 2 10 7 14 5 15	
Diversity	F=2.42; p 0.1232	9 10 11 7 8	Selected stations in and adjacent to 004 and 005 discharges
Abundance	F=16.5; p 0.0004	7 9 10 11 8	
Richness	F=10.6; p 0.0020	9 10 7 11 8	

McMahon (1979) and McMahon and Williams (1984) determined that aerial their efficiency in valve closure. Doherty et al. (in press) reported that Corbicula maintain this closure activity for 2 weeks in low dose, high dose chlorine exposures. Substantial mortality occurred after the initial 14 days of chlorination in tests with constant and low, followed by high exosure. This behavioral modification probably accounted for the lack of observed toxicity in all short-term tests.

Mudalia which lacks this avoidance mechanism did respond differently to zinc exposure in flow-through conditions at Glen Lyn by having an increased toxicity when compared with laboratory exposures. Snails at Glen Lyn could have injested metals accumulated in the periphyton (chapter 2 reported accumulation occurred after 6-h of exposure) which was not a contributing factor in laboratory exposures. Mudalia has consistently proven to be sensitive to acute effects with zinc exposure in all prior laboratory and field artificial stream exposures (Chapter 3). The acute responses of Mudalia in all longer-term exposures to zinc were supported by its inability to avoid lethal exposure in shorter term tests.

INFLUENCE OF METAL INTERACTIONS AND PH

Cellulolytic activity was sensitive to low levels of cadmium (0.012 mg/L) and zinc (0.025 mg/L), both alone and

in combination. Cadmium and zinc concentrations in water were often below detection limit in all experiments yet metal uptake by both snails and clams was apparent. At low zinc concentrations (i.e., 0.025-0.05 mg/L) Belanger et al. (1986b) found that clams did not abbreviate siphoning activity and therefore fed on zinc-laden algae which contributed a major portion of the total observed body burden of metal. Cellulolytic activity measurements which coincided with these measurements in the work by Belanger et al. (1986b) confirmed this behavior (Farris et al., in review). Clams apparently did not avoid cadmium and zinc at exposure levels used in this study, except at 0.1 mg/L cadmium did clams fail to accumulate higher body burdens.

Mudalia cellulolytic activity exhibited patterns of decline that were consistent with accumulation of zinc and cadmium. There was no effect upon cellulolytic activity or metal accumulation attributed to interactions between zinc and cadmium in snails. The overiding effect of cadmium on cellulolytic activity of snails was unaffected by zinc addition. Exposures of snails to cadmium in combination with zinc resulted in cellulolytic responses similar to those exposed to cadmium alone. A heightened sensitivity to longer term cadmium exposure of has been cited previously for freshwater snails. Spehar et al. (1978) determined a 28-day LC50 for Physa integra to be .01 mg Cd/L, which was approximately 11 times lower than the 7-day cadmium LC50 of 0.11

mg/L. Holcombe et al. (1984) have reported adverse effects in Aplexa embryos, larvae, and adults, based on delayed hatch, survival, and reduced growth, respectively, at cadmium levels of 0.004-0.007 mg/L. These effect levels are about 12 to 19 times lower than the 96-hr LC50 of 0.093 mg/L for this species. Long-term snail tests, such as these involving cellulolytic activity, show adverse effects of cadmium at much lower levels than do acute tests with freshwater snails.

Corbicula cellulolytic activity was sensitive to field exposures of cadmium at low levels in the first 10 days of this study. These results were consistent with those of earlier studies (Chapter 3) in which cellulolytic activity was not significantly reduced to higher levels of zinc (i.e. 0.5-1.0 mg/L) since clams avoided the overiding feeding response. This avoidance behavior resulted in significant reductions in activity occurring by day 20 or 30 as clams could not remain closed. McMahon (1979) reported that Corbicula can maintain this avoidance response for 10-15 days.

The contribution of food associated metal is further substantiated by the absense of any detectable cadmium in the water. The availability of metals by other routes however was evidenced by accumulation to steady state, depuration, and patterns that reflected clams attempting to "handle" these exposure levels. Significant reductions in activity seen in clams exposed to 0.012 cadmium and 0.025 zinc combination and 0.025 mg/L cadmium without zinc addition by day

20 were followed by increased activity levels by day 30. This substantial increase in activity was not due to any aberrant change in control activity since the unrelativized data indicates a 200-500% increase in activity from day 20 to 30 in clams exposed to these levels. Such an increase could be directly related to the depuration which occurred at these same exposures. Graney et al. (1983) attributed such alterations in uptake of cadmium in Corbicula to shifts in the ambient environmental conditions (i.e., pH, food availability, TSS). This may or may not be the case at lower concentrations in the present study. These results fail to address any other connection between this obvious increase and a causative factor except to provide evidence of two highly coincidental changes.

INFLUENCE OF ZINC AND PH

Cellulolytic responses in Corbicula were more sensitive to pH than in Mudalia. Responses to 0.05 mg/L zinc at ambient pH were consistent for both Corbicula and Mudalia to previous Glen Lyn exposures (Chapter 3). Alkaline and acidic pH, however, resulted in Corbicula having significantly reduced activity in all four exposures tested, compared to only alkaline pH and zinc and acidic pH alone, having any significant affect upon Mudaliaby day 30.

Both Corbicula, and Mudalia responded to acidic conditions with significantly increased cellulolytic activity in the first 20 days on. This was consistent with the observation that pH optima of cellulases occur at pH 4-6 (Sinsabaugh et al. 1985) Following 20 days of exposure to low pH, lethal limits for both molluscs were reached. Okland and Okland (1980) observed that freshwater snails (Gastropoda, as a group) are generally not found below pH 6.0, although some species occur at low density down to pH 5.2 if calcium concentration was low. Garve (1980) concluded that the safe pH for populations of Helisoma to be above 6.0 based on reduced egg production and egg hatching success at pH 5.65.

Accumulation of zinc in Corbicula and Mudalia exposured to alkaline pH and zinc were consistent with trends seen by Graney et al. (1984) for Corbicula exposed to cadmium and alkaline pH. This contradiction in uptake related to pH was attributed to the possibility that: (1) inorganic and organic complexes can form at higher pH and exhibit greater availability to suspension feeders where complexation has enhanced availability by reducing the amount of substrate absorption; (2) lower pH may have stressed molluscs to the extent that feeding behavior was diminished; and/or (3) reduced pH may alter mucus production, causing metal uptake to be reduced. Any and all of these explanations could be applicable to both Corbicula and Mudalia at the exposure concentrations tested.

APPLICATION OF CELLULOLYTIC ACTIVITY TO IN-STREAM MONITORING

Cellulolytic activity of Corbicula from selected stations in the Clinch River confirmed that this index was applicable to artificial stream and field exposure validation of a complex effluent. Activity levels from clams caged at stations 8 and 11 were significantly reduced below those found upstream of the power plant outfalls and Dumps Creek. Total metal content during the sampling period was measured by American Electric Power. Zinc in the effluent was found to be 2 to 8 times higher at stations 8, 11, and 15 than at upstream stations. All measured total zinc during the river exposure ranged in values comparable to exposures used previously in Glen Lyn 30-day artificial stream studies (0.05-0.1 mg/L). These in-stream results agree with levels of zinc that affected cellulolytic activity in mollusks tested in artificial streams described in Chapter 3.

Cellulolytic activity was more sensitive in Corbicula from the instream monitoring than effects seen in macroinvertebrate assemblages collected during the 28-day monitoring. The Hester-Dendy survey indicated significant effluent effects on the structure of macroinvertebrate assemblages immediately downstream of outfalls 004 and 005. Compared to river water bioassays conducted by AEP (e.g., acute Daphnia tests), results from the macroinvertebrate colonization data were more sensitive to the effluent.

Others have substatiated the application of more sensitive parameters coupled with in-stream monitoring to predict the effects of metals in complex effluents (Miller et al. 1986; VanHassel and Gaulke, 1986). Recommendations from these studies included (1) more emphasis on site selection in invertebrate field collections with respect to defining control, impact, and recovery zones; (2) evaluation of effluent impacts incorporating native organisms from receiving systems that are known to elicit a sensitive reaction; (3) greater emphasis on incorporation of site-specific reactions rather than standard LC50 determinations alone.

SUMMARY AND CONCLUSIONS

A series of artificial streams was used to evaluate cellulolytic activity in molluscs as a stress indicator to selected constituents of power plant effluents. The Asiatic clam (*Corbicula fluminea*) and a phytophagous snail species (*Mudalia dilatata*) were used to distinguish the effects of behavioral adaptation and food uptake upon metal accumulation. Long-term (30-day) tests were conducted in artificial streams under controlled laboratory conditions and at a field site receiving river water. Stress indices from these two potential monitors are examined seasonally and compared to more conventional biological responses used in monitoring (growth, metal accumulation, and macroinvertebrate community structure).

Laboratory artificial stream exposures generally were inconclusive for indicating any direct correlations with zinc induced stress except to indicate that starvation-induced stress occurred during long-term holding with both clam and snail populations. Dietary requirements were better met in field-laboratory experiments as evidenced by both cellulolytic activity and accumulation data even though difficulty increased in maintaining target metal concentrations.

Corbicula detected acutely toxic levels of zinc and remained closed, thus avoiding zinc exposure and zinc-laden

algae during earlier intervals of the studies (days 1-20). The valve closure response enabled clams to be more resistent to acutely lethal levels in all exposures to zinc and cadmium. Cellulolytic activity was sensitive to all exposure levels of zinc (0.025 - 1.0 mg/L) and cadmium (0.012 - 0.1 mg/L). Patterns of significantly reduced cellulolytic activity were most often apparent following longer term exposures. These results were in agreement with metal accumulation patterns seen in all field-laboratory artificial stream exposures. Recovery ability in clams was reflected by depuration of metals and associated increased cellulolytic activity.

Mudalia were more sensitive to metal exposures than clams, and their inability to effectively depurate or avoid metal exposures was evident during the first 10 days of most studies. This information has suggested that snails acted as more suitable indicators of short-term stress (5-10 days), while clams were better suited for examination of long-term effects (exceeding 20 days duration). Only the 0.025 mg/L zinc exposure had no significant effect upon cellulolytic response in snails.

Metal uptake and cellulolytic activity appeared to be unaffected by the presense of zinc and cadmium combinations in comparison with responses to singular cadmium or zinc exposures. These data were difficult to interpret, owing to the

fact that at low metal exposures, zinc and cadmium were most often non-detectable in the water.

Cellulolytic responses of Corbicula were found to be more sensitive to the influence of pH than responses of Mudalia. Alkaline and acidic pH resulted in significantly reduced enzyme activity in Corbicula in all zinc and pH controlled combinations, compared to only alkaline pH and zinc and acidic pH alone, having significant effects upon snails.

Compared to the effects seen in macroinvertebrate assemblages collected during 28-day monitoring at the Clinch River Plant, cellulolytic activity for in-situ clams was more sensitive in detecting demonstrable biological effects within the receiving system. Reductions in cellulolytic activity at stations monitored for total zinc content were consistent with effects seen at comparable exposures to zinc in site specific artificial streams.

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CURRICULUM VITAE

Jerry Lin Farris

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Little Rock, AR

MARITAL STATUS: Married August 16, 1980, Mary K. Martin
Children: Whitney Karlin, March 26, 1983

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EDUCATION:

Sylvan Hills High School, North Little Rock, Arkansas
Arkansas State University, State Univ., Arkansas.
B. Sc. Degree, Zoology, 1979.
University of Olkahoma Field Biological Station,
Kingston, OK. Algal Ecology and Field Ecology,
Summer Session, 1980.
Arkansas State University, State Univ., Arkansas.
M. Sc. Degree, Biology, 1981.
Virginia Polytechnic Institute and State University,
Blacksburg, Virginia. Ph. D. Degree,
anticipated completion, 1986.

RELEVANT CURRICULUM: Transcripts are available
upon request.

Literature and History of Biological Science
General and Field Botany
Invertebrate Zoology and Vertebrate Zoology
Genetics
Speciation
Embryology
Histology
Comparative Anatomy
Natural History of Vertebrates
Cytology
Organic Chemistry
Enzymology
Animal Physiology
Insect Physiology
Advanced Comparative Animal Physiology
Advanced Microbiology of Aquatic Systems
Comparative Ethology
General Entomology

Insect Taxonomy
Aquatic Entomology
Advanced Aquatic Insect Ecology
Ichthyology
Fish Health and Diseases
Aquatic Biology
Limnology
Algal Ecology
Field Ecology
Issues in Human Ecology
Ecosystem Dynamics
Environmental Science-Water
Hazard Evaluation of Toxic Chemicals
Fortran
Biometry I, II, and III
Nonparametric Statistical Methods

POSITIONS HELD:

Arkansas State University: (Biology Department)

Graduate Teaching Assistant - Invertebrate Zoology, Histology, comparative Anatomy, Botany, Embryology, 1979-1980. Duties included lecture and exam preparation, laboratory supervision and assignment of course grades.

Laboratory and Field Technician - 1979-1981. Responsibilities included: 1) field investigations of population dynamics and distribution of macroinvertebrates in rice fields of NE Arkansas, EPA Biological Control Monitoring Project; 2) environmental inventory of macroinvertebrates for the Harding Creek portion of Lower Spring River Watershed, Lawrence County, AR., U.S. Soil Conservation Service; 3) environmental inventory of macroinvertebrates for the Caney Creek Project, Arkansas, U.S. Corps of Engineers.

Virginia Polytechnic Institute and State University: (Biology Department)

Graduate Teaching Assistant - Aquatic Ecology, General Biology, Principles Biology, Honors Laboratory, 1982-1984. Duties included lecture and exam preparation, laboratory supervision and assignment of grades.

Graduate Teaching Associate, 1983-1984. Duties include scheduling and supervision of 30 plus Graduate Teaching Assistants, conducting weekly lab training sessions for the GTAs, modifying lab exercises as appropriate and acting as liason between the staff and laboratory technicians.

Graduate Research Assistant, 1984-1986. Responsible for ongoing research being conducted at the Glen Lyn Power Plant field site and laboratory bioassays conducted at the University Center for Environmental Studies Ecosystem Simulation Laboratory.

MEMBERSHIPS:

North American Benthological Society
Sigma Xi
Society of Environmental Toxicology and Chemistry

RESEARCH INTERESTS:

Use and development of field-derived toxicity information for comparison with laboratory-generated estimates. The importance of factors influencing the responses of organisms in the laboratory and field is dependant upon whether the goal of the test is to predict actual effects, estimate safe levels, or determine relative toxicity. Optimal test conditions can elucidate the degree of difference attributable to either field or laboratory responses.

Modifications in standardized toxicity testing protocol for acute and chronic tests incorporating :hp1.Daphnia:ehp1. and fathead minnows. The toxicity of an effluent or single chemical to a given species in a specific receiving system is known to be better estimated by toxicity tests using the species and receiving water in question than by those using surrogates. Specificity of test organism and test conditions can be designated by development of either applicable test adjustments and/or incorporation of site specific organisms and diluent.

The influence of heavy metal speciation on toxicity, metal accumulation and detoxification processes of aquatic macroinvertebrates. Physicochemical factors are known to affect modes of uptake and bioavailability of metals. Inclusion of those recognized factors (hardness, pH, TSS) into toxicity testing and biomonitoring will affect predictability from test methodology as now applied toward hazard assessment.

Suitability of physiological and biochemical indicators of sublethal stress to supplement conventional methods of hazard assessment. Those sensitive functional measurements that have been shown to be simple, rapid and cost effective require quantification to in-vivo responses at exposure concentrations that are not unrealistic.

OTHER PROFESSIONAL ACTIVITIES:

Consulting aquatic ecologist for Hercules Inc., Radford Army Arsenal Project, for conducting surveys of fish communities in the New River, Radford, Virginia, 1983.

Consulting aquatic ecologist for Celanese Corporation,

Celco Plant, for evaluation of Asiatic clam, (:hpl.Corbicula:ehpl. :hpl.fluminea:ehpl.) invasion evaluation, and control strategies, Narrows, Virginia, 1983-1985.

Consulting aquatic ecologist for Celanses Corporation, Celriver Plant, for evaluation of Asiatic clam (:hpl.Corbicula:ehpl. :hpl.fluminea:ehpl.) invasion evaluation, and control strategies, Rock Hill, South Carolina, 1983-1985.

Consulting aquatic ecotoxicologist for Consumers Power Company in evaluating potential synergism between thermal effluent, heavy metals and dioxin, a review of the literature and site specific data, Jackson, Michigan, 1984.

Consulting aquatic ecologist for Procter and Gamble Environmental Safety Department, Cincinnati, Ohio on development of artificial streams for use in laboratory and site specific evaluation of toxicants, 1984.

Invited lecturer for Borg-Warner Chemicals Corporation in carrying out hazard evaluation of potentially toxic chemicals in their biological workshop for nine plants in the USA, Parkersburg, West Virginia, Jan. 11-13, 1985.

Consulting aquatic ecotoxicologist for Borg-Warner Chemicals Corporation in evaluating potential synergism between heavy metals and phenolic compounds, Morgantown, West Virginia, 1984-1986.

Consulting aquatic ecotoxicologist for Borg-Warner Chemicals Corporation in evaluating environmental impact statements and predicting hazard assessment of industrial effluents from a new plant siting, Parkersburg, West Virginia, 1985.

Consulting macroinvertebrate taxonomist for Appalachian Power Company in conducting surveys of benthic macroinvertebrates in the Clinch River, Virginia, 1985.

PAPERS PRESENTED AT SCIENTIFIC MEETINGS:

Farris, J. L., S. E. Belanger, D. S. Cherry, A. E. Linkins, and J. Cairns, Jr. 1985. Cellulase activity associated with chronic zinc exposure in laboratory and field artificial streams. Annual Meeting of the Society of Environmental Toxicology and Chemistry, St. Louis, Missouri.

Belanger, S. E., J. L. Farris, D. S. Cherry, and J. Cairns, Jr. 1985. Response of Corbicula fluminea to low levels of zinc in artificial stream systems. Annual Meeting of the Society of Environmental Toxicology and Chemistry, St. Louis, Missouri.

Farris, J. L., F. G. Doherty, and D. S. Cherry. 1984. Use of an improved artificial stream system for testing the control of Asiatic clams (Corbicula fluminea) with chlorine. Annual Meeting of the Society of Environmental Toxicology and Chemistry, Arlington, Virginia.

Farris, J. L., and G. L. Harp. 1982. Aquatic macro-invertebrates of three acid bogs on Crowley's Ridge in Northeast Arkansas. Annual Meeting of the North American Benthological Society, Ann Arbor, Michigan.

Farris, J. L., and B. L. Kimmel. 1980. Double isotropic labelling applied to the detection of autotrophic and microheterotrophic responses to nutrient enrichment. Annual Meeting of the Great Plains Limnology Society Meeting, Fayetteville, Arkansas.

PUBLICATIONS:

Thesis:

Farris, J. L. 1981. Aquatic macroinvertebrates of three acid bogs on Crowley's Ridge in Northeast Arkansas. M. S. Thesis, Arkansas State University. Arkansas. (George L. Harp, Major Advisor).

Farris, J. L. 1986. Cellulolytic responses to heavy metal accumulation in Corbicula fluminea and Mudalia dilatata. Ph. D. Dissertation. Virginia Polytechnic Institute and State University, Blacksburg, Virginia. (Donald S. Cherry and John Cairns, Jr., Major Co-Advisors).

Journal Articles:

Farris, J. L. and G. L. Harp. 1982. Aquatic macro-invertebrates of three acid bogs on Crowley's Ridge in Northeast Arkansas. Arkansas Academy of Science Proceedings 36:23-27.

Belanger, S. E., J. L. Farris, D. S. Cherry, and J. Cairns, Jr. 1985. Sediment preference of the freshwater Asiatic clam, Corbicula fluminea. The Nautilus 99(2/3):66-73.

Belanger, S. E., J. L. Farris, D. S. Cherry, and J. Cairns, Jr. 1986. Growth of Asiatic clams, Corbicula sp., during and after zinc exposure in

field-located and laboratory artificial streams. Archives of Environmental Contamination and Toxicology 15 (In Press).

Doherty, F. G., J. L. Farris, D. S. Cherry, and J. Cairns, Jr. 1986. Control of the freshwater fouling bivalve Corbicula fluminea by halogenation. Archives of Environmental Contamination and Toxicology (In Press).

Papers in Review or in Preparation:

Farris, J. L., S. E. Belanger, D. S. Cherry, and J. Cairns, Jr. Chronic zinc exposure reduces cellulolytic activity and growth of Corbicula in artificial streams. submitted to Environmental Toxicology and Chemistry.

Farris, J. L., S. E. Belanger, D. S. Cherry, and J. Cairns, Jr. Interaction of aerial exposure and chlorination for control of the Asiatic clam, Corbicula sp. For submission to The Nautilus.

Farris, J. L., D. S. Cherry, F. s. Colwell, R. B. Genter, S. E. Belanger, and J. Cairns, Jr. Community responses to low levels of zinc in site-specific artificial stream microcosms. For submission to Canadian Journal of Fisheries and Aquatic Sciences.

Farris, J. L., A. E. Linkins, D. S. Cherry and J. Cairns, Jr. Enzymatic response to chronic toxicity in the cellulase system of Mudalia dilatata and Corbicula fluminea. For submission to Environmental Toxicology and Chemistry.

Belanger, S. E., D. S. Cherry, J. L. Farris. Role of aeration in acute toxicity protocol of fathead minnows to phenolic effluent. For submission to Environmental Pollution.

Cherry, D. S., J. H. Van Hassel, S. E. Belanger, J. L. Farris, and J. Cairns, Jr. A site-specific variance demonstration for power plant discharges at the Glen Lyn Plant, Virginia. Invited Paper to Hydrobiologia.