Impact Zone Delineation for Biological Assessment of Power Plant Effluent Effects on Snail Populations in the Clinch River

by

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ABSTRACT

The impact of a power plant discharge (Clinch River Plant, CRP, Carbo, Virginia) on resident snail populations was assessed. In 1988, snail absence below the plant, was attributed to plant discharges rather than naturally occurring habitat limitations. Habitat limitations for the two dominant snail species, *Leptoxis praerosa* and *Pleurocera unciale* were defined before power plant impact was assessed. Eleven physicochemical parameters (i.e., flow rate, substrate type, silt accumulation, depth, water chemistry and food biomass parameters) were measured at selected sites and compared to snail density. Flow rate, substrate type and periphyton biomass were the most influential parameters in determining *Leptoxis* density; while periphyton biomass was the most influential for *Pleurocera*. Cluster analysis also linked *Leptoxis* density with river structure and flow. Other variables linked to *Pleurocera* density were flow rate, river structure and silt. Although *Leptoxis* is most prolific in riffle/shoal areas and *Pleurocera* in slower riffle-pool interfaces, these two species often coexist. This research suggests that habitat partitioning between these two species is influenced most by flow rate. Greatest density of *Leptoxis* occurred at flow rates of 20-30 cm/sec. Frequency of occurrence was greatest at 20-100 cm/sec. *Pleurocera* occurred most frequently at flow rates of 20-30 cm/sec with greatest density at 25-45 cm/sec.

Measurements of impact of the CRP effluent (i.e., toxicity, metals {mainly copper} bioaccumulation in aufwuchs and snails, and cellulase enzyme activity impairment) were summarized by using zone delineation. Habitat parameters were measured below plant discharges and upstream, and

compared with water column Cu, snail tissue Cu and aufwuchs Cu measurements. Habitat selection was strongly influenced by effluent but the role of waterborne metals concentration and habitat alterations (e.g., periphyton changes and bioconcentration) was unclear. Feeding studies were conducted to estimate impact of aufwuchs bioconcentration of metals on snails. *Leptoxis* significantly bioconcentrated Cu when fed aufwuchs containing 564 (±269) ug Cu/g in artificial stream feeding studies, but no cellulase impairments were seen in these studies. No foodborne bioconcentration was found from aufwuchs containing up to 20,000 (±18,400) ug Zn/L. These results suggest that though foodborne uptake of Cu may occur, water column Cu concentrations may have to be an order of magnitude higher for impairment to occur through injestion than through waterborne exposures.

Acute and chronic effects of both whole effluent and Cu on *Leptoxis* were measured in laboratory and artificial stream exposures. The 96-hr LC_{50} was 95% effluent (containing 148 ug Cu/L)in flow-through exposures, but in static stirred exposures, 100% effluent (105 ug Cu/L) was not toxic. The lowest-observable effect concentration (LOEC) from 30-day exposures was 10% effluent (22 ug Cu/L) causing cellulase activity impairment (70% of control activity) and bioconcentration (300 ug Cu/g). Constituents of effluent other than Cu were believed to contribute to impairment effects since no impairment was found in 30-day $CuSO_4$ dosings of up to 25 ug Cu/L. The LOEC for Cu from 30-day $CuSO_4$ dosings ranged from 17-35 ug/L and the no-observed effect concentration (NOEC) was 12 ug Cu/L. The EPA water quality criteria concentration (17 ug Cu/L) was questionable for *Leptoxis* in long-term exposures (114-day), causing enzyme impairment and mortality.

Chronically toxic conditions to *Leptoxis* occurred on the left side of the river for 0.7 km downstream of discharge, where the water column contained 42 ug Cu/L, while acutely toxic conditions occurred in the immediate mixing zone. Artificial stream impairment tests were substantiated in the river except in lower reaches of the impairment zone (left side of river, 0.7-0.9 km below cooling tower discharge), where snail absence was attributed to periphyton Cu bioconcentration (242 ug Cu/g). Functional recovery (of enzyme activity) was found at the next acceptable habitat downstream (Station 14A), so the area of impact extended 0.9 km downstream of the discharge on the left side of the river. It was concluded that zone delineation by simultaneously evaluating structural and functional aspects of environmental change is a better approach to impact assessment than approaches that only use functional measurements.

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CHAPTER ONE: INTRODUCTION

RESILIENCY OF THE CLINCH RIVER STUDY AREA

Freshwater ecosystems may be influenced by historical events (e.g., former discharges and ecoaccidents) or ongoing discharges (including non-point sources). The ecosystem at the time of assessment, may be recovering from one or more of these influences, or may be permanently altered as a result. Recovery of an ecosystem after a stress has been removed, may depend on the inertia (i.e., the ability to resist displacement of structural and functional characteristics) and elasticity (i.e., the ability to snap back from ecological displacement) of that particular ecosystem (Cairns, 1977); loss of biological integrity occurs when displacement exceeds the range of oscillations characteristic of that ecosystem. An ecosystem lacking inertia may adapt to the stress. The degree of adaption or resistance to change may influence the present state of the ecosystem and its response to further anthropogenic stress (Cairns and Niederlender, 1989).

Interest in incorporating natural ecological elasticity into risk assessment has increased in recent years, largely because of highly publicized ecoaccidents (Cairns, 1991). Factors involved in determining elasticity include existence of nearby epicenters for reinvasion, transportability or mobility of dissemules (i.e., life stages with adequate mobility for reinvasion), condition of the habitat following the disturbance, presence of residual toxicants, chemical/physical

environmental quality following pollutional stress (e.g., absence of algae may alter the O_2:CO_2 ratio), and management strategies to assist recovery (Cairns, 1991). The riverine ecosystem presented in this study was recovering from both historical episodic events and a discharge that had been recently altered to decrease toxicity.

STUDY SITE

The Clinch River, which originates in southern West Virginia, flows through southern Virginia before it discharges into the Tennessee River west of Knoxville, Tennessee. Historically, the Clinch River has supported one of the world's richest molluscan faunas. Since the Virginia State Water Control Board, along with environmental groups, has been concerned with preserving that richness, emphasis has been placed on pollution control into the Clinch River.

One of the first upstream industries to discharge into the Clinch River is a coal-burning power plant (American Electric Power's Clinch River Plant; CRP) at Carbo, Virginia. The CRP has been responsible for two large episodic events affecting Clinch River communities. An alkaline excursion was reported to be responsible for eliminating bottom dwelling fish-food organisms for approximately 5-6 km and snail and mussel populations for 18 km, following a CRP fly-ash pond spill at the CRP into the Clinch River at Carbo, Virginia in 1967 (Anonymous, unpublished). Approximately 216,600 fish were killed in

Virginia and Tennessee by the episode. Insect communities showed downstream recovery (i.e., further downstream stations had higher density and diversity) in 1969, but molluscan communities were not recovered for at least 30 km below the spill site (Crossman, 1973). Differences in reinvasion and recolonization potentials of the two groups of organisms stressed the importance of monitoring molluscan populations since they were slower to recover.

In 1970, before molluscan populations had recovered to prior density, and acid spill occurred at the CRP (Crossman, 1973). Approximately 5,300 fish were killed by the spill. After the spill no surviving mayfly or molluscan species were found for 18 km below the spill. Recovery, again was comparatively slow for molluscs. Within six weeks, diversity of arthropod benthic organisms had recovered, but molluscan species had not.

The research presented was part of a larger study on the ongoing effects of the CRP on molluscan populations. Since many mussels were endangered and could not be removed in large quantities for toxicity studies, snails and clams were used more extensively for these tests. Snails feed on attached forms of algae and detritus, and should be sensitive indicators of environmental stress. This research focused on the effects of CRP effluent on indigenous snail populations.

SNAILS IN THE STUDY AREA

A study conducted by Messinger in 1976 reported three genera of snails in this area of the river - *Goniobasis, Pleurocera* and *Helisoma*. In 1985, snails of four families were found in the Clinch River and Dumps Creek, near the Clinch River Plant (CRP), Hydrobiidae, Pleuroceridae, Physidae and Ancylidae (Appalachian Power Company, 1985). Historically, *Io* (which is currently endangered), has been found in the study area (Adams, 1899). The river is a third order stream with numerous riffle, run, and pool areas both above and below the study area, facilitating comparison of habitat, so that habitat selection effects could be distinguished from avoidance responses to plant effluents.

POWER PLANT DISCHARGES

Copper (Cu), zinc (Zn), and other metals are often present in power plant effluents. These metals may come from coal ash or cooling tower discharges. Coal ash siltation has been shown to alter aquatic biota, with accompanying acidic pH excursions and elemental (arsenic, cadmium, chromium, copper, selenium and zinc) concentrations and bioaccumulation (Cherry, *et al.*, 1984). Metals may be enriched on fly ash particles from electrostatic precipitators, producing 25-60% mortality (at 4.3 to 20.5 mg/L total suspended solids) in rainbow trout, *Oncorhynchus mykiss* (formerly, *Salmo gairdneri;* Cherry *et al.*, 1987). Since the Clinch River received no direct discharge from CRP coal ash ponds, studies focused on cooling tower discharges. Steam (used to drive

turbines in the production of electricity) is condensed for reuse. Clinch River water was used at the CRP to wash over the condenser pipes (made of a copper alloy) and accept the heat from the steam, promoting condensation. The river water was then cooled by trickling over wooden gratings in cooling towers, and returned to the river. This process had two sources of metals contamination. First, though various copper alloys are present in the condenser pipes, and Cu-leaching rates are lower now than in the past, some leaching still occurs. Zinc and other metals (e.g., Pb in some older plants) also may leach from the solder used to join the pipes. Second, when the river water is cooled, evaporation occurs that may concentrate leached metals, along with those naturally present in the river. Not surprisingly, concentrations of copper and zinc in cooling tower blowdown of the CRP were an order of magnitude higher than levels found upstream of the plant discharge (Van Hassel and Gaulke, 1986), averaging 857 ug Cu/L and 390 ug Zn/L in the blowdown and 30.1 and 39.1, respectively, upstream of the plant (1977-87) (more advanced instrumentation has shown that ambient copper concentrations in the Clinch river range from 3-7). After the cooling tower pipes were replaced in 1987 copper concentrations in cooling tower blowdown were decreased to 100-150 ug/L (data from acute tests, presented in Chapter 4).

MECHANISMS OF TOXICITY AND SUBLETHAL EFFECTS

Copper exposures

Mechanisms of toxicity and sublethal effects of both copper and zinc depend upon the level of exposure. Copper-induced changes in osmoregulation in freshwater fish species is thought to be a primary cause of mortality at acute concentrations, as well as an effect from chronic exposures (Heath, 1987). Decreased plasma osmolality is associated with a rise in blood volume (Courtois and Myerhoff, 1975) and tissue water content (Heath, 1984) suggesting increased influx of water through the gills from changes in gill permeability to water (Heath, 1987). Similarly, swelling of gill leaflets, followed by necrosis and sloughing off epithelium of the osphradium and gills occurred in whelks (*Busycon canaliculatum*) exposed to sublethal concentrations of copper (Betzer and Yevich, 1975). Gill damage appears to be a generalized effect from sublethal copper exposures.

Sublethal copper exposures may have effects on energy metabolism (Heath, 1987). Routine oxygen consumption in bluegill (*Lepomis macrochirus*) was initially stimulated and then inhibited, both being dose-dependent (O'Hara, 1971). Heath (1987) attributes the initial effect to simple irritation of the mucous membranes of the oral cavity, causing increased activity, while prolonged exposures depressed metabolism. This depressed metabolism was attributed to a reduction in muscle metabolism, due to inhibition of energy

metabolism enzymes, decreased muscle tone, or decreased spontaneous activity - chiefly the latter two, though the mechanisms (probably involving feeding rates) were not well understood. The major implication of this depressed metabolism is decreased growth rates. Decreased growth rates have also been linked to depressed digestive enzyme activity in some molluscs (Farris *et al.*, 1988).

Copper accumulation in tissues also may produce effects. Accumulation in the liver of juvenile rainbow trout, *Oncorhynchus mykiss*, resulted in interruptions of the plasma membrane and degeneration of mitochondria (Leland, 1983). Since bioconcentrated copper may cause effects it is difficult to separate these effects from direct effects from water-borne copper. Furthermore, it is difficult to determine which effects are from water-borne copper and which are from food-borne copper.

Zinc exposures

Internal hypoxia is thought to be the cause of death in freshwater fish, from acute zinc exposures (Heath, 1987). At high-level zinc exposures (24-hr LC_{50}, 40 mg Zn/L)Skidmore (1970) linked gas exchange rate reduction in rainbow trout, from zinc induced gill epithelium damage, to death due to tissue hypoxia. Hypoxia may result in blood acidification through lactoacidosis (Holeton and Randall, 1967) and intracellular acidosis (Wood *et al.*, 1982 and Turner *et al.*, 1983). Decreases in blood pH may reduce oxygen carrying

capacity due to the Boar shift (Smith, 1982). However, increases in hemoglobin oxygen affinity resulting from erythrocytic swelling (Nikinmaa, 1983) has been shown to counteract this reduction in trout. Therefore, it is unclear if this shift in blood pH contributes to toxicity. Similarly, the role of other changes, such as plasma ion stability changes (Skidmore, 1970) is not well understood.

Effects of exposure to lower zinc concentrations (96-hr LC_{50}) may be markedly different from high-level effects. Alterations in gill tissue and/or mucus production may result in blood alkalosis from increased CO_2 (Sellers *et al.*, 1975). The authors suggest that mucus production may interfere with gas exchange but gill tissue damage may not necessarily cause a decrease in the partial pressure of blood oxygen (PaO_2) and correspondingly increase anaerobic metabolism. Since PaO_2 is the primary controlling factor for fish ventilatory activity (Smith and Jones, 1982), this increased ventilation may compensate for much of the decrease in O_2 exchange. Consequently, blood acidosis may not occur to offset the accumulation of HCO_3. However, exposures of *Salmo* to higher levels (1.4 mg Zn/L) resulted in the initiation of anaerobic metabolism (Sellers *et al.*, 1975). Therefore, threshold levels of sublethal zinc exposures may dictate which physiological systems are initiated.

Membrane changes caused by these exposures may have other effects. Calcium is essential to the integrity of the cellular membrane, intracellular cements and stabilization of branchial permeability (Isaia and Masoni, 1976;

and Potts and Flemming, 1971). Watson and Beamish (1980) found that low-level exposure to zinc stimulated production of Ca-ATPase and several other ion-ATPases *in vivo* but inhibited production *in vitro*.

EFFECTS OF COPPER AND ZINC ON SNAILS

Toxicity to snails

Toxicity of copper and zinc varies widely among snail taxa, and because of this it is important to obtain information on endemic snails, or closely related species, in impact predictions. Many species, including *Leptoxis praerosa* and *Pleurocera unciale*, are not represented in the literature. Copper LC_{50} values for various snail species, at water hardness levels of 50-250 mg/L $CaCO_3$, vary from 0.04 to 1.7 mg/L (USEPA, 1984). Copper LC_{50} values for pulmonate snails range from 39 (Arthur and Leonard, 1970) to 108 (Wurtz and Bridges, 1961) ug/L. Those for prosobranch snails ranged from 210 to 1700 ug/L (Arthur and Leonard, 1970; Rehwoldt *et al.*, 1973; Paulson *et al.*, 1983; Cairns, *et al.*, 1978).

Zinc LC_{50} values ranged from 0.5 to 4.4 mg/L (USEPA, 1980). These values ranged from 303 (in soft water, 20 mg $CaCO_3$/L) to 7100 ug/L for pulmonates (Cairns and Scheier, 1968; Academy of Natural Sciences, 1960; Wurtz and Bridges, 1961; Wurtz, 1962; Nebeker *et al.*, 1986) and 14,000 to 20,200 ug/L for a prosobranch (Rehwoldt *et al.*, 1973).

Effects of sublethal concentrations of copper and zinc on snails

Currently, a few standard methods are available for sublethal testing (e.g., Fathead minnow, *Pimephales promelus* and *Ceriodaphnia dubia*; USEPA, 1989). Comparisons are still being made to assess the feasibility of these and other tests to accurately determine sublethal effect levels. Some generalizations may hold true among different types of organisms (e.g., effects upon gills may be similar in fish and prosobranchs). At sublethal concentrations, copper and zinc may cause changes in snail physiology, including enzyme activity. Farris (1986) reported that zinc significantly decreased cellulolytic enzyme activity in the snail, *Mudalia dilatata*. Cellulases act upon cellobiose to cleave glucose, a useable form of energy for snails. Cellulase activity was linked to growth in the clam, *Corbicula fluminea* (Farris, 1989), but it is unclear if this is a cause and effect relationship or if both simply reflect decreased metabolic activity. Pennack (1989) concluded that nutrients are derived chiefly from noncellular organic matter ingested with cellulose organic matter. This suggests that cellulolytic enzyme activity may not necessarily have a direct effect on growth.

Other responses that snails may exhibit when stressed by elevated concentrations of copper and zinc include decreased feeding and growth rates (Arthur and Leonard, 1970), increased mucus sloughing (Paulson *et al.*, 1983) and changes in rates of reproduction. Increased mucus sloughing, when induced by a prior alkaline stress, seemed to afford the snail some protection

from a subsequent copper stress. Changes in mucus production and other physiological processes, may serve as a buffer from some toxicants.

Effects upon the snail's habitat may weaken the organism or alter the effects of copper and Zn. For example, heavy metals may affect microbial processes (Flemming and Trevors, 1988). Since microorganisms in the sediment play an essential role in the cycling of carbon, regeneration of inorganic nutrients, and nutrient transformations (Swift *et al.*, 1979), changes induced by these metals may affect the amount of available carbon and nutrients. This may affect production of periphyton, as well as quality of detritus, both important sources of food for snails.

FACTORS THAT MODIFY TOXICITY OF COPPER AND ZINC

Copper and zinc are important trace elements. Copper is an essential micronutrient and is necessary for a wide range of metabolic processes. There are at least thirty Cu-containing enzymes. Some function as redox catalysts (e.g., cytochrome oxidase, nitrate reductase), others are dioxygen carriers (e.g., haemocyanin) (Weser *et al.*, 1979). Zinc is essential to the formation of nucleic acids, DNA polymerase and RNA polymerase and therefore, necessary for normal cell differentiation and growth. The concentration of zinc in cells can govern metabolic processes, such as, carbohydrate, fat, and protein metabolism and nucleic acid synthesis or degradation - through initiation and/or regulation of enzyme activity (Rand and Petrocelli, 1985). Although both metals are

essential, each can be toxic to aquatic plants, invertebrates and fish (Moore and Ramamoorthy, 1984). Both copper and zinc are toxic to molluscs as illustrated by their use both separately and in combination as toxicants in biofouling measures against the Asiatic clam, *Corbicula* (Cherry *et al.*, 1980).

Several factors exist that may alter copper and zinc availability for reaction. Solubility affects availability, and copper is moderately soluble and relatively abundant in the earth's crust (Forstner and Wittman, 1979). World median concentrations of copper reported by Bowen (1985) were 3 ug/L(range 0.2 to 30 ug/L) in uncontaminated fresh-water systems. Most cupric salts dissolve readily in water to the free cupric cation (Cu^{2+}) in the hydrated form, ($Cu(H_2O)_6^{2+}$) (Leakie and Davis, 1979). The form of the metal (ionic, complexed, precipitated), and its bioavailability, depends on environmental factors such as pH, redox potential (E_h), soil and sediment type, water hardness and organic content (as reviewed by Flemming and Trevors, 1989).

Inorganic and organic materials in the water can bind to the metals and reduce or enhance their availability; and therefore, their effect upon an organism. Natural waters and sediments are chemically complex (Florence and Batley, 1980), containing a mixture of metals (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Mn^{1+}, Zn^{2+}, Fe^{3+}), inorganic (OH^{-}, Cl^{-}, SO_4^{2-}, HCO^{-}, PO_4^{3-}) and organic ligands (urea, amino acids, organic acids, humic and fulvic compounds) in solution. These dissolved, suspended and settleable materials are important to the availability of copper and zinc.

Hardness and alkalinity affect reactivity of copper and zinc. Alkaline earth cations and trace metals compete for membrane transport sites (Lee, 1973). Chloride ion uptake in the freshwater pulmonate, *Lymnaea stagnalis*, under sodium-free conditions, coincided with equivalent net calcium ion uptake (DeWith *et al.*, 1986). Sodium ion uptake for *L. stagnalis* coincided with almost equivalent apparent net hydrogen ion excretion, under chloride-free conditions.

Hardness may alter toxicity appreciably since carbonate ions and their equivalents bind to the metals decreasing their availability (Jenne and Luoma, 1977). Precipitation or dissolution is determined by the concentration of the ions and solubility product constants (Sylva, 1976). The two major inorganic complexing ligands in most natural freshwaters are the hydroxy (OH^-) and carbonate (CO_3^{2-}) (as reviewed by Flemming and Trevors, 1989). The predominant hydroxy- or carbonate-copper species depends on the concentrations of OH^- (pH) and carbonate ions present (water hardness). Free Cu^{2+} was reduced from 11 to 3% of the total dissolved copper when hardness was increased from 50 to 250 mg/L at pH 7.5 (Stiff, 1971).

Products of biological decomposition that react with heavy metals, may affect availability. Again, complexes formed may be pH-dependent. Copper and zinc form complexes with many organic and inorganic ligands. Copper interacts strongly with sulfur forming relatively insoluble sulfides. Humic materials in freshwaters bind more than 90 percent of total copper (Moore and

Ramamoorthy, 1984). Schnitzer and Kerndorff (1981) reported that at pH 7, copper and zinc precipitated >95 and 67 percent of available fulvic acids, respectively, while at pH 6 the values were 83 and 17 percent, and at pH 8 both copper and zinc precipitated >90 percent of the fulvic acids. Recognition that interaction with chemical components affects toxicity is manifested by the use of water hardness measurements in developing water quality criteria and standards (e.g., USEPA, 1984).

Rather than decreasing activity, other elements or compounds may have additive or synergistic effects. Rodgers *et al.* (1980) showed that Cu, in combination with Zn, however, was responsible for most of the toxicity to *Corbicula*, indicating a lack of synergistic activity. In contrast, Sprague (1964) reported that copper and zinc were synergistic in eliciting an avoidance response in fish. Synergistic effects of other constituents in the water column may contribute to variability among molluscs (Couthtrey and Martin, 1977), with respect to metals concentration versus body burden (Graney, 1980).

Suspended and settleable materials may bind heavy metals. Bound metals may be temporarily less accessible to snails, but may be retained in an area and released with changes in pH. The toxicity of zinc to *Daphnia magna*, a filter feeder, varied with the type of suspended solids in the water column (Hall*et al.*, 1986). Snails are grazers and detritivores, rather than filter feeders. The aufwuchs-bound metals they consume may be even more available than sediment-bound metals and more confined to impact zones than

phytoplankton-bound metals.

Flemming and Trevors (1989) review adsorption and complexation of copper with components of the sediment. Carbonates and sulfides in the sediment may bind Cu, removing it from solution, but retaining it in the ecosystem. In addition, copper may adsorb to both living and nonliving (especially Fe and Al hydrous oxides) particulate matter. Kosalwat *et al.* (1987) found that copper did not bioconcentrate in the midge, *Chironmomus decorus* as much when complexed with substrate as in aqueous form.

Bioconcentration of metals may vary widely among taxa and may be influenced by physicochemical factors such as pH. Cherry *et al.* (1984) listed pooled bioconcentration factors (BCF's) (biotic/aquatic concentration) for aquatic plants and invertebrates at various pH levels. For plants, the copper BCF values were 18 and 10 at pH 7.4 and 6.5, respectively, and rose to 40 at pH 5.4. Invertebrate copper BCF values ranged from 79 to 67 without as much correlation to pH. Zinc BCF values varied more widely than those given for copper ranging from 4.1 to 22 in plants and 4.6 to 38 in invertebrates. Invertebrates inhabiting polluted freshwaters had copper bioconcentrations of 5-200 ug/g (as reviewed by Moore and Ramamorthy, 1984).

Since this potential for bioaccumulation exists, organisms may possess certain abilities to regulate rates of uptake of heavy metals via excretory mechanisms. These mechanisms have been reviewed by Bryan (1976). Among aquatic plants, Bryan found little evidence of heavy metal excretion or

regulation and suggested that losses occur mainly by diffusion. Among molluscs, however, regulatory mechanisms included excretion as granules from the kidneys of scallops (Bryan, 1973), excretion as spheres pinched off digestive cells, as in *Cardium edule* (Owen 1955), ejection of iron particles from mantle and storage and ejection of foreign materials by leucocytes in oysters (Yonge 1926; Takatsuki 1934). However, Bryan (1976) concluded that though regulatory mechanisms are diverse, they are generally weak in bivalve and gastropod molluscs.

The type of organism and its respective life history may be important to copper and zinc toxicity. Sensitivities of aquatic invertebrates to copper may depend upon their surface to volume ratio, respiratory rates, and flow rates over gill surfaces; as these variables increase, copper uptake is facilitated (Hobson *et al.*, 1979). Toxicity of copper and zinc may vary greatly among similar taxa. Among snail taxa, two orders are found in freshwater. Prosobranchs have an operculum on the foot that can be closed during short-term environmental stress. Pulmonates were once terrestrial, and have lost gills and operculum (Clark, 1981). Though pulmonates may lack the protection of an operculum, they are not as susceptible to changes in dissolved oxygen concentration as gilled snails, and do not experience gill damage and xenobiotic uptake through gill epithelium. Arthur and Leonard (1970) reported 96-hr copper LC_{50} values of 1.7 mg/L for the operculate, *Campeloma decisum*, and 0.039 mg/L for the pulmonate, *Physa integra*. *Campeloma* was observed

to remain tightly closed in its shell at copper concentrations greater than 1 mg/L. The six-week, no-effects concentration showed no significant difference between the two snails, suggesting that the operculum only affords the snail a short-term protection. According to Bryan (1976), there is no evidence that the entry of metals can be prevented by any animal through rapid permeability changes, though organisms such as bivalves can temporarily prevent entry by closing the shell.

Differing responses among species, to heavy metals, may be due to physiological differences. Wurtz (1962) suggested that snails containing hemoglobin (*Helisoma* spp.) are more tolerant of zinc than those containing hemocyanin (*Physa* spp.). *Helisoma campanaulatum* had 96-hr zinc LC_{50} values of 1.27 and 0.87 mg/L in hard and soft water (100 and 20 mg $CaCO_3$/L), respectively, while *Physa heterotropha* had respective values of 0.4 and 0.3 mg/L. However, since these differences in sensitivity are small, and other physiological differences exist between the two snails, the toxicological role of respiratory pigment is not clear.

Test organism state of health has also been shown to affect copper and zinc toxicity. Guth *et al.* (1977) found evidence that zinc resistance in the snail (*Limnaea stagnalis*) is reduced when it is parasitized. Food availability often fluctuates seasonally and may affect the health of the organism. Periphyton production depends on available sunlight and nutrients. Available sunlight varies seasonally with the position of the sun and riparian shading.

Seasonal changes temperature, food availability, life stage and other variables may affect the toxicity of copper and Zn. An increase in temperature may affect organism susceptiblity by changing the rate of enzyme activity or oxygen-carrying capacity of the water. For example, chlorine was shown to be significantly more toxic at higher temperatures (test values ranged from 6 to 32°C) to the snails *Goniobasis virginica, Nitocris carinata* and *Physa heterostropha* (Gregg, 1974). For gill-breathing snails, decreased oxygen concentrations can increase vent rate, thus, increasing contact and entrance of metals.

Life stage of test organisms may influence waterborne and food-borne entrance of metals. *Amnicola* eggs survived at higher copper and zinc concentrations than adults (Rehwoldt *et al.*, 1973). The 24-hr and 96-hr copper LC_{50} values for *Amnicola* eggs were 4.5 and 9.3 mg/L, respectively, and 1.5 and 0.9 mg/L for adults. The 24-hr and 96-hr zinc LC_{50} values were 28.1 and 20.2 mg/L for eggs and 16.8 and 14.0 mg/L for adults. *Amnicola* eggs are sealed in capsules that may protect them to a certain degree from environmental stress. Thomas *et al.* (1985) found that feeding niches of juvenile and adult *Biomphalaria glabrata* (Say), a freshwater pulmonate snail, differed markedly. Juveniles ate less living macrophyte tissue and fewer species of large diatoms but much larger quantities of decaying plant material than adults. Since many snail species attain much of their adult size within a year, the existence of different feeding regimes among life stages suggests

seasonal differences in heavy metals uptake.

In summary, biotic factors influencing copper and zinc availability and toxicity include bioaccumulation, physiological and anatomical traits, life stage, and state of health of test organisms. Abiotic factors affecting availability and toxicity include season (temperature, food availability and life stage), pH, dissolved oxygen, dissolved materials (eg. hardness and alkalinity), suspended matter, sediment and possibly, synergistic effects with other elements or compounds.

OBJECTIVES

The objectives of this research were: 1) to define habitat preference ranges for dominant snails in the Clinch River near the CRP; and 2) to determine the area of snail habitat affected by plant discharges. By defining preference ranges, theoretical snail distribution without influence from discharges, could be estimated. By comparing this with actual snail density in those areas below CRP discharge, the area of impact could be estimated. Then, using toxicological data collected in laboratory, artificial stream, and in-river studies, the area lost to the snails could be attributed to changes caused by the discharge.

EXPERIMENTAL DESIGN

Two pulmonates, *Ferrissia rivularis* and *Physella* sp., and two

prosobranchs, *Leptoxis praerosa* and *Pleurocera unciale* were present in the study area. Since *Leptoxis* and *Pleurocera* were the dominant taxa, habitat studies were conducted to determine the microhabitat of each. Dominant microhabitat variables were determined by regression analysis of measured habitat variables vs. snail density. Further definition of microhabitat was determined by defining preferred ranges within dominant variables.

Structural and functional changes in snail populations were then assessed within areas where microhabitat conditions existed. Structural changes were assessed by comparing population density below CRP discharges to those found in similar habitat at upstream stations. Functional changes in snail populations induced by CRP effluent were assessed in laboratory and artificial stream tests and validated with in-river studies. Concentrations of the effluent and dominant constituents (copper and zinc) causing acute toxicity, bioconcentration, impairmants (growth and cellulolytic enzyme activity) and habitat changes were determined.

RELEVANCE OF THIS RESEARCH

Federal legislation to restore and maintain biotic integrity in aquatic ecosystems (e.g., Water Pollution Control Act of 1966, Federal Water Pollution Control Act Ammendments of 1972 - PL92-500, and Clean Water Act of 1977 - PL95-217) has been set and the accompanying methodology to test system integrity, is still being developed. Maintenance of chemical, physical, and

biological integrity of water resources in the United States are mandated by the Clean Water Act of 1977. Current legislation, in both the USEPA and state water control agencies, acknowledges natural water quality variabilities in and among water bodies. Since these variabilities affect biological and chemical responses, the ability for ecosystems to assimilate perturbants may be site specific (Cairns, 1977), and monitoring practices should reflect this.

In the past, limitations in experimental design and resources have necessitated regulation based on chemical monitoring. The assumption was that these nonbiological measurements could be used to estimate biotic integrity, and the assumption was made that meeting physicochemical standards for water quality is sufficient to ensure biological integrity (Karr and Dudley, 1981). These measurements, however, may be inadequate (USEPA, 1985, Thruston *et al.*, 1979; Gosz, 1980; Karr and Dudley 1981), so federal and state agencies have increased the emphasis on biological measurements. USEPA has called for biological criteria in its water quality standards program and for the adoption of biological criteria into state water quality standards by 1993. Current permitting practices (Fig. 1) combine both technology-based (chemical) data and water quality-based (biological) data to assess potential hazard of discharges. When priority water bodies are established (e.g., endangered species habitat) permit requirements must reflect the existing circumstances. Otherwise, information obtained from chemical and biological data comparisons are used to set permit requirements. The complexity of

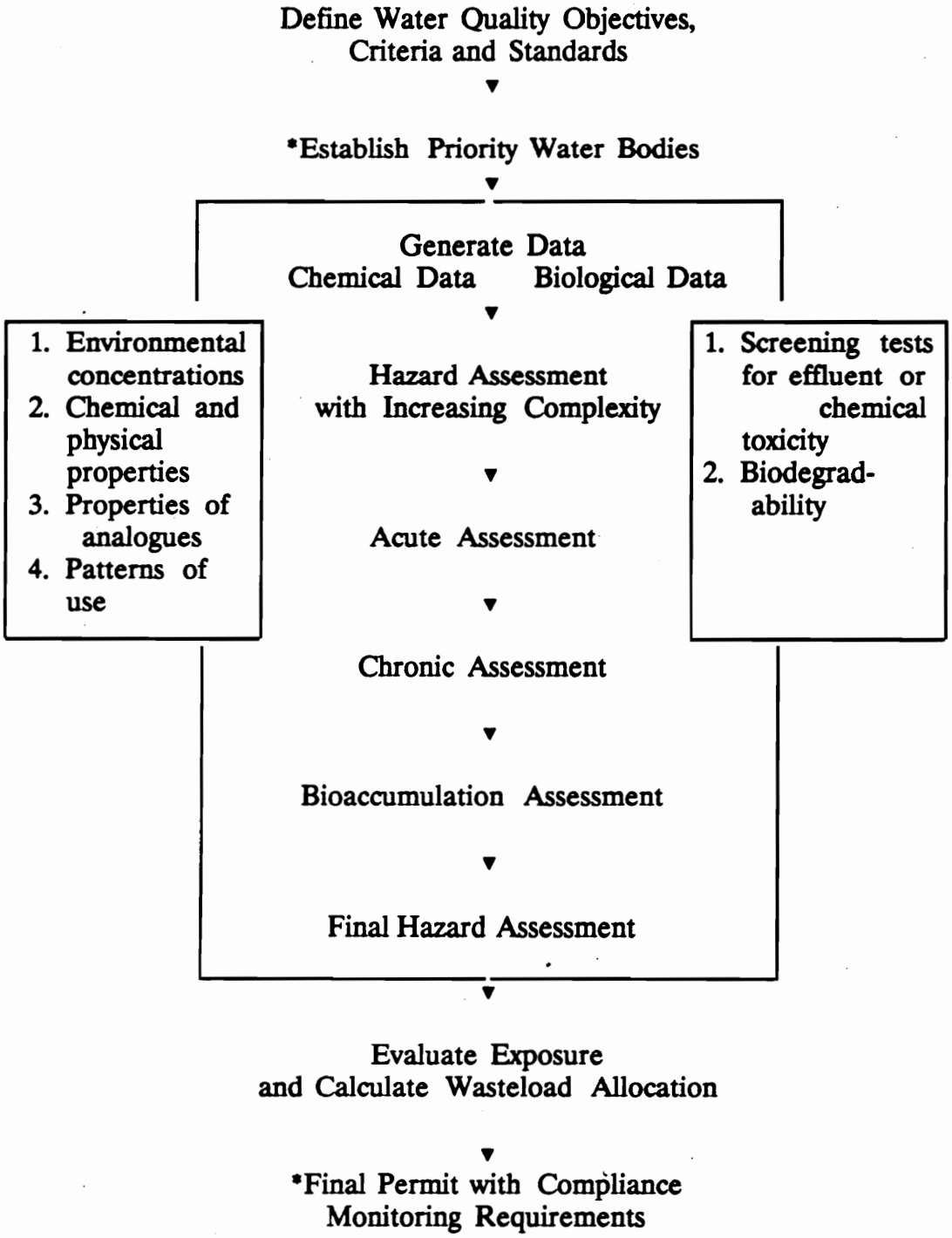


Figure 1. Overview of water quality-based program (after Tooby, 1978).

assessment depends on the impact of the discharge. For example, if the discharge passes the acute tests, permit requirements may be based upon this information and further assessments are not necessary.

Tools to measure biological quality are still being developed. Some criticisms of currently developing techniques include expense, time consumption, gear sensitivity and unequal applicability to every situation. Karr *et al.* (1986) summarized methods historically used for biological assessment. They include indicator species, diversity indices (combining number of species with abundance or biomass), and relative abundance of desirable species. One currently popular approach is the Index of Biotic Integrity (IBI)which uses an array of biological metrics based on either fish or insect populations to estimate biotic integrity.

The IBI approach analyzes structural differences of a single group of organisms between the area being assessed and an unimpacted area of similar habitat. This approach certainly gives more information on biotic integrity than some of the earlier methods and is a good measurement of structural integrity of the biota being sampled. Weaknesses with this approach include lack of information on environmental gradients, functional variables and other types of organisms. First, no mention is made of downstream changes in the structural variables measured. Since only structural variables are measured, no information is obtained on the functional changes below discharges. For example, fish may swim into an area and forage. However, breeding may not

occur, or if it does, more sensitive life stages may not survive. Second, when the community structure is affected, no information is given on mechanisms of effect. Third, in some situations, fish or insect communities may not reflect the impact upon other biota. For example, below CRP discharges, fish population structure was not affected (Van Hassel, 1989), but clam populations were eliminated for 0.5 km (Belanger, *et al.*, 1990) and snail and mussel populations were severely affected for 0.9 km downstream. The IBI should be useful in comparing relative health of streams from the same region or in monitoring long-term trends in health of a particular stream; and is one step toward a comprehensive measure of biological integrity in aquatic systems. It seems to be more accurate, consistent, and sensitive, than the Shannon Weiner Species Diversity Index; but, even so, no overall assessment or final management decision should hinge on any single index, but on all the information and expertise available (Angermeier and Schlosser, 1987).

Zone delineation through ecosystem monitoring has not been used extensively by researchers. Since this type of holistic approach incorporates laboratory and artificial stream toxicity and impairment tests, field validations, and habitat measurements to assess impact zones (Barbour and Plafkin, 1988), it should provide a more accurate assessment of impact than previously described methods, which only evaluate structural aspects of communities. The proposed zones (below the immediate mixing zone) include a toxic zone; a chronic impairment zone, characterized by avoidance by some species, or,

bioconcentration or impairments when organisms inhabit this zone; and a recovery zone, characterized by structural and functional recovery (Fig. 2). The presented research will aid in evaluating this of type of assessment in providing information on actual structural and functional effects on populations.

Before effects on populations may be assessed, habitat tolerences/preferences must be specified, unless historical data are available for comparison. Otherwise, population density measurements are difficult to interpret, especially when distributions are patchy. Since historical data were unavailable for snail populations in the study area, habitat preferences were evaluated. Information was available on the life history of genera, *Leptoxis* (Miller-Way and Way, 1989; Aldridge, 1982) and *Pleurocera* (Morrison, 1954; Goodrich, 1940; Dazo, 1965), but habitat preferences for species, *L. praerosa* and *P. unciale* (the dominant species in the study area) were not found.

Cellulolytic enzyme activity and growth were chosen for use as impairment measurements. Cellulase activity has been shown to be impaired by exposure to some heavy metals (Farris, *et al.*, 1988 and 1989), but more validations of this measurement in laboratory and field studies are needed to further establish the feasibility and limitations of using this impairment measurement.

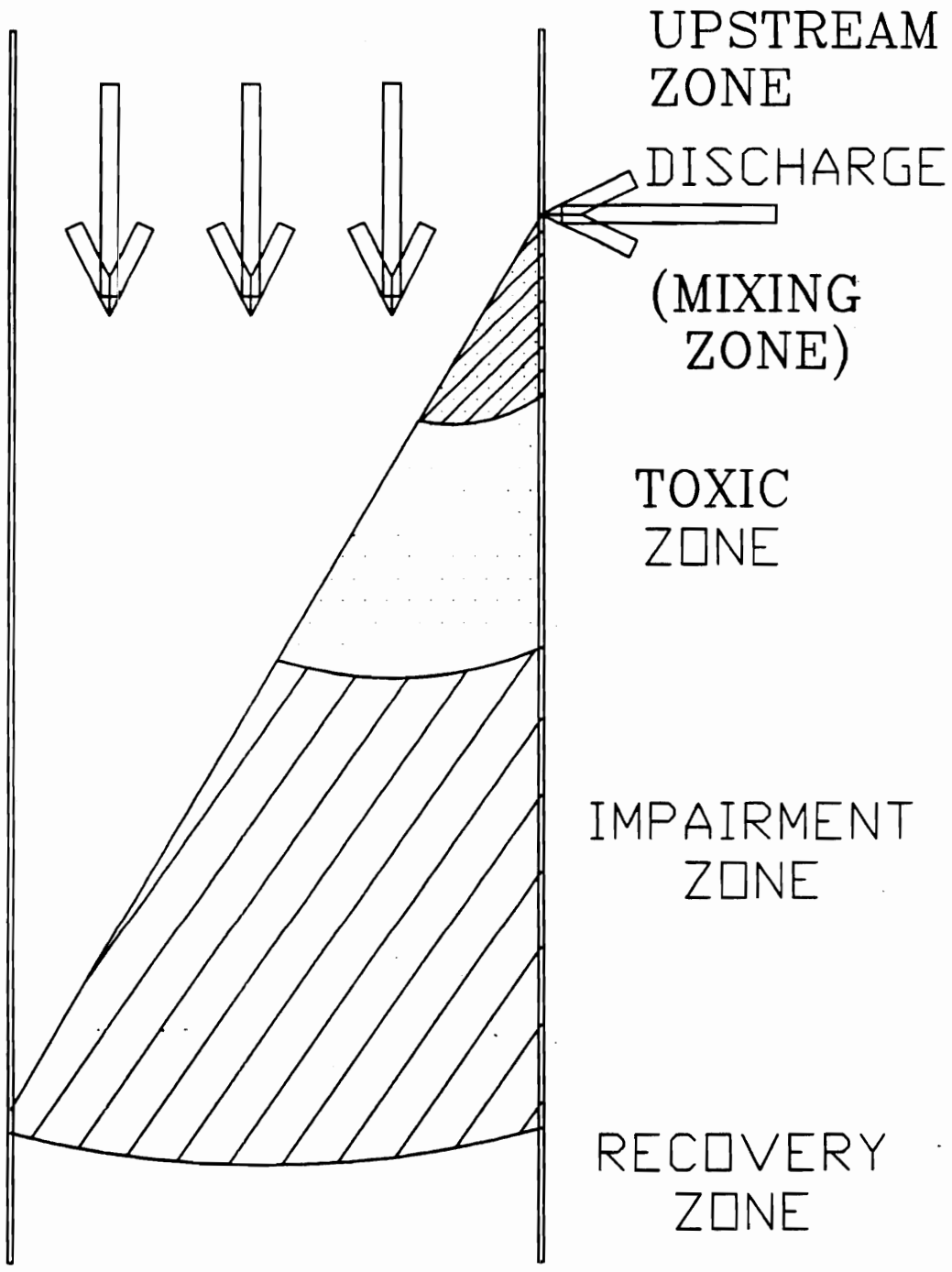


Figure 2. Proposed impact zones and their relative location to a discharge point.

CHAPTER TWO: HABITAT DEFINITION

INTRODUCTION

Most pleurocerids inhabitant lotic environments. Although information has been gathered on ecological niches of some species, lotic species have been underrepresented (as reviewed by Gordon, 1987). Gordon found that out of over 100 species of Pleuroceridae, ecological niches for only 13 had been published. Since that estimate, information on a few other species has been published (Gordon, 1987; Miller-Way and Way, 1989), but information is still lacking for many pleurocerids. Some generalizations may become clearer as more niches are defined.

The subfamily Pleurocerinae is distinguished by dioecious reproduction, males lacking intromittent reproductive structures, and oviparous females with an egg-laying sinus in the right side of the foot through which eggs are deposited (Morrison, 1954). Two representatives, *Leptoxis praerosa* (Say) and *Pleurocera unciale* Reeve, coexist in the Clinch River, Virginia. The obective of this study was to determine physicochemical variables that influence the distributions of *Leptoxis* and *Pleurocera.*

Leptoxis praerosa is the genotype for *Leptoxis* Rafinesque, 1819 (Morrison, 1954). Distribution of this species is widespread, with populations occurring in the Cumberland, Duck, Ohio and Tennessee river drainages. Though no life history for this species has been published, other publications

indicate that the genus is typically semelparous and biennial with a life span of approximately two years (Miller-Way and Way, 1989; Aldridge, 1982). One representative, *L. dilatata*, reportedly laid eggs in late April through early May (Miller-Way and Way, 1989).

Pleurocera unciale unciale (Reeve) inhabits the upper tributaries of the Tennessee River in Virginia and eastern Tennessee (Goodrich, 1940). No life history for this species has been published. One researcher, however, reported that a closely related species, *P. acuta*, mated mainly during fall (some mating occurred during early spring) and deposited eggs in the spring. The normal life span was 3 years and sexual maturity was reached in 2 years (Dazo, 1965). Although pleurocerids generally feed on red and green algae, desmids and diatoms, large quantities of decaying vegetative material and extremely fine sand grains were found in the stomach contents of *P. acuta* in addition to algae, indicating facultative detritivory (Dazo, 1965).

METHODS

Habitat characterization

Two habitat studies were conducted to include both non-random (Study I) and random sampling (upstream stations, 1A-2, Study II). In Study I (June, 1988), eight upstream sites were selected, four sites of favorable habitat (presence of both species) and four of poor habitat (absence of snails). In

Study II (July, 1988), three riffles and three pools were chosen (Stations 1A, 1B and 2, Fig. 3). Bank-to-bank transects were randomly positioned at each pool and riffle.

Triplicate measurements of snail density and physicochemical variables (except for single measurements of water quality variables in Study II) were taken at each site in Study I, and at left, middle and right banks of each transect in Study II. Density was estimated by quantitative Surber square foot sampling (APHA, 1986). Snails were enumerated and identified on site. Voucher specimens were identified by Dr. Robert Dillon, College of Charleston, S.C., as *Leptoxis praerosa* (Say), *Pleurocera unciale* (Reeve), *Ferrissia rivularis* (Say) and *Physella* sp. Physicochemical measurements included alkalinity, pH, ash-free dry weight (AFDW), silt (ash weight), chlorophyll a, hardness (Study II only) (APHA *et al.*, 1986), current velocity (General Oceanics flowmeter Model 2030R), macrohabitat (pool or riffle), substrate type (gravel, cobble, sand, silt, and/or bedrock - ordered from least to greatest complexity in the regression analysis), and percent algal cover (visual observation). Stepwise regression was used to distinguish influential habitat variables and gradient analysis was used to estimate optimum values of selected variables (SAS, 1985).

To further define velocity preferences (for *Leptoxis* and *Pleurocera*) transects were chosen in midriffle, throughout the riffle-shoal area, and into the

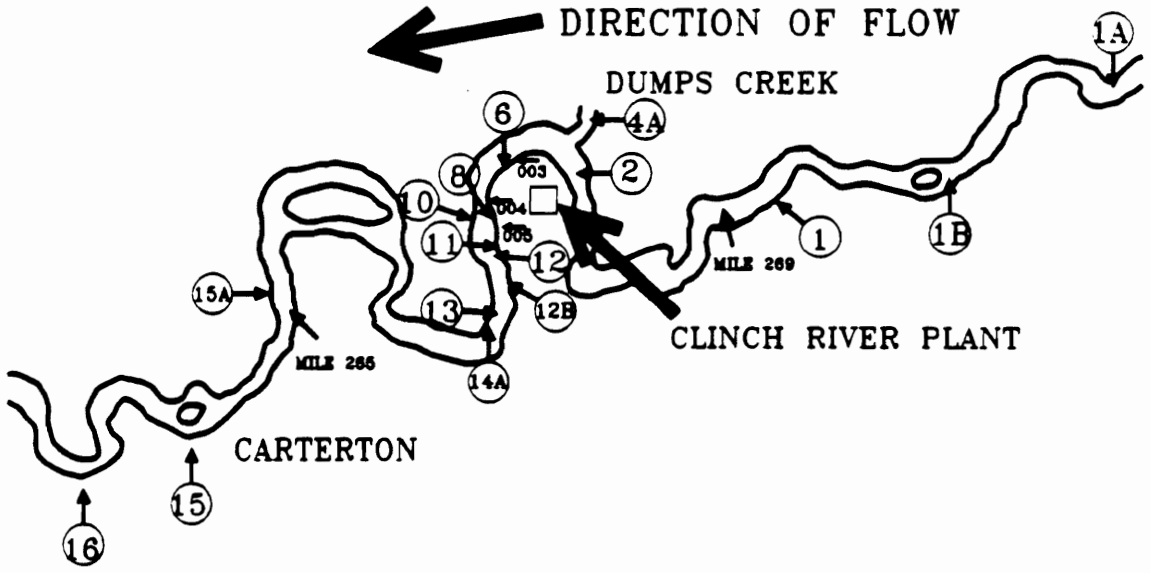


Figure 3. Map of Clinch River in the vicinity of the CRP showing sites where *Leptoxis* populations were sampled and caged snails were exposed. River flow is from east to west.

pool at Station 1. Current velocity and snail density were measured at three meter intervals along each bank-to-bank transect.

RESULTS

Snail microhabitat

Stepwise regression analysis in Studies I and II had similar results for both species. The most influential variables in determining *Leptoxis* density (in descending order of significance) were aufwuchs biomass (AFDW), bed velocity and substrate type in Study I; and substrate type, chlorophyll a production, and surface velocity in Study II (Table 1). Variables important for *Pleurocera* were macrohabitat, bed velocity and aufwuchs biomass in Study I; and algal cover, chlorophyll a production and surface velocity in Study II.

In Study I, substrate type was similar throughout most sites (Appendix B, Table B1). However, sites with highest *Leptoxis* density did not contain visible amounts of sand and silt. Lowest snail density ($<1/0.1m^2$) was associated with highest silt accumulation (388-643 mg/cm^2). Similarly, the relationship between snail density and periphyton biomass was not clear. Highest chlorophyll a production was associated with lowest snail density.

In Study II, highest density of both species was associated with substrate containing gravel and cobble (Appendix B, Tables B2a-B2c). Highest density was also associated with depths of <20 inches. Low snail density was

associated with silted pools; greatest amounts of silt were found in pools. Relationships between snail density and food variables were not always clear. Periphyton biomass was generally less where snails were found. Highest chlorophyll a production was associated with high snail density. No relationship was seen between snail density and water quality variables.

Velocity preference

Leptoxis preferred higher surface and bed velocity than *Pleurocera* in Studies I and II. Highest *Leptoxis* density was associated with current velocity of 98 to 126 cm/sec, while highest *Pleurocera* density was associated with bed velocity of 23 to 126 cm/sec, and highest *Pleurocera* density with velocity of 37 to 75 cm/sec in Study II.

Gradient analysis (a statistical procedure for determining the location along a gradient most associated with the dependent variable) showed that *Leptoxis* consistently preferred a higher velocity (58-98 and 91-98 cm/sec, respectively in Study I and Study II than *Pleurocera* (45-46 and 36-55 cm/sec, respectively; Table 2). *Leptoxis* preference for higher velocities was also seen in the 1989 density study (using multiple transects; Table 3). *Pleurocera* was found at lower current velocity (0-10 cm/sec) than *Leptoxis*, while *Leptoxis* was found at higher velocity (>110 cm/sec) than *Pleurocera* (<91 cm/sec). Overall, *Leptoxis* occurred in higher density than *Pleurocera*, with highest

Table 1. Stepwise regression analysis for models of snail density using physicochemical variables from habitat studies as explanatory variables in selected and random sites. Also included are preference ranges, where greatest density were found - June-September 1988.

Variables in best regression model	F value	P value	Preference ranges
Leptoxis			
Study I			
Ash-free dry weight of aufwuchs, mg/cm^2	16.63	0.0006	5-50
Bed velocity, cm/sec	13.04	0.0017	90-130
Substrate type[a]	8.67	0.0080	GCSa
	$r^2=0.7604$		
Study II			
Substrate type[a]	11.84	0.0184	GCSaS
Chlorophyll a	9.05	0.0298	0.02-0.03
Surface velocity, cm/sec	5.55	0.0651	30-65
	$r^2=0.8007$		
Pleurocera			
Study I			
River structure	11.92	0.0025	Riffle
Bed velocity, cm/sec	5.15	0.0344	10-85
Ash-free dry weight of aufwuchs, mg/cm^2	1.88	0.1859	3-50
	$r^2=0.3905$		
Study II			
Algal cover, %	31.14	0.0025	5-20
Chlorophyll a	11.80	0.0185	0.01-0.23
Surface velocity, cm/sec	10.01	0.0250	5-45
	$r^2=0.9251$		

[a] G=gravel, C=cobble, Sa=sand, S=silt

Table 2. Optimum conditions for each snail, as determined by evaluation of locations along environmental gradients associated with highest snail densities (gradient analysis).

Variable	Optimum Value	
	Leptoxis	*Pleurocera*
Selected sites (n=24)		
Depth, cm	27	40
Surface velocity, cm/sec	98	55
Bed velocity, cm/sec	91	36
pH	8.27	8.22
% periphyton cover	14	22
Ash-free dry wt., mg/cm^2	70	41
Random sites (n=54)		
Depth, cm	27	40
Surface velocity, cm/sec	59	46
Bed velocity, cm/sec	58	45
pH	8.22	8.21
% periphyton cover	6	20
Ash-free dry wt., mg/cm^2	2	2

Table 3. Snail density (SD) measured at various current velocities (1989).

Current Velocity cm/sec	n	Density *Leptoxis* snails/0.1m ^{2}	Density *Pleurocera* snails/0.1m^2
0-10	6	0(0)	1(1)
11-20	4	24(49)	0(0)
21-30	4	54(40)	8(13)
31-40	2	34(13)	25(13)
41-50	4	24(16)	10(7)
51-60	7	38(16)	4(4)
61-70	4	17(7)	5(1)
71-80	3	14(6)	2(2)
81-90	2	16(18)	2(2)
91-100	1	61(-)	0(-)
101-110	0	-	-
111-120	1	24(-)	0(-)

density at current velocity of 91 to 100 cm/sec. *Pleurocera* highest density occurred at much lower current velocity (31-40 cm/sec).

DISCUSSION

General considerations

Leptoxis was generally more abundant throughout the study area than *Pleurocera*. Gordon (1987) also found *Leptoxis* to be the most abundant and productive snail taxa in an area of the Arkansas River. In the Clinch River study area, most *Leptoxis* reproduction occurred from July to October, and most *Pleurocera* reproduction occurred from September to October. Both snails had a somewhat bimodal reproduction pattern, with some reproduction also occurring in May. Similarly, population density and reproductive potential peaked during mid-summer, but reproduction occurred throughout the year for *Elimia potasiensis*, *Pleurocera acuta* and *Leptoxis arkansensis* (Gordon, 1987).

Overall, current velocity, food variables, and macrohabitat were the most influential variables associated with snail density. *Leptoxis* preferred higher velocity than *Pleurocera* in Studies I and II, as well as in the 1989 multiple-transect study. Highest *Leptoxis* density was found at current velocity of 98 to 126 cm/sec and 23 to 126 cm/sec in Studies I and II, respectively. When additional measurements were taken along velocity gradients in the 1989 multiple-transect study, results agreed with the high end of the first estimates.

In this study greatest density was found at 91 to 100 cm/sec velocity. *Leptoxis* was also found uniformly throughout velocity gradients from approximately 11 to 100 cm/sec, with highest density (>20 snails/0.1m^2) from 11 to 60 cm/sec. One explanation for this bimodal density pattern is that larger snails may prefer velocity of 11 to 50 cm/sec, while younger snails may prefer faster velocity. Another explanation is that all snails may prefer faster velocity, but be limited by their ability to maintain their position on substrate at faster velocity. In the 1989 transect study, at highest velocity, adult *Leptoxis* were confined to the undersurface of stones, somewhat protected from the current, but smaller individuals were found on all sides. The explanation may be found in a discussion by Hynes (1970). He illustrated that current velocity is reduced in the boundary layer 1-3 mm thick on the tops of stones because of friction between water and substrate. Animals having a dorso-ventrally flattened shape, which exist within this space were protected from the current. Therefore, upper-threshold current limitations may be size-dependent if animals are not dorso-ventrally flattened. *Leptoxis* has a globular shape, so young snails may be within the 1-3 mm layer, but adult snails may be taller and thus, more exposed to the current and unable to maintain their position when currents are greater than 100 cm/sec. Microcurrents close to substrate were not determined in this study because of instrument limitations.

Shell shape, as well as size, may account for *Pleurocera* absence from fastest currents. Since *Pleurocera* is larger and has a longer, more fusiform

(conical), shell shape than *Leptoxis*, it appears that current velocity may play a large role in niche separation between the two snails. Gordon (1987) also suggested that smaller, more compact *Leptoxis arkansensis* preferred or tolerated higher current velocity than larger, more elongated *Pleurocera acuta*. Both Gordon's findings and results of this research agree with Goodrich's (1924) observations on the general ecology of *Leptoxis* and *Pleurocera*. *Leptoxis* habitat was characteristically faster, mid-channel habitat, while *Pleurocera* habitat was typically considered to be "quiet water." Gordon observed juveniles of *Elimia* and *Pleurocera* inhabiting habitat more suitable to *Leptoxis*, suggesting an age-dependent habitat shift (also see Moore, 1964 and Dussart, 1987). This was seen in *Pleurocera*, but much more pronounced in *Leptoxis* than *Pleurocera*, in this research. Smaller *Leptoxis* inhabited current velocities in which adults were confined to the underside of cobbles and boulders, presumably for protection from velocity.

Similarly, investigators of pleurocerid ecology have noted strong correlations between substrate, current velocity, and species occurrences (Goodrich, 1922; Krecker, 1924; Houp, 1970; Harman, 1972; Krieger and Burbauck, 1976; Ross and Ultsch, 1980; Hawkins *et al.*, 1982; Gordon, 1987). In general, current velocity and substrate have been shown to be major determinants of benthic habitat for macroinvertebrates (Cummins, 1962; Williams, 1981; Nowell and Jumars, 1984; Gordon, 1987). Gordon (1987) found that current velocity, along with substrate particle size, was a primary

discriminator in niche divergence among three pleurocerids. *Leptoxis arkansensis* exhibited preferences for mid-channel habitats with faster currents and coarse substrates. *Elimia potosiensis* and *Pleurocera acuta* exhibited preferences for edge and backwater habitats with low velocity and fine-particle substrates.

Substrate type

Substrate type was a significant factor in determining density. *Leptoxis* was associated with gravel and cobble, and sites with highest density had little silt. Although substrate was not a significant factor in choice of habitat, *Pleurocera* appeared to prefer microhabitats with cobble and gravel, but was more tolerant of silt than *Leptoxis*. Similarly, Houp (1970) found substrate to be the most important factor in *Pleurocera acuta* habitat preference, indicating that sand is necessary for reproduction since it is used to coat eggs. No strong associations with sand were seen in this study, but *Leptoxis* was often found where current velocity was strong and most sand was swept away. Solid substrate for oviposition is needed (Branson, 1961; Jones and Branson, 1964; Dazo, 1965; Bickel, 1968; Hynes, 1970; Mancini, 1978; Diamond, 1982) and young are typically found on coarse substrates.

Although substrate was not a significant factor in *Pleurocera* choice of habitat, it appeared to prefer microhabitats with cobble and gravel, but was more tolerant of silt than *Leptoxis*. Although Ross and Ulrich (1980) found

substrate preference to be a possible source of niche separation between closely related species, this probably was not true in this research. Substrate was very uniform throughout riffle/shoal macrohabitat in the study area.

Periphyton

Although periphyton biomass and chlorophyll a production were influential in both *Leptoxis* and *Pleurocera* distributions, optimum biomass was not clear. A complication in linking food-quantity measurements to snail habitat preference is that grazing may affect periphyton assemblages (Sumner and McIntire, 1982; Cuker, 1983; Lamberti and Resh, 1983; Lamberti and Moore, 1984; McAuliffe, 1984; Hart, 1985; Steinman *et al.*, 1987, 1989; McCormick and Stevenson, 1991). Since a grazer-density dependency factor is involved in the relationship between algal biomass and *Leptoxis* density, a direct correlation may be difficult, depending upon the biomass measurement. A suggestion is that algal biomass may be influential in habitat choice when algal growth is inhibited. Periphyton growth, under optimal conditions, becomes more limited by substrate for attachment. Grazing also may affect the periphyton species present in the assemblage, grazing may either enhance succession (Sumner and McIntire, 1982; Breitburg, 1985; Tuchman and Stevenson, 1991) or arrest succession (Underwood and Jernakoff, 1987; Jernakoff, 1983; MacLulich, 1986; Tuchman and Stevenson, 1991), depending on the selective grazing of that snail. Since, in addition, periphyton

communities are affected by current velocity and other diverse conditions in lotic environments (Cole, 1983; McCormick and Stevenson, 1991), algal variables may be very site specific.

Interaction among habitat variables increases the difficulty in discerning the influence of each on snail density. For example, in the Clinch River study area, riffle/shoal locations were characterized mainly by bedrock and cobble with very little sand or silt, whereas pool locations had sand and silt deposits covering most stones. River structure and substrate were also connected to depth and current velocity; pool locations were characteristically deeper and slower than riffle locations. Water depth and current velocity influence the distribution of some pleurocerids (Houp, 1970; Foin, 1971), and the influence of each may be interconnected. For example, Foin (1971) suggests that depth limitation of the pleurocerid *Oxytrema proxima* depends on current velocity necessary for provision of sufficient oxygen around the gills. The extent of influence of each of these variables may vary among species, so defining how much these factors and their interactions affect pleurocerid distribution is difficult. For example, Gordon (1987) found respiration rates of *Pleurocera* significantly lower than for *Elimia* and *Leptoxis*. *Elimia* and *Pleurocera* were ecologically separated by dissolved oxygen gradient. By comparing respiration rates and pleurocerid distribution patterns in other rivers, Gordon suggested that *Elimia* preferred habitats intermediate to *Leptoxis* and *Pleurocera*.

Water chemistry

The importance of water chemistry variables in influencing molluscan distribution has been recognized for many years (Boycott, 1936; Clarke and Berg, 1959; Hunter, 1957). Water chemistry variables may affect distribution when comparing one riverine system to another since some pleurocerids have minimum pH and buffering tolerances (Dillon & Benfield, 1982). Localized variability in ambient pH (range of 8.04-8.35) and alkalinity (range of 140-154 mg $CaCO_3$/L), however, was not limiting to *Leptoxis*, and only a weak association was found between pH and *Pleurocera* density. Similarly, water hardness was not a limiting factor in the Clinch River study area, ranging from 114 to 216 mg as $CaCO_3$/Lthroughout the study area, well in excess of the 20 mg as $CaCO_3$/Lthreshold reported to be limiting to molluscs (Macan, 1949).

Habitat definition

In summary, in the Southwestern Virginia reaches of the Clinch River, *Leptoxis* was associated with gravel and cobble, sites with highest density had little silt. *Pleurocera* appeared to prefer microhabitats with cobble and gravel, but was more tolerant of silt than *Leptoxis*. Although the relationship was not clear, higher snail density were associated with greater algal cover, and highest periphyton productivity, but organic biomass was generally lower where snails were found (probably because of grazing). No relationship was found between water quality variables and snail density, under conditions studied. In general,

Leptoxis preferred riffle/shoal areas with fast flows, and *Pleurocera* preferred riffle/shoal areas with moderately fast flows.

CHAPTER THREE: SNAIL DENSITY AND HABITAT CHANGES

INTRODUCTION

Using molluscs as monitors

Since life history and physiological differences exist among taxa, impact may depend on type of organism, location in the river, detection and avoidance capabilities, or other factors involving uptake and depuration. Indigenous molluscs are often appropriate monitors of power plant discharge impact because of their sensitivity to copper and other associated metals (Wurtz, 1962; Rehwoldt *et al.*, 1973; Cairns *et al.*, 1976; Coughtrey & Martin, 1977; Guth *et al.*, 1977; Cherry *et al.*, 1980), sessile life form, long and complex life histories, position in the food web, and accessibility (Cairns *et al.*, 1971).

Most rivers in the United States support molluscan populations that can be used as monitors of pollutants. Pollutant effects on periphyton and detritus may affect gastropods that graze on these materials. Since periphyton assemblages may be affected by heavy metals exposure, and gastropods are capable of moving among localized patches of conditions, their distribution patterns may reflect an avoidance reaction to environmental stress and may be a highly visible and easily traceable indicator of impact.

Since habitat variables may affect snail distributions and metal uptake (Phillips, 1976), habitat preferences must be defined to locate suitable habitat

before population shifts may be attributed to a discharge, or anything other than natural habitat variability. Habitat definition for dominant snail species is present in Chapter 2. Effluent impact on habitat selection are discussed in this chapter.

Food alterations

Snails remaining in areas where habitat (e.g., food) has been altered may be affected, but the contribution of food-borne heavy metals to bioconcentration or impairment in molluscs is still unclear. Further, some physiological variables, such as cellulolytic enzyme activity, may be altered by food variability (Farris *et al.*, 1989) as well as heavy metal toxicity (Farris *et al.*, 1988). Food may vary in quantity, as well as quality. For example, changes in species composition of algal assemblages may result from heavy-metal stress (Patrick, 1978). Copper may depress cell division and photosynthesis in some algal species more so than others (Stauber and Florence, 1987). Correspondingly, altered food quality (Anderson and Cummins, 1979; Fuller *et al.*, 1988) may result from changes in species composition.

Localized alterations in stream conditions may have an impact upon the food source for gastropods and other grazers and collector-gatherers (Fuller *et al.*, 1986; Brooks and Rumsby, 1965). In addition to having an altered species composition, algae may bioconcentrate heavy metals (Les & Walker, 1983). Metal concentrations measured in periphyton scrapings are actually aufwuchs

metals concentrations, since metals may accumulate in other parts of the aufwuchs assemblage, as well as in live algal cells. They may adsorb onto materials composing these assemblages, such as dead cell material (Jackson, 1978) and bacterial cells (Beveridge, 1984). Copper precipitation and adherence may depend on pH (Stiff, 1971; Sylva, 1976). Phosphates and carbonates may form precipitates with Cu, if present in sufficient quantities (Stiff, 1971). Adsorption of copper to living and non-living particulate matter may be substantial, and has been shown to be significant in removing dissolved copper from polluted surface waters (Ramamoorthy and Kushner, 1975). Metals bind to absorbent species by associations ranging in strength from weak van der Waals forces to strong covalent bonding, coprecipitation with ferromanganese oxides and incorporation into clay crystal lattices (Ramamoorthy and Rust, 1978).

METHODS

Study area

Three stations, characterized by long riffles, were chosen upstream (1A, 1B, and 2), and four downstream (Stations 13, 15A, 15, and 16), of a cooling tower discharge (Fig. 3, Chapter 2). The river between Stations 13 and 15A was characterized by deep pools and river bends, below which cooling tower effluent is well mixed with river water, as evident from conductivity and copper

measurements. Triplicate measurements using a Surber sampler (APHA, 1986) were taken at the left, middle and right banks of pool and riffle transects at each station. Snails were enumerated and identified on site. Voucher specimens were identified by Dr. Robert Dillion, College of Charleston, S.C., as *Leptoxis praerosa* (Say), *Pleurocera unciale* (Reeve), *Ferrissia rivularis* (Say) and *Physella* sp.

Snail density was determined in June and October, 1988 and monthly (river conditions permitting) from April, 1989 to March, 1990, to evaluate seasonal population stability. In June 1988 sampling, a Surber sampler was used at one transect across riffle and pool areas at each station. Only riffle transects were used in the October, 1988 sampling. Three replicates were taken at the left bank, middle and right bank of each transect. Three replicates were also taken at the left bank of the riffle and pool at each station in 1989-90 monthly sampling. Since the river floor in pools was often inaccessible during winter, density was measured by counting numbers on one-square-foot pieces of slate placed on the river bed for at least one month. Density was estimated for four size classes of *Leptoxis* (<3mm, 3-7mm, 7-10mm and >10mm, total shell length) and *Pleurocera* (<4mm, 4-9mm, 9-15mm and >15mm, total shell length) and for three size classes of *Ferrissia* (<1mm, 1-2mm and >2mm, shell width).

Microhabitat analysis

CRP effluent influences on snail habitat were evaluated by defining habitat preferences for dominant snails (Chapter 2) and determining amount of acceptable habitat lost due to CRP impact. Stations 1A, 1B, 2, 13, 15A, 15 and 16 were sampled. Copper concentrations in the water column, aufwuchs and snail tissue were measured (as described below) in addition to habitat variables described in Chapter 2 - flow, depth, substrate type, chlorophyll a, ash free dry weight, periphyton cover, silt, alkalinity and pH.

Metals in the study area

Water samples were collected at left bank, middle and right bank for both transects at each of the seven stations used in density and habitat studies. Samples were transported to VPI&SU, filtered through 0.45 um porosity membrane filters, and preserved with 150 ul of 50% Baker Instra-analyzed HNO_3 per 50 ml of sample. Copper was analyzed using flame atomic absorption spectrophotometry (Perkin Elmer 1100).

Three snails were collected from left bank, middle, and right bank of each riffle transect. Snails were dissected within 24 hours of collection. Soft body parts were dried at 45°C in a Fisher Isotemp Oven, Series 100, Model 126G, to dryness (usually 24-48 hours). Dried samples were weighed and digested in HNO_3 to sample clarity and cessation of obvious color change (usually 24-48 hours). Samples were cooled and 50 ul of 30% hydrogen peroxide was added. Samples were reheated to steam production for 45

minutes, cooled and diluted to 20 ml using deionized water (Valdes *et al.*, 1982). Copper was analyzed from snail samples collected at each station, and zinc and Pb from Stations 1B and 13.

Three periphyton samples were collected from standard surface areas (3.2 cm^2) of rocks at left bank, middle, and right bank of riffle transects and transported to VPI&SU for metal analysis. These samples were dried at 45°C, digested, and analyzed as described for snails.

Food-borne uptake studies

A 30-day artificial stream study was conducted in 1989 using dosed and undosed artificial streams (at Glen Lyn Power Plant, Virginia and the Ecosystem Simulation Laboratory, ESL, Virginia Tech, respectively) to evaluate effects of food-borne uptake of copper and zinc on snails. In the Glen Lyn test (July 3-August 3, 1989), artificial streams were dosed with metals. Raw (New River) water was used as the dilution water and target concentrations were 0, 8.6 ug Cu/L, 17.2 ug Cu/L, 47 ug Zn/L, 94 ug Zn/Land 8.6 ug Cu + 47 ug Zn/L. Because of space limitations at GL, six artificial streams at the ESL (July 8-August 8, 1989) received dechlorinated tap (New River) water. No metals were added to the streams, so the only source of metals was the food. Approximately 20 cobbles (with attached periphyton) were collected from New River or Wolfe Creek (Glen Lyn, Virginia) every five days and placed in the streams at Glen Lyn. After 5-day exposures in these dosed streams, the metal-

contaminated cobbles were transported to the ESLand placed in the respective stream for that treatment (numbered 1-6, corresponding to stream numbers in dosed streams). Five periphyton scrapings were taken in each treatment; scrappings were taken when cobbles were transferred from dosed to undosed streams.

The test was originally designed to last for much longer (to correspond to the 114-day study, Chapter 4). Control mortality (>10%) was observed in the undosed study and the test was ended prematurely. Apparently, snails in the dosed streams decreased feeding rates, so that when cobbles were transferred to undosed streams enough food remained to feed those snails. Snails in the control streams at Glen Lyn, however, fed at a rate which did not leave sufficient food when cobbles were transferred to the control stream at ESL, and snails starved.

Two snails, *Leptoxis praerosa* and *Mudalia dilatata* were used. *Leptoxis* were collected from the Clinch River upstream of the CRP and acclimated by gradually increasing the ratio of New to Clinch river water over the course of 10-14 days. *Mudalia* were collected from the New River, upstream of the Glen Lyn plant. One hundred individuals of each species were added to each artificial stream at Glen Lyn and ESL. When snail mortality was suspected, confirmation was made by pricking the sensitive foot region followed by a 15 minute recovery period in control water (Paulson *et al.*, 1983).

Water chemistry samples were collected from each artificial stream every

5 days for metal analyses and every 10 days for other water chemistry measurements (pH, hardness, alkalinity and conductivity; APHA, 1986). Water samples were filtered, preserved and analyzed for copper and zinc concentration, using flame atomic absorption spectrophotometry (with a graphite furnace attachment). On day-30, snails were removed and preserved for cellulase activity (Farris *et al.*, 1988) and bioconcentration (as previously described for snails collected from the CRP study area). Three aufwuchs samples were collected from standard surface areas (3.2 cm^2) of three cobbles added to each stream. These samples were dried, digested and analyzed for metal bioconcentration (as described for snail tissues). Temperature was measured at 5-day intervals in the New River study.

A second feeding study was conducted at the CRP (August 16-September 15, 1990). Ceramic tiles were precolonized with algae for 4-8 weeks by placing them in plastic trays supplied with a constant flow of Clinch River water. The artificial stream assembly was described by Farris *et al.* (1988). At 2-3 day intervals (3 days per week) three colonized tiles each were randomly placed in clear 15L polycarbonate bell jars containing copper and/or zinc treatments prepared by mixing metal sulfates with Clinch River water. Each bell jar contained 1L of treatment solution and was covered with clear plastic to reduce evaporation and contamination. They were then placed under the greenhouse tarp, to allow algal growth to continue while tiles were being soaked. Then tiles were removed from the soak solutions and snapped into

each feeding chamber of feeding trays (Fig. 4). *Leptoxis*, collected from the Clinch River, was used in the Clinch River study. Ten *Leptoxis* were added to each chamber of feeding trays. Snail mortality was checked as described above.

Water samples for metals analysis were collected from each artificial stream after each tile change and at the beginning and end of the test for other water chemistry measurements (pH, hardness, alkalinity and conductivity). Water samples were transported in ice-filled coolers to VPI&SU for analysis. Water samples were also filtered, preserved and analyzed for copper and zinc concentration, as described above (using a graphite furnace attachment).

On day-30 all surviving snails were preserved for cellulase analysis (as previously cited). Snail tissues were homogenized in a phosphate buffer and centrifuged to separate supernatant from pellet. The supernatant was used to measure enzyme activity. In this study, the pellet was rinsed into a tared glass vessel, dried, digested and analyzed for metal bioconcentration (as previously described for snail tissue collected from the CRP study area). One periphyton scrapping (1.0 in^2) was collected from each tile before transfer to a feeding chamber. These samples were dried, digested and analyzed for metal bioconcentration.

Temperature was monitored constantly in the Clinch River test by placing a max-min thermometer in an artificial stream, recording the high and low values and resetting the instrument on each tile-change day.

RESULTS

Snail density in the study area

In the June 1988, *Leptoxis* and *Pleurocera* were absent ($<1/ft^2$) from the left bank at Station 13 (Fig. 5 and 6), the first station below the cooling tower discharge. Two *Ferrissia* (Fig. 7) were found at the left bank of Station 13. *Leptoxis*, *Pleurocera* and *Ferrissia* were at upstream density levels at the next downstream stations (Stations 15A-16). Station 15A had significantly higher *Leptoxis* and *Pleurocera* density and number of taxa at the left bank of the pool, and overall density at the left bank of the riffle in the early summer sampling study (Appendix A, Tables A1 and A2). The proportion of juvenile snails was larger (personal observation) at Station 15A than other stations; perhaps, resulting from a surge from reproduction, rather increased population density. Data from early fall sampling showed that Station 15A did not have higher density than other stations (Appendix A, Table A3). Monthly sampling May, 1989 to March, 1990 showed some variability in measured cohort proportions among stations, but trends in reproductive cycles could be generalized when data from all stations were combined (Fig. 8). *Leptoxis* reproduction occurred mainly June-October (though the smallest size class was not found in June, a time delay was assumed for attainment of a detectable size for the sampling method used). *Pleurocera* reproduction occurred primarily in September and October, but to some extent also from April to August.

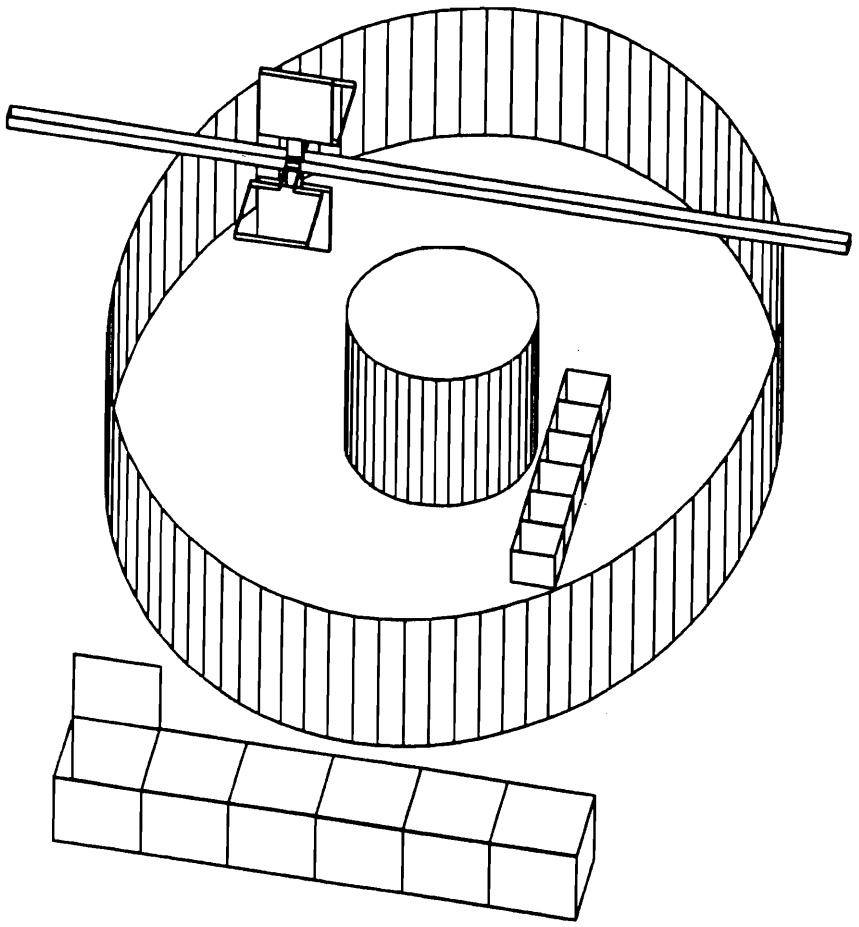


Figure 4. Feeding chambers used in the 30-day artificial stream study at the CRP. Precolonized ceramic tiles were placed in divided chambers in screened-off sections of plastic trays containing ten *Leptoxis*/chamber. Trays were then positioned behind paddlewheels in artificial streams, 1990.

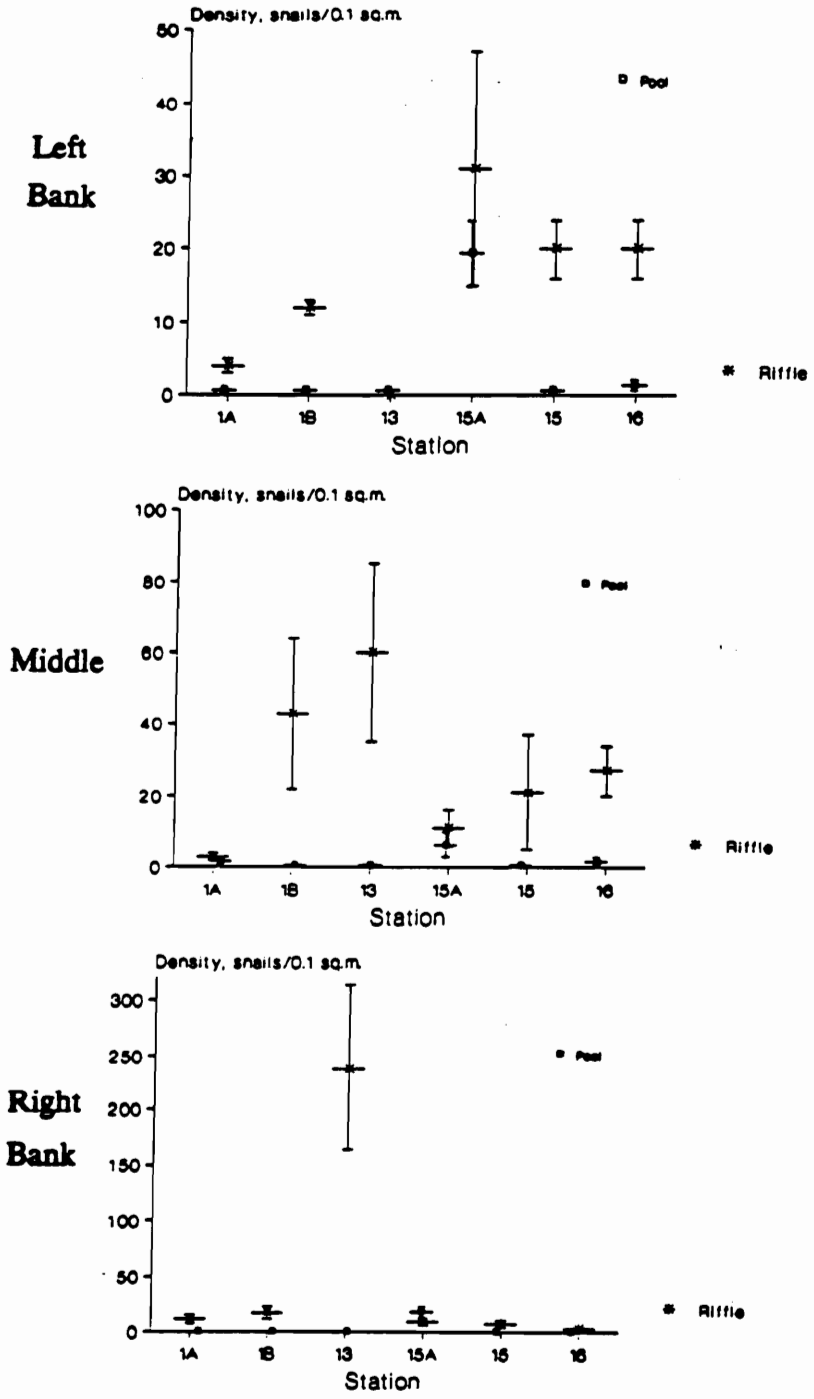


Figure 5. Density of *Leptoxis* per 0.1 m^2 in pools and riffles at six sampling stations in June, 1988.

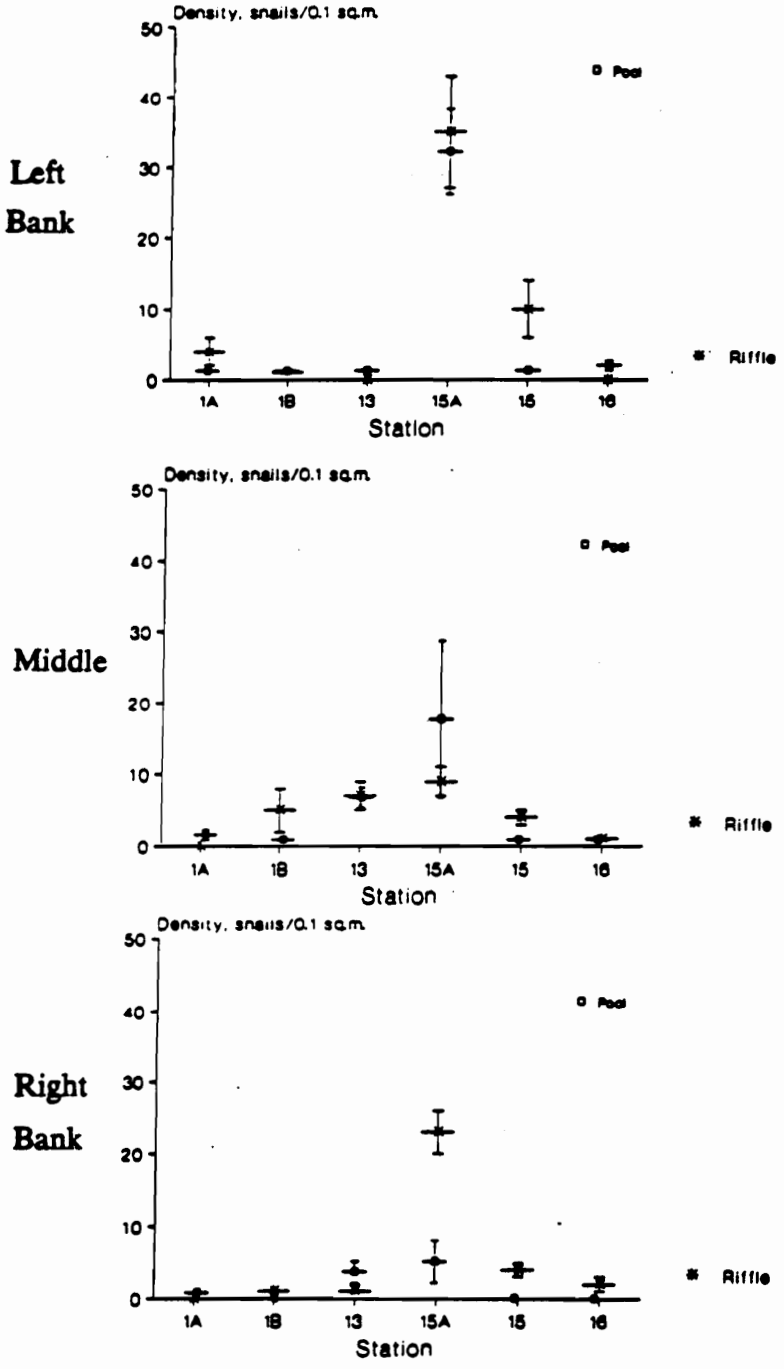


Figure 6. Density of *Pleurocera* per 0.1 m^2 in pools and riffles at six sampling stations in June, 1988.

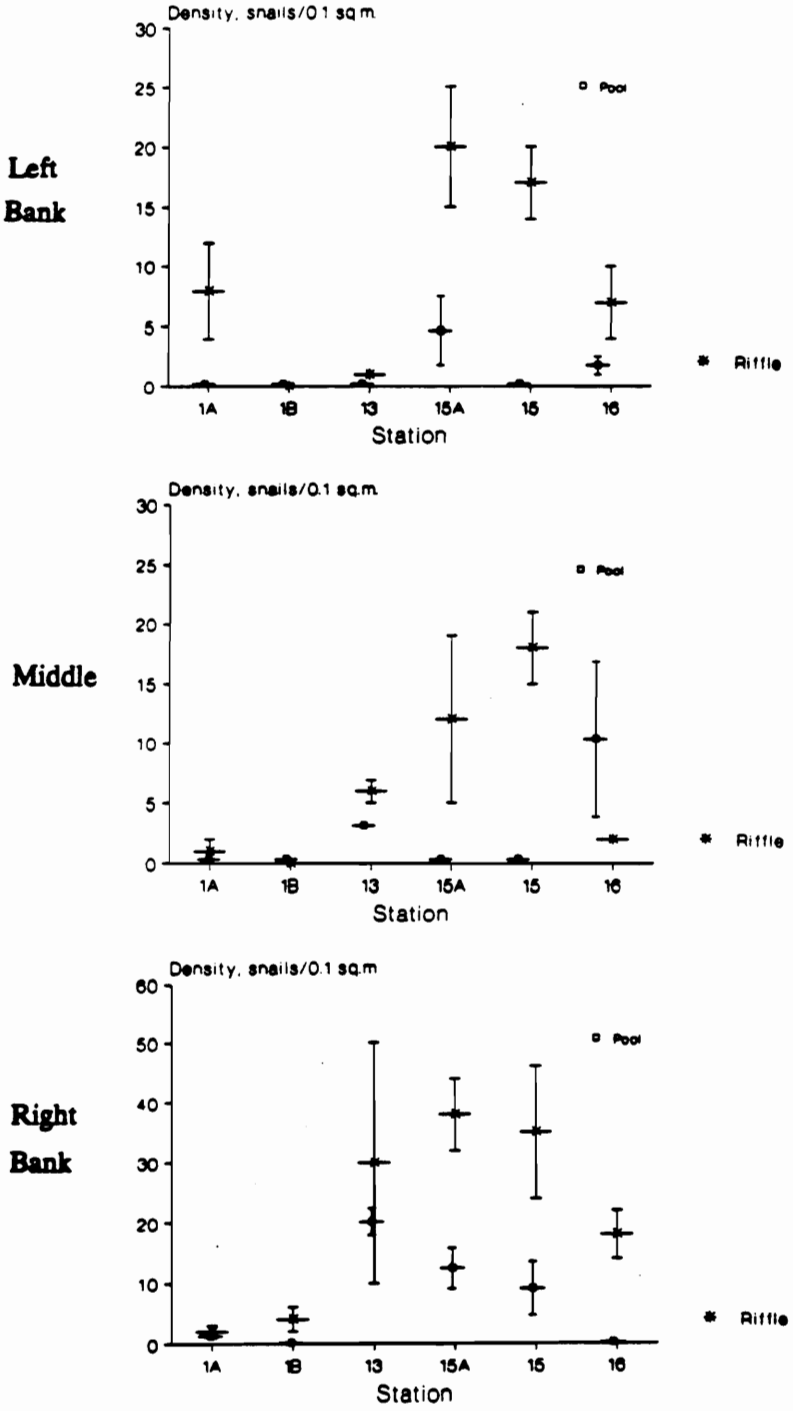


Figure 7. Density of *Ferrissia* per 0.1 m^2 in pools and riffles at six sampling stations in June, 1988.

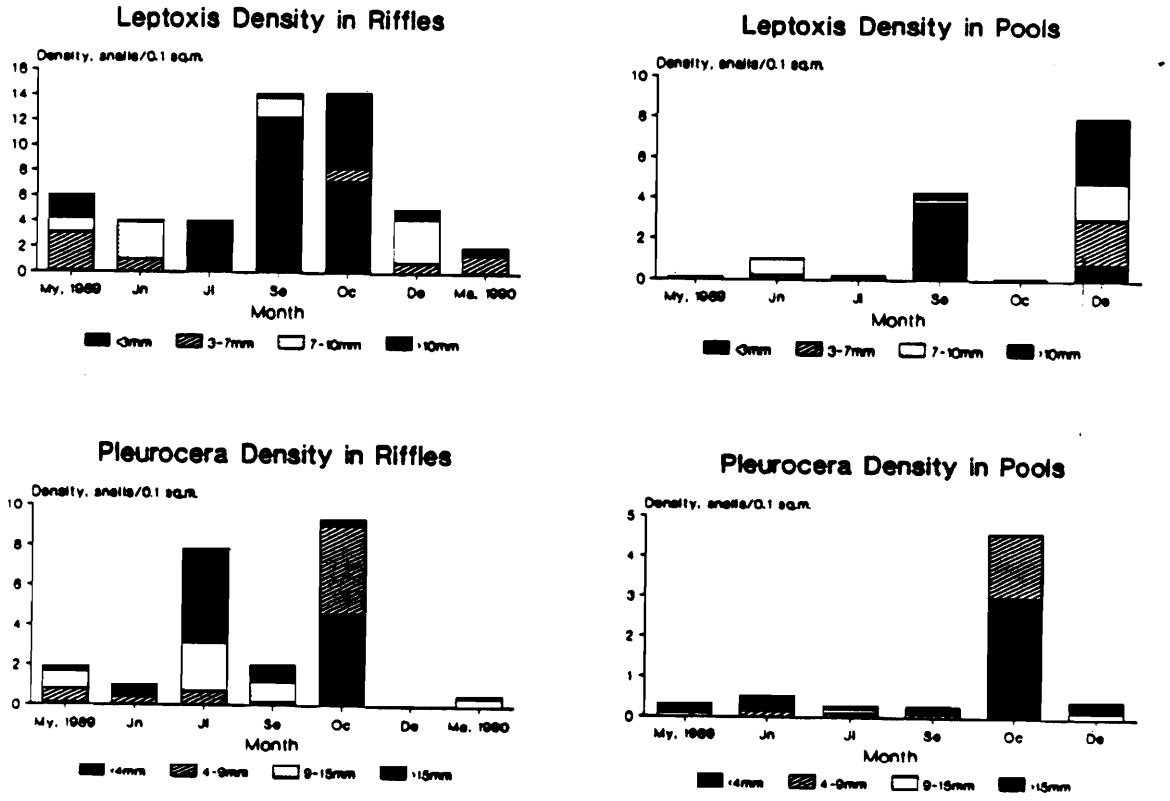


Figure 8. Average density of four size classes of *Leptoxis* and *Pleurocera* from monthly surber sampling at the left bank of riffle and pool locations at Stations 1A, 1B, 2, 13, 15A, 15 and 16, 1989-90.

Snail populations showed some recovery following the 1987 cooling tower changes that lowered copper concentrations in the discharge by from >800 to <150 ug Cu/L(see Introduction). *Pleurocera* began colonizing the left bank of Station 13 by July, 1989, and *Leptoxis* by the following December (Fig. 9). By March, 1990 density of both species had returned to upstream levels. Downstream stations had slightly higher density than upstream stations in spring and summer, but not as much in fall and winter months.

Impact on habitat

Microhabitat analysis of stations throughout the study area showed that variables significantly associated (presented in decreasing order of significance) with *Leptoxis* density (stepwise regression, SAS, 1985) were water copper concentration, pH, alkalinity, substrate type, *Pleurocera* copper bioconcentration, aufwuchs copper bioconcentration and algal cover (Table 4). Variables significantly associated with *Pleurocera* density were alkalinity, aufwuchs copper bioconcentration, and algal cover.

Metals in the environment

Copper concentrations in river water were slightly elevated at the left bank of Station 13 (from 27 ug/Lupstream to 38 ug/Lat Station 13; Table 5) in August, 1988, but the difference was not significant. These measurements (using flame atomic absorption; detection limit 10 ug Cu/L;AWWA, 1985) may

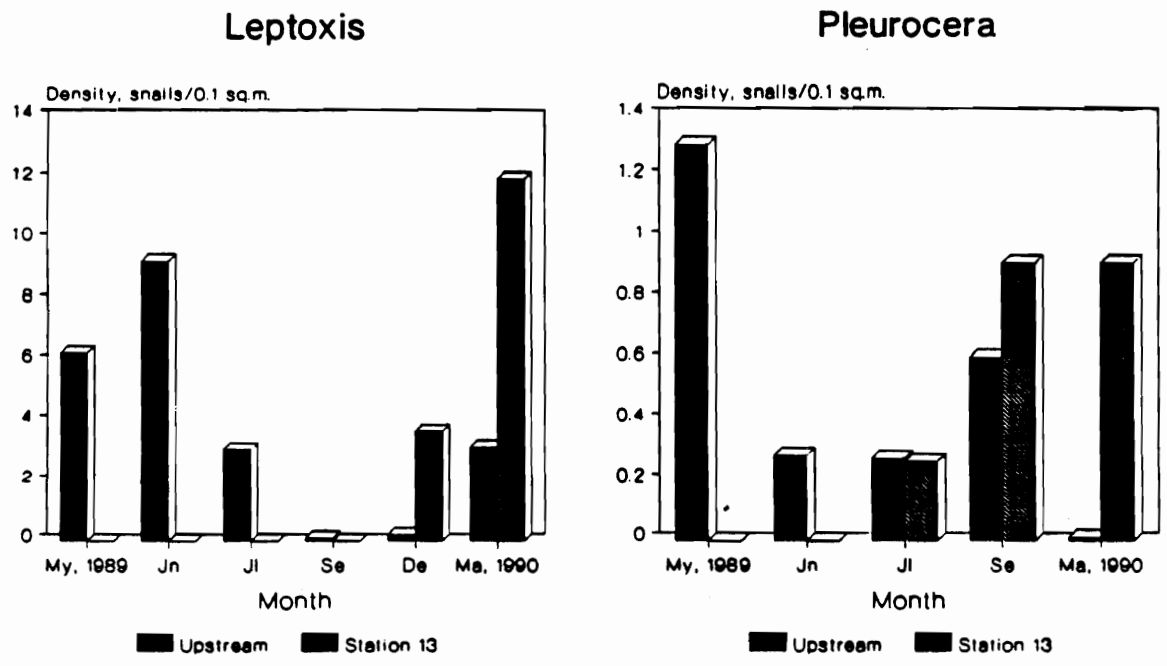


Figure 9. *Leptoxis* and *Pleurocera* population density from left bank riffles at Stations 1B and 13, 1989-90.

Table 4. Stepwise regression analysis for *Leptoxis* and *Pleurocera* density vs. physicochemical variables from random sites (HSS, Stations 1A, 1B, 2, 13, 15A, 15 and 16) - July-September 1988.

Variables in best regression model	F value	P value	Preferred ranges
Leptoxis			
Substrate[a]	17.82	0.0018	GCSA
Alkalinity, mg/L	23.70	0.0007	95-135
pH	24.47	0.0006	8.2-8.5
Algal cover, %	5.96	0.0348	0-20
Pleurocera Cu bioconcentration, ug/g	15.45	0.0028	<60
Aufwuchs Cu bioconcentration, ug/g	13.35	0.0044	<90
Water Cu bioconcentration ug/L	25.20	0.0005	<27
$R^2=0.8586$			
Pleurocera			
Alkalinity, mg/L	14.71	0.0018	125-135
Aufwuchs Cu bioconcentration, ug/g	9.93	0.0071	<70
Algal cover, %	7.82	0.0143	0-40
$R^2=0.6129$			

[a] G=gravel, C=cobble, Sa=sand, S=silt

Table 5. Means (SE) of metals in water samples and periphyton scrapings collected from Stations 1A (upstream) and 13 (0.9 km below discharge) - August, 1988.

	Metals		
	Cu	Zn	Pb
Water samples, ug/L			
Station 1A (n=12)	27	9	ND
(Ambient levels)	(3)	(2)	
Station 13 (n=6)			
Left	38	18*	ND
	(4)	(2)	
Mid.	35	18	ND
	(3)	(7)	ND
Right	25	14	ND
	(0)	(2)	
Aufwuchs scrapings, ug/g			
Station 1A (n=9)	53.6	133	124
	(2.5)	(43)	(73)
Station 13 (n=3)			
Left	259*	132	32
	(25)	(31)	(4)

*Significantly different from Station 1A ($P<.05$) (t-test)
ND - Not detectable using flame atomic absorption spectrophotometry

be sensitive enough at these concentrations to show gross differences but measurements below 30 ug/L are questionable. Copper concentrations were still slightly higher at the left bank of Station 13 than the right bank (8.0 and 1.8 ug/L, respectively) in September, 1989 (Table 6). Since a more sensitive technique (graphite furnace; detection limit 0.02 ug Cu/L; Perkin-Elmer, 1982) was used to measure copper in the 1989 samples, these data are considerably more accurate.

Copper accumulation in the aufwuchs at Station 13 (259 ug/g) was significant, higher than at an upstream station (54 ug/g; Table 5); neither zinc or lead accumulation was measured. Zinc concentrations in the water column at the left bank of Station 13 (28 ug/L) were significantly higher then the upstream station (from 9 ug/L). Lead concentrations were not detectable in the water samples taken at Station 13 or the upstream reference station. Ambient concentrations (upstream stations) of copper, zinc and lead in *Leptoxis* body tissue ranged from 37 to 190 ug Cu/g; 127 to 156 ug Zn/g; and 62-88 ug Pb/g (Table 7). No *Leptoxis* were found at the left bank of Station 13 (0.9 km below cooling tower discharge) for comparison. *Leptoxis* did not have elevated concentrations of copper at Station 15A, the next downstream station sampled. Ambient (upstream) metal concentrations in *Pleurocera* tissue ranged from 28 to 160 ug Cu/g, 108 to 119 ug Zn/g and 64 to 77 ug Pb/g (Table 8). Although snails were absent from the area where discharge was channeled by river flow to the left bank, at Station 13, a few *Pleurocera* and *Ferrissia* were

Table 6. Graphite furnace atomic absorption spectrophotometry analysis of water samples collected from the discharge zone of the CRP (September 21, 1989) (n=1).

Location	Cu, ug/L	Zn, ug/L
Station 6	52.4	26
Station 8		
-at discharge	19.4	12
-left bank downstream 22m	12.3	11
-left bank downstream 75m	12.2	14
Station 13		
-left bank upstream 560m	11.8	9
-left bank upstream 375m	10.2	7
-left bank upstream 190m	9.2	10
-midchannel upstream 560	2.6	5
-left bank	8.0	8
-middle	1.6	19
-right bank	1.8	10
Station 15		
-left bank	1.4	16
-middle	1.4	13
-right bank	2.4	19
Station 16		
-left bank	1.8	6
-right bank	1.9	18

Table 7. Metals analysis, ug/g, of soft body tissue from *Leptoxis praerosa* (Say). Values represent means of three replicates (SE) - August, 1988.

Station	Bank	Cu	Zn	Pb
Station 1A	L	84.2 (50.3)	-	-
	M	134.8 (83.6)	-	-
	R	37.1 (12.5)	-	-
Station 1B	L	100.3 (5.9)	156.2 (15.0)	87.6 (7.3)
	M	111.1 (12.9)	127.4 (19.8)	62.0 (11.6)
	R	104.9 (29.8)	164.2 (42.7)	70.4 (18.9)
Station 2	L	54.2 (6.2)	-	-
	M	137.6 (45.7)	-	-
	R	190.2 (31.7)	-	-
Station 13	L	No snails	No snails	No snails
	M	56.2 (7.1)	130 (17.8)	92.8 (20.7)
	R	63.3 (18.9)	108 (26.8)	101 (12.8)
Station 15A	L	66.8 (1.6)	-	-
	M	32.0 (5.3)	-	-
	R	34.1 (8.5)	-	-
Station 15	L	63.1 (8.7)	-	-
	M	119.3 (10.7)	-	-
	R	89.2 (n=1)	-	-
Station 16	L	79.0 (8.9)	-	-
	M	89.6 (7.8)	-	-
	R	74.9 (46.1)	-	-

Table 8. Metals analysis, ug/g, of soft body tissue from *Pleurocera unciale* (Reeve). Values represent means of three replicates (SE) - August, 1988.

Station	Bank	Cu	Zn	Pb
Station 1A	L	43.6 (7.5)	-	-
	M	No snail	-	-
	R	No snail	-	-
Station 1B	L	No snail	No snail	No snail
	M	99.4 (9.7)	119.3 (4.2)	77.1 (n=1)
	R	99.1 (15.0)	108.0 (11.3)	63.6 (12.2)
Station 2	L	160.1 (26.6)	-	-
	M	137.1 (28.2)	-	-
	R	27.5 (17.4)	-	-
Station 13	L	58.4 (6.3)	114.9 (11.8)	51.7 (3.2)
	M	41.0 (7.1)	57.0 (14.9)	58.7 (11.5)
	R	38.9 (3.8)	74.2 (3.5)	59.6 (11.0)
Station 15A	L	104.1 (40.9)	-	-
	M	107.0 (28.5)	-	-
	R	117.3 (22.3)	-	-
Station 15	L	127.7 (10.5)	-	-
	M	100.7 (18.9)	-	-
	R	114.8 (43.0)	-	-
Station 16	L	40.1 (24.6)	-	-
	M	69.3 (14.4)	-	-
	R	94.6 (6.3)	-	-

found on the right of discharge flow. Copper and zinc bioconcentration in those *Pleurocera* were slightly higher than for middle and right bank measurements but not when compared to upstream bioconcentration. Stations further downstream (15A, 15 and 16) also did not have bioconcentration greater than upstream measurements.

Food-borne uptake studies

At water hardness of 24 to 38 mg $CaCO_3$/L (1989 study; Appendix B, Table B1), water-borne uptake, but no significant food-borne uptake, was seen at concentrations tested (up to 17 ug Cu/L and 94 ug Zn/L, nominal concentration). *Leptoxis* significantly bioconcentrated copper in 8.6 ug Cu/L (7±2 ug Cu/L measured concentration; Appendix B) and combination treatments (17 ug Cu and 94 ug Zn, nominal; 5±2 ug Cu and 56±32 ug Zn/L, measured concentrations; Table 9; Appendix B, Table B2). No zinc bioconcentration or cellulolytic enzyme activity impairment were detected in *Leptoxis* in dosed or undosed exposures. However, bioconcentration of zinc in *Leptoxis* was significantly reduced in all zinc-dosed treatments.

Mudalia exhibited significant cellulase impairment when exposed to 8.6 ug Cu/L (7±2 ug Cu/L, measured concentration), 94 ug Zn/L (66±43 ug Zn/L, measured concentration) and a combination of 17 ug Cu and 94 ug zinc (5±2 ug Cu + 56±32 ug Zn/L, measured concentration). *Mudalia* zinc bioconcentration was significant (61 ug Zn/g) from the combination dosed

Table 9. Means (SD) of cellulolytic enzyme activity (n=6), copper and zinc bioconcentration (n=3) in *Leptoxis* and *Mudalia* from 30-day dosed and undosed feeding study (Study I) at Glen Lyn and ESL, respectively (July 3-August 8, 1989).

Stream	Treatment, ug/L # %	*Leptoxis* Cellulase Activity, ug/g	*Leptoxis* Cu Biocon., ug/g	*Leptoxis* Zn Biocon., %	*Mudalia* Cellulase Activity, ug/g	*Mudalia* Cu Biocon., ug/g	*Mudalia* Zn Biocon.,
Dosed Streams							
1	Control	100(25)	4(7)	192(261)	100(47)	22(10	4(8)
2	8.6 Cu	94(68)	8(4)*	-	75(46)*	25(10)	-
3	17.2 Cu	78(46)	7(11)	-	79(24)	30(14)	-
4	47 Zn	90(33)	-	13(27)*	-	-	-
5	94 Zn	91(79)	-	17(15)*	40(16)*	-	66(35)
6	17 Cu+94 Zn	97(60)	31(16)*	28(50)*	29(15)*	34(8)	61(20)*
Undosed Streams							
1	-	100(94)	108(113)	187(110)	100(28)	72(16)	76(9)
2	-	70(42)	68(26)	-	217(89)	135(75)	-
3	-	22(22)	48(15)	-	230(54)	96(21)	-
4	-	90(91)	-	55(10)	-	67(37)	82(22)
5	-	111(111)	-	56(20)	-	44(17)	111(52)
6	-	62(23)	57(12)	54(18)	-	-	-

*Significantly different from control ($P<0.05$) according to Duncan's multiple range analysis (SAS, 1985; Appendix B).

treatment. No significant cellulase activity impairment or bioconcentration were found in the undosed stream treatments. *Leptoxis* copper bioconcentration was significant, compared to control snails, after feeding for 30-days from tiles with aufwuchs bioconcentration of 564 (±269) ug Cu/g (Table 10, Fig. 10), from the highest copper treatment (500 ug Cu/Lsoak) tested. No cellulolytic enzyme activity impairment was seen, however, in this treatment or any of the treatments tested. Table B3 (Appendix B) shows that approximately 25-55%of copper was removed from the soak solution during the 2-3 day soak period. Zinc was not bioconcentrated in *Leptoxis*, although, in both the 2000 ug Zn/L and 50 ug Cu + 2000 ug Zn/Ltreatments, aufwuchs bioconcentration of zinc was significant (20,300± 18,400 and 17,600± 13,700 ug/g, respectively). Tiles soaked in zinc solutions removed approximately 76-85% of the zinc during the soak period Appendix B, Table B3). Some elevation of copper and zinc was measured in streams containing feeding trays compared to a control.

DISCUSSION

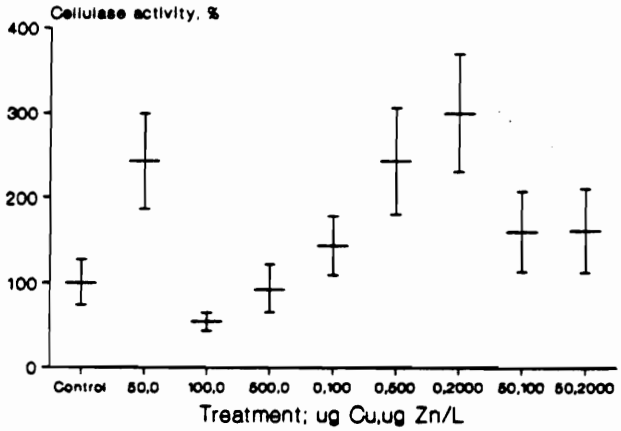
Population structure

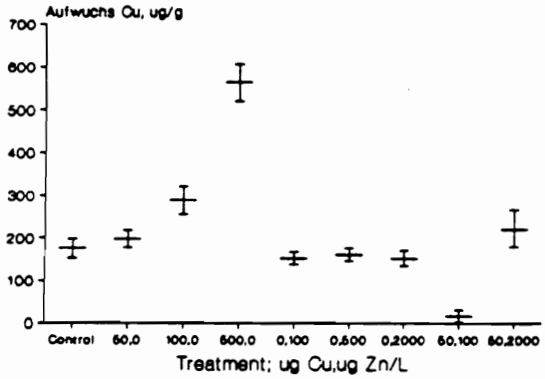
It took approximately two years for snails to begin recolonizing an area characterized as acceptable habitat (left bank of Station 13) after the copper stress was reduced. Within 1-3 months after reinvasion was observed, density had returned to upstream levels. This was not surprising when factors

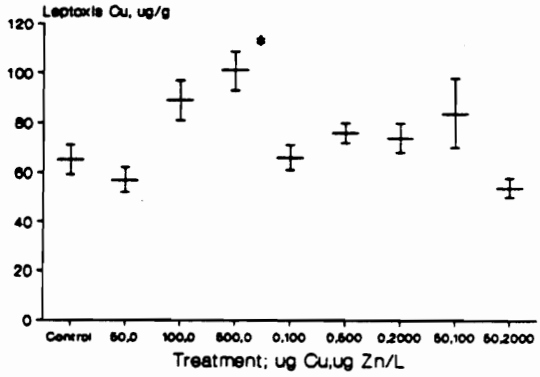
Table 10. Means (SD) of cellulolytic enzyme activity (n=18) copper and zinc bioconcentration in *Leptoxis* (n=18) and aufwuchs (n=39) from 30-day feeding study using precolonized ceramic tiles (August 16-September 15, 1990).

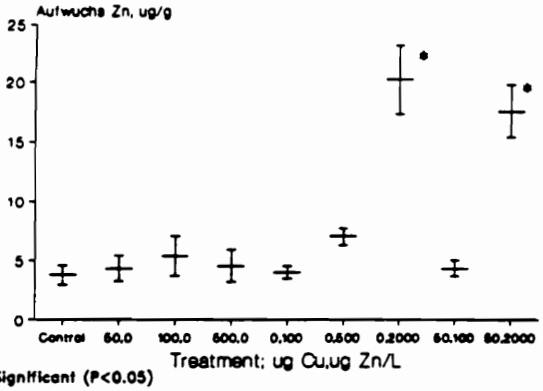
Chamber #	Tile-soak treatment,	Cellulase Activity, %	Cu content, ug/g Aufwuchs	Cu content, ug/g *Leptoxis*	Zn content, ug/g Aufwuchs	Zn content, ug/g *Leptoxis*
1	Control	100(113)	174(149)	65(25)	3800(5200)	866(982)
2	50 Cu	243(237)	197(133)	57(21)	4360(6680)	985(1150)
3	100 Cu	54(44)	289(208)	89(39)	5380(10,300)	1410(2360)
4	500 Cu	93(117)	564(269)	101(33)*	4580(8540)	634(137)
5	100 Zn	144(146)	151(95)	66(22)	4030(3250)	1110(1400)
6	500 Zn	243(264)	159(95)	76(18)	7060(4210)	841(545)
7	2000 Zn	299(290)	151(112)	74(27)	20,300(18,400)*	1570(2870)
8	50 Cu+100 Zn	160(197)	16(87)	84(59)	4380(4270)	2450(3470)
9	50 Cu+2000 Zn	161(206)	222(277)	54(18)	17,600(13,700)*	1310(1680)

*Significantly different from control ($p<0.05$) from Duncan's multiple range analysis (SAS, 1985).









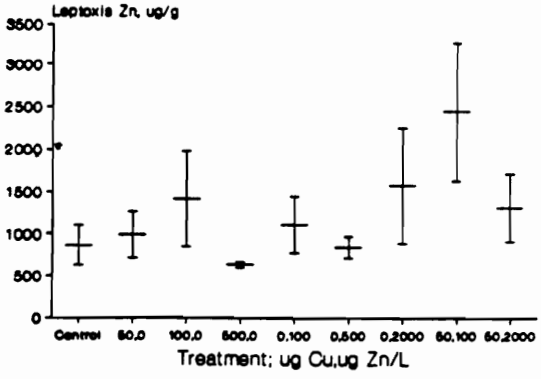


Figure 10. ***Leptoxis*** **cellulase activity, periphyton and** ***Leptoxis*** **copper and zinc bioconcentration (SE) from 30-day feeding study at the CRP, 1990.**

important in recovery, as defined by Cairns (1977), are evaluated for the Clinch River study area. They included: 1) existence of nearby epicenters for reinvading organisms; 2) transportability or mobility of disseminules; 3) general present condition of habitat following pollution stress; 4) presence of residual toxicants; 5) chemical-physical water quality following pollutional stress; and 6) management or organizational capabilities for immediate and direct control of damaged area. The first two factors did not present problems in the Clinch River study area, because upstream sites had healthy snail populations and snails were available for reinvasion. Snails are relatively mobile because of their ability to drift downstream. The reasons recolonization took two years were probably due to the condition of habitat and residual toxicants. The residual copper left in the ecosystem was significant and resulting changes to the habitat lingered. In this case, although copper in the water column was reduced to lower levels in summer 1987, copper remained significantly elevated in periphyton (259 ug/g, compared to ambient levels of 54 ug/g, one year later. The algal assemblages were visibly altered. Although no quantitative algal studies were performed, a cursory examination showed that Station 13 had a higher proportion of bluegreens to greens and diatoms. Since periphyton copper concentration was a significant factor in snail distribution (for both *Leptoxis* and *Pleurocera*), changes in habitat including bioconcentration of copper were thought to be responsible for snail avoidance of Station 13. These factors may have been more important in avoidance of the area than water-

borne metals (or other water quality variables), but the relationship is not well defined. Water-column copper concentration did not appear to be significantly elevated at Station 13 in 1988. However, these values measured by flame atomic absorption showed Station 13 concentrations to be 38 ug Cu/L, compared to ambient concentrations of 27 ug Cu/L. Upstream measurements were later shown to be approximately 1-5 ug Cu/L, using more advanced measurement techniques (i.e., graphite furnace and ICP). Whether the concentration at Station 13 was actually lower than 38 is not known. Measurements taken the next year, 1989 (two years after copper reductions) showed concentrations to be approximately 9 ug Cu/L. Concentrations of zinc measured in 1988 were significantly elevated at Station 13 (28 ug Zn/L) above upstream levels (9 ug Zn/L). In 1989, zinc concentrations at the left bank of Station 13 were reduced to upstream levels (8 ug Zn/L). Since zinc concentrations were elevated in 1988 and back to ambient concentrations in 1989, it is reasonable to assume that copper concentrations had similar patterns, since both are rough measurements of effluent concentration. Therefore, avoidance of the left bank of Station 13 may be attributed to metals concentrations in the water column and periphyton and associated changes in habitat.

In 1988, snails were prolific at the middle and left banks of Station 13, but absent at the left bank. This absence was attributed to the CRP effluent flow which was released upstream and channeled by river currents to the left

bank. The two *Ferrissia* found on the left bank at Station 13 in early summer were attached to submerged macrophyte (*Heteranthera dubia*) which may have channeled effluent flow away from the limpets. In 1988, downstream recovery of snail populations occurred at Stations 15A-16. Similarly, Clements *et al.* (1988) found that abundances of dominant insects were significantly reduced in August, 1986, from the 004 discharge downstream to Station 13, but recovered at Station 15A (the next downstream station sampled).

Snails began recolonizing the left bank at Station 13 by late summer, 1989, and showed full recovery of population density by March, 1990. The largest size class for *Leptoxis* was not as dense as in upstream stations, however. This was not surprising since this area was beginning to recover temporally and the larger snails were second-year individuals.

The absence from the left bank of Station 13, seen in 1988, may have been due to alterations of food, actual effects from feeding on the copper-contaminated aufwuchs, or avoidance. Feeding studies showed that in Clinch River water (water hardness, approximately 150 mg $CaCO_3$/L) much higher aufwuchs copper concentrations are needed to produce bioconcentration of copper in *Leptoxis* or impairment (as measured by cellulolytic enzyme activity). In 1988, aufwuchs contained 259 ug Cu/g. Aufwuchs containing 289 ug Cu/g did not produce measurable copper bioconcentration in *Leptoxis*. The next highest aufwuchs bioconcentration tested (564 ug Cu/g) produced significant bioconcentration (100 ug Cu/g) in *Leptoxis*, but no measurable cellulolytic

enzyme activity impairment. In these studies, 100 ug Cu/L produced some bioconcentration (289 ug/g) in aufwuchs but 500 ug Cu/L to produce the level of aufwuchs bioconcentration (564 ug Cu/g) that produced significant copper bioconcentration in *Leptoxis* (101 ug Cu/g). Water column concentrations were only 38 ug Cu/L in 1988, lower than the 50 ug Cu/L soak treatment that did not result in aufwuchs or *Leptoxis* bioconcentration. However, perhaps the time needed to produce bioconcentration may have been greater than the 30-days used in the feeding study.

Any food-borne contribution by zinc was unlikely since zinc concentrations in the water column (28 ug Zn/L) were much less than those (2000 ug Zn/L) producing significant aufwuchs zinc bioconcentration (17,600-20,300 ug Zn/g). No bioconcentration of zinc or impairments were seen when *Leptoxis*. At a lower water hardness (approximately 30 mg $CaCO_3$) no food-borne uptake or impairments were seen in *Leptoxis* or *Mudalia* at levels tested (aufwuchs soaked in up to 17 ug Cu/L and 94 ug Zn/L within 30-days. Unfortunately, higher metal levels were not tested in the lower water hardness study, so, although water hardness is well known to affect toxicity of metals (Lee, 1973, DeWith *et al.*, 1986) the effect of water hardness on food-borne uptake could not be determined in this study.

Changes in habitat

Localized variability in ambient pH (range of 8.04-8.35) and alkalinity

(range of 140-154 mg $CaCO_2$/L) were not limiting to snails upstream. Downstream, however, these variables (pH and alkalinity ranges of 8.20-8.40 and 84-132 mg $CaCO_2$/L, respectively) were influential to the distribution of both species. This suggests an avoidance of areas where effluent is measurable but does not confirm that avoidance is due to either variable. For example, such a limited pH increase (0.28 pH units) associated with the effluent, probably did not influence density, but may indicate avoidance of other constituents of the effluent, such as copper concentration.

Food-quality degradation

Bioconcentration of copper by aufwuchs was a significant factor in both *Leptoxis* and *Pleurocera* habitat choice. This suggests avoidance of the area with elevated aufwuchs copper concentration (259±43 ug/g). Although input from food may be a major source of metals in gastropod tissues (Young, 1977), levels of food-borne copper causing snail detection and avoidance have not been determined. Learned behavior, however, is possible in snails (reviewed by Zakharov and Balaban, 1987). Alternatively, avoidance of Cu-laden periphyton may involve Cu-induced changes in food quality. Metal-induced changes in algal assemblages have been documented, and nutritional value of algae varies among species (Anderson and Cummins, 1979). Food quality may affect growth rates (Rietsma *et al.*, 1988), toxicity (Belanger *et al.*, 1989), reproduction rates (reviewed by Rollo and Hawryluk, 1988) and distribution

(Lamberti and Resh, 1983).

Food-borne uptake

In the dosed and undosed streams study, food-borne metals from cobbles treated with up to 17 ug copper and/or 94 ug Zn/L did not result in bioconcentration or cellulase impairment; however, in combination, waterborne exposures caused copper and zinc bioconcentration, respectively, in *Leptoxis* and *Mudalia*. This concentration also caused significant cellulase impairment in *Leptoxis*. The lowest copper treatment concentration producing bioconcentration in *Leptoxis* from food-borne uptake (in the precolonized tile study) was 500 ug Cu/L. Though zinc bioconcentration in periphyton was significant when treated with 2000 ug Zn/Lsolution, both separately and in combination with Cu, *Leptoxis* did not bioconcentrate zinc or have cellulase impairment. Since treated tiles were rinsed vigorously before snails were fed, a portion of the metals adhering to loosely-attached particulate matter may have been removed, as well as some metal-laced periphyton, making the aufwuchs bioconcentration measurements. somewhat lower, and, perhaps, more reflective of actual periphyton bioconcentration.

Results from both studies show that though food-borne uptake may occur (as seen here only in Cu), water column concentrations required to treat periphyton may be an order of magnitude higher than waterborne exposures (17 ug Cu and 94 ug zinc from the dosed exposures) causing impairments.

There are confusing findings by others; findings in other studies have both supported and contradicted these findings. Jimenez *et al.* (1987) found that food-borne uptake of an organic contaminant did not contribute significantly to the total uptake in the bluegill sunfish (*Lepomis macrochirus*). Young (1977) found, however, that the food chain was a major source of Zn and Fe uptake in a carnivorous marine dogwhelk, *Nucella lapillus*. Phillips (1976) found uptake of copper by the mussel, *Mytilus edulis*, to be so erratic and affected by environmental variables (e.g., salinity, temperature, metals) that he suggested this mussel not be used as an indicator of copper in the environment.

Leptoxis and *Mudalia* both were affected by the combination treatment, though each bioconcentrated a different metal. Differences in metal bioconcentration between closely related molluscan species have been documented (Segar *et al.*, 1971) and associated with dietary differences (Young, 1977). Both species in this study have similar feeding habits, accounting for similarities found in these results.

CHAPTER FOUR: ZONE DELINEATION

INTRODUCTION

Monitoring power plant effluent effects by standard laboratory bioassays may have limited environmental application (Kimball & Levin, 1985; Mount *et al.*, 1985; Cairns, 1983; Weber, 1981). Laboratory screening procedures may indicate an effluent's toxicity to organisms (USEPA, 1989). Their accuracy, however, in predicting effects at higher levels of biological organization may be limited (Cairns and Orvos, 1989). Often more site-specific assessment techniques are needed. For example, delineation of zones of impact through ecological monitoring accounts for differences in each ecosystem and its indigenous populations (Barbour and Plafkin, 1988) and, thus, may be a more direct assessment of actual environmental effects.

Effects on snail density below Clinch River Plant (CRP) effluent discharges were measured and compared to snail sensitivity to effluent and its constituents (i.e., copper and Zn). Snail sensitivity was measured by acute toxicity, sublethal effects (cellulolytic enzyme activity and growth impairment) and avoidance of areas where habitat variables were altered from effluent exposures. From definition of suitable habitat for dominant taxa (*Leptoxis* and *Pleurocera*) (described in Chapter 2) changes in snail density could be attributed to CRP effluent (and associated metals) concentrations (Chapter 3). Once habitat preferences were defined, areas of measurable impact were

distinguished from areas of naturally occurring unsuitable habitat. The overall objective of this portion of the research was to define in-river zones where snails experienced acute toxicity or impairment from effluent exposures. The roles of bioconcentration of copper and food-quality degradation were examined to provide further information which may prove useful for monitoring and early warning before populations are severely affected.

METHODS

Acute toxicity

Two 96-hr flow-through tests were conducted in artificial streams at the Clinch River Plant (September 23-27 and October 27-31, 1988), and one 96-hr static-stirred test was conducted in 15-L polycarbonate containers at Virginia Tech (October 12-16, 1988). Clinch River water was the diluent in each of these tests. Flowthrough tests at the Clinch River had constant flow of Clinch River water from upstream of the CRP. For the static test at VPI&SU, Clinch River water was collected upstream of the CRP and transported to VPI&SU. Lighting and temperature were controlled as described by USEPA (1989). In the flow-through tests, artificial streams (Chapter 3) were housed under a greenhouse tarp, to facilitate periphyton growth. Clinch River water was pumped into fiberglass troughs and gravity-fed to artificial streams. Flow rates were measured and delivery tubes cleaned on a regular basis when tests were

being conducted.

Plant effluent was continuously pumped into a fiberglass headbox, aerated to minimize effects of plant chlorination procedures, and gravity fed to artificial streams. Effluent flow rates into the artificial streams were controlled by altering the size of openings in delivery pipes to yield the desired effluent concentration in each artificial stream. Flow rates were measured and delivery pipes cleaned periodically throughout the test (frequency of maintenance varied with river conditions). Effluent concentrations of 10, 20, 40, 60, 80 and 100% were used in both flow-through tests. Animals tested at 100% were placed directly into the effluent headbox. Artificial streams were mixed with fiberglass paddlewheels.

For determinations of metal toxicity, stock solutions prepared in 50-100L plastic containers were delivered to artificial streams by peristaltic pumps. Pump rates were measured for each pump head before tests were begun. Concentrations of stocks were determined from diluent and toxicant flow rates. Primary stocks were made by dissolving metal salts ($CuSO_4 \cdot 5H_2O$ and $ZnSO4 \cdot 7H_2O$) in deionized water. Stock solutions were diluted to 50 or 100L with Clinch River water. Measured amounts of copper and zinc in water samples collected from the first flow-through study were markedly less than target concentrations. Experiments conducted at VPI&SU showed that at the concentrations used in the study, metals (especially copper) precipitate probably by binding to constituents of the Clinch River water (i.e., $CaCO_2$ and

$MgCO_3$). To reduce precipitation of metals in the stock solutions, Clinch River water was filtered through an ion-resin column prior to use in the tests. Recovery of copper was improved. Target concentrations of copper and zinc used in the study were determined from LC_{50} values for snails found by other investigators (USEPA, 1980 and 1984). Target copper concentrations for both flow-through studies were 0, 0.01, 0.05, 0.10, 0.40, 0.60 and 1.00 mg/L. Target zinc concentrations for both studies were 0, 1.0, 5.0, 10.0, 13.5, 15.0 and 20.0 mg/L. Target concentrations for combination treatments, in both studies were 0.05 ug Cu + 5.0 ug Zn/L;0.40 ug Cu + 5.0 ug Zn/L;0.05 ug Cu + 13.5 ug Zn/L;and 0.40 ug Cu + 13.5 ug Zn/L.

Snails from the Clinch River (*Leptoxis praerosa* and *Pleurocera unciale*) were used in both flow-through tests. Ten of each species were added to each artificial stream and not fed during the test. Snail mortality was determined by probing the sensitive foot region followed by a 15-minute recovery period in control water (Paulson *et al.*, 1983).

Water samples were collected at the end of each test and transported in ice-filled coolers to VPI&SU for analysis. Water quality measurements, pH, conductivity, alkalinity, and hardness, were conducted according to standard methods (APHA, 1986). A portion of each water sample was filtered, preserved, and analyzed as previously described.

Clinch River water and effluent, for use in the 96-hr static test, were collected in 10L polypropylene carboys and transported to VPI&SU. Two

prosobranchs, *Leptoxis praerosa* and *Pleurocera unciale*, were collected from the Clinch River, transported to VPI&SU and held in aerated coolers overnight for temperature equilibration to 22±2°C. Pulmonates, *Ferrissia rivularis* and *Physella* sp., were also used in this test. Rocks with attached *Ferrissia* were collected from the Clinch River and held in aerated coolers overnight. Because of low population density in the Clinch River study area (only three individuals found in seven stations), *Physella* was collected from the New River. For comparison with studies previously conducted at VPI&SU, *Mudalia dilatata*, was also collected from the New River. *Physella* and *Mudalia* were acclimated to Clinch River water for four days prior to testing by gradually increasing the ratio of Clinch to New River water.

Ten individuals of each species, except *Ferrissia*, were added to each test container. Numbers of *Ferrissia* added to test containers depended on numbers of individuals attached to each cobble, since preliminary investigations showed that *Ferrissia* shells were often damaged when individuals were removed from rock surfaces. Snails were not fed during the test except for algae on cobbles with attached *Ferrissia*. Mortality for the larger snails was determined as previously described. Mortality for *Ferrissia* and *Physella* was determined by lack of observable movement when viewed through a dissecting microscope.

Target effluent concentrations were 0, 10, 20, 40, 60, 80 and 100%. Target copper concentrations were 0, 0.01, 0.05, 0.10, 0.40, 0.60 and 1.00 mg/L. Target zinc concentrations were 0, 1.0, 5.0, 10.0, 13.5, 15.0 and 20.0

mg/L. Target concentrations for combination tests, in mg/L, were 0.05 mg Cu + 5.0 mg Zn/L;0.40 mg Cu + 5.0 mg Zn/L;0.05 mg Cu + 13.5 mg Zn/L; and 0.40 mg Cu + 13.5 mg Zn/L. Metal stocks were made by dissolving metal salts ($CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$) in deionized water. Measured amounts of effluent or metal stocks were added to each 15L cylindrical polycarbonate container and diluted to a final volume of 5L with Clinch River water.

Temperature and dissolved oxygen were measured at the beginning of the test and daily throughout the test duration. When dissolved oxygen concentration began to fall after the first day, containers were aerated for the remainder of the test. Water samples were collected at the end of each test. Water quality measurements included pH, conductivity, alkalinity and hardness (using standard techniques previously referenced). A portion of each water sample was filtered, preserved and analyzed for metals as previously described. Measured metals concentrations were used to calculate LC50s (Finney, 1971).

Two 96-hr static-stirred tests (plus one range-finding test) were conducted in 1989 in the artificial streams at the CRP. Test procedures were similar to those used in the 1988 studies. Water quality samples were collected at beginning, middle and end of each test. When copper concentrations were less than 50 ug/L graphite furnace atomic absorption spectrophotometry was used for increased sensitivity at copper concentrations below 50 ug/L (detection limit for graphite furnace and flame techniques are 0.02 and 10 ug Cu/L,respectively; Perkin-Elmer, 1982; AWWA, 1985). Target

concentrations used in the May 23 - May 27 test were 0, 10, 60, 80 and 100% CRP effluent; 17, 34, 68, 150, 500 and 1000 ug Cu/L; 47, 94, 200, 1000, 5000 and 10000 ug Zn/L; and a combination of 17 ug Cu + 47 ug Zn/L. In the test conducted May 31 - June 4 these concentrations were used, plus additional concentrations of 2000 ug Cu/L, 20,000 ug zinc and 34 ug Cu + 94 ug Zn.

Impairment tests

Cellulolytic enzyme activity was measured to determine the lowest-observed effect concentration (LOEC) and the no-observed effect concentration (NOEC) for whole effluent and Cu. The LOEC is defined as the lowest concentration of a toxicant which produces a significant impairment (USEPA, 1989). The NOEC is the highest concentration not producing a significant impairment. Cellulolytic enzyme activity (Farris *et al.*, 1988) was measured in six snails per treatment for each species tested (*Leptoxis* and *Pleurocera*). Copper bioconcentration (Valdes *et al.*, 1982) was measured in three snails of each species per treatment. Snails were collected for analysis following 14-, 20- and 30-day flow-through artificial stream exposures at the CRP, June 28-September 16, 1988, and a second 30-day study, August 31-September 30, 1990. Products of endocellusase and exocellulase activity were expressed as a percentage of control values. Two replicate streams per treatment were used. Periphyton growth on stream walls provided food.

Water chemistry and aufwuchs scrapings were collected from each stream, and flow rates were adjusted every 5 days in the 1988 studies and every 2-3 days in the 1990 study. Target concentrations for the 14-day exposures were 6, 12, and 25 ug Cu/L and 0, 1, 5, 10, and 20% CRP effluent. For 20- and 30-day, 1988-exposures, target concentrations were 6 and 12 ug Cu/L and 0, 1, 10, 20, and 40% CRP effluent. For the 1990 exposures, target concentrations were 0, 12, 17, 35 and 75 ug Cu/L. Copper concentrations were measured using an inductively coupled plasma analyzer (detection limit 6 ug Cu/L, AWWA, 1985) for the 14- and 20-day studies and flame atomic absorption spectrophotometry in the 30-day study.

Growth in *Leptoxis* and *Pleurocera* also was used to assess sublethal effects of total effluent, copper and zinc in the 14-day study. Aperture width and total shell length were measured using digital calipers. Measurements were made on the first and last day of the study. Aperture widths of *Leptoxis* were measured after exposure to target concentrations of 0, 1, 5, 10 and 20% effluent and 6, 12 and 25 ug Cu/L; shell length of *Leptoxis* after exposure to 0 and 20% effluent and 0 and 25 ug Cu/L; shell length of *Pleurocera* after exposure to 0, 1, 5, 10 and 20 percent effluent and 0, 6, 12 and 25 ug Cu/L; and aperture width of *Pleurocera* after exposure to 0 and 20% effluent and 0 and 25 ug Cu/L.

The relationship between eventual fate of cellulolytic enzyme activity and bioconcentration of copper and zinc were determined in a long-term mortality

study conducted in artificial streams at the CRP (June 13 - October 15, 1989). *Leptoxis* and *Mudalia* were exposed to six treatments (control, 8 ug Cu/L, 17 ug Cu/L, 24 ug Zn/L, 47 ug Zn/L and 17 ug + 47 ug Zn/L) for 114 days. On day 40 and every 12 days thereafter, six *Leptoxis* (and six *Mudalia* on select days) were removed for analysis of cellulolytic activity and bioconcentration of copper and zinc. Three nylon mesh bags containing 10 *Leptoxis*, each, were placed in each stream for mortality determination each time snails were removed for lab analyses.

Soft body parts of each of the six snails were homogenized in phosphate buffer. The homogenate was centrifuged to separate solid and liquid portions. Cellulolytic enzyme activity was determined from the supernatant. Bioconcentration was determined on the pellet of each snail homogenate, rather than whole digested bodies, as previously described. The purpose for this change in procedure was to determine both metals uptake and cellulolytic enzyme activity in each individual. For days 40 and 58, copper and zinc were measured in the supernatant of homogenates to compare metals concentrations present (and variability) in supernatant and pellet, and determine feasibility of estimating bioconcentration of copper and zinc from the pellet.

In-river validation

Fifteen snails were placed in nylon mesh (2 mm^2 mesh size) bags (cages) containing precolonized cobble from the Clinch River. Cages were attached an

iron stakes at Stations 1, 2, 4A, 6, 8, 10, 11, 12, 12B, 13, 14A, 15A, and 15 (Fig. 3, Chapter 2; August 11, 1988). After 40 days, mortality was determined as in acute tests, and, when survival allowed, six snails from each cage were collected for cellulolytic enzyme activity and three for copper bioconcentration analyses. Duncan's Multiple Range Analysis (SAS, 1985) was used to determine if means of measurements at selected stations were different from those at control stations. Attempts to repeat the study in 1989 failed due to persistent flood conditions producing sediment shifts, damaging caged snails.

RESULTS

Acute toxicity

LC_{50} values for whole effluent were 95->100% for *Leptoxis* and 90->100% for *Pleurocera* (Table 11). Copper concentrations in CRP effluent varied and effluent in flow-through tests had higher copper concentration (148 ug Cu/L) than in static tests (105 ug Cu/L; Appendix B, Table B4). Water hardness and conductivity were correspondingly higher in effluent used in the flow-through test (Appendix B, Table B4), indicating a more concentrated effluent. Pulmonates were more sensitive to copper (LC_{50}, 33 ug Cu/L, both genera tested), zinc (LC_{50}, 59-144 ug Zn/L), total effluent (LC_{50}, 60% for *Physella*), and a combination of copper (LC_{50} 8ug Cu/L, combined with 640-820 ug Zn/L) and zinc (LC_{50}, <500 ug Zn/L, combined with 80-362 ug Cu/L), than

prosobranchs (Table 11). Of the two dominant snails in the CRP study area (both prosobranchs), *Leptoxis* was more sensitive to copper and zinc treatments. The copper LC_{50} value for *Leptoxis* was generally within the range of 90-150 ug/L. For *Pleurocera* copper LC_{50} values occurring most often ranged from 160-370 ug/L. The copper LC_{50} for both pulmonates was 33 ug/L. The zinc LC_{50} values for *Leptoxis* and *Pleurocera* ranged from 1210-700 and 3590-8680 ug Zn/L,respectively. Pulmonate zinc LC_{50} values ranged from 59-144 ug Zn/L, more than an order of magnitude less than prosobranch LC_{50} values.

The two pulmonates had similar sensitivities to both total effluent and combinations of copper and zinc. In copper and zinc combination tests, zinc additions of <1000 ug/L, reduced the copper LC_{50}, attributing most of the toxicity to Cu. Again, pulmonates were more sensitive than prosobranchs to this combination treatment, with LC_{50} values for both species tested <28 ug Cu/L. Additions of >1360 ug Zn/Lreduced the copper LC_{50} to 10-15 ug/L. This zinc concentration is within the range of zinc LC_{50} values for *Leptoxis* but much less than those for *Pleurocera*. Generally, *Pleurocera* LC_{50} values in combination treatments decreased in dose-dependent fashion with increasing concentrations of the second metal, for both copper and zinc. The toxic effects of the metals appear to be additive at the concentrations tested. Differences in LC_{50} values were not seen between static stirred (VPI&SU)and flow-through (CRP) tests in these studies (Appendix C, Tables C1 and C2).

Table 11. Summarized LC_{50} values from 96-hr static and flow-through test conducted at CRP and VPI&SU, 1988-89.

Prosobranchea			Pulmonata	
Leptoxis	*Pleurocera*	*Mudalia*	*Physella*	*Ferrissia*
Effluent, %				
95->100	90->100	>100	60	-
Cu, ug/L				
27-146	87-364	112	33	33
Zn, ug/L				
1210->10,600	3590->10,600	850	59	114
Combined metals				
Cu, ug/L				
Combined with:				
640-820 ug Zn/L				
>106	106	67	<28	<28
1360-1500 ug Zn/L				
10	10	-	-	-
7980-8860 ug Zn/L				
<15	<15	-	-	-
Zn, ug/L				
Combined with:				
10-15 ug Cu/L				
1360	1360	-	-	-
50-60 ug Cu/L				
-	5010	-	-	-
46-106 ug Cu/L				
-	816	-	-	-
80-326 ug Cu/L				
-	200	639	<500	<500

Impairment

Cellulolytic enzyme activity in *Leptoxis* appeared to be reduced (though not significantly) in 14-day exposures up to 20% effluent (28±2 ug Cu/L), (Fig.11). Cellulolytic enzyme activity in *Leptoxis* was significantly impaired in both 20- and 30-day exposures to 40% effluent (52±2 ug Cu/L) and 10% effluent (20±3 ug Cu/L), respectively (Fig. 12 and 13). The LOEC for effluent, therefore, was 10% and the NOEC was 1%. Effluent in 20-day exposures had slightly higher copper concentration (229 ug Cu/L) than in 14- and 30-day exposures (176-191 ug Cu/L), along with higher water hardness and conductivity (see Appendix B, Table B5).

Although no significant changes were detected, $CuSO_4$ exposures resulted in reduced cellulolytic enzyme activity in 14-day studies (up to 15±2 ug Cu/L; Fig. 11) and 20-day studies (up to 10±2 ug Cu/L; Fig. 10), and increased cellulolytic enzyme activity in the 30-day study (up to 25±6 ug Cu/L; Fig. 13).

Total shell length appeared to be a more sensitive measure of growth impairment than aperture width in the 14-day study. Table 12 shows that aperture width measurements did not follow a dose-response pattern in tests with *Leptoxis*, but shell length measurements did show a such pattern. *Leptoxis* growth was impaired (significantly less than the control according to Duncan's analysis) from 14-day exposures to 20% CRP effluent (28±2 ug Cu/L), while *Pleurocera* was impaired by the 10% effluent (20±2 ug Cu/L)

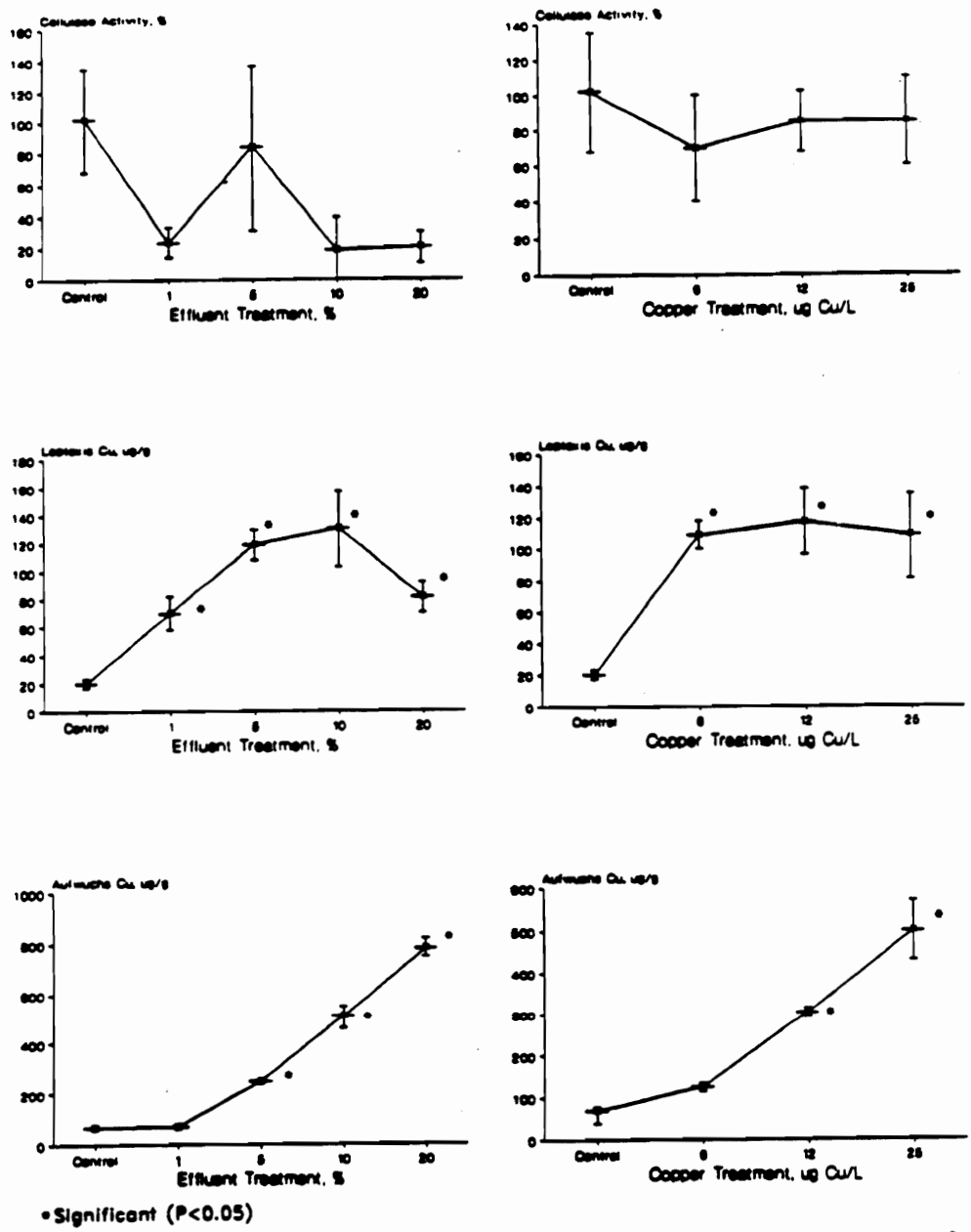


Figure 11. Periphyton bioconcentration, *Leptoxis* bioconcentration, and cellulolytic activity (SE) from 14-day study, using artificial streams, 1988 (*Significance was tested at $p<0.05$ and experimental values were compared with their respective controls).

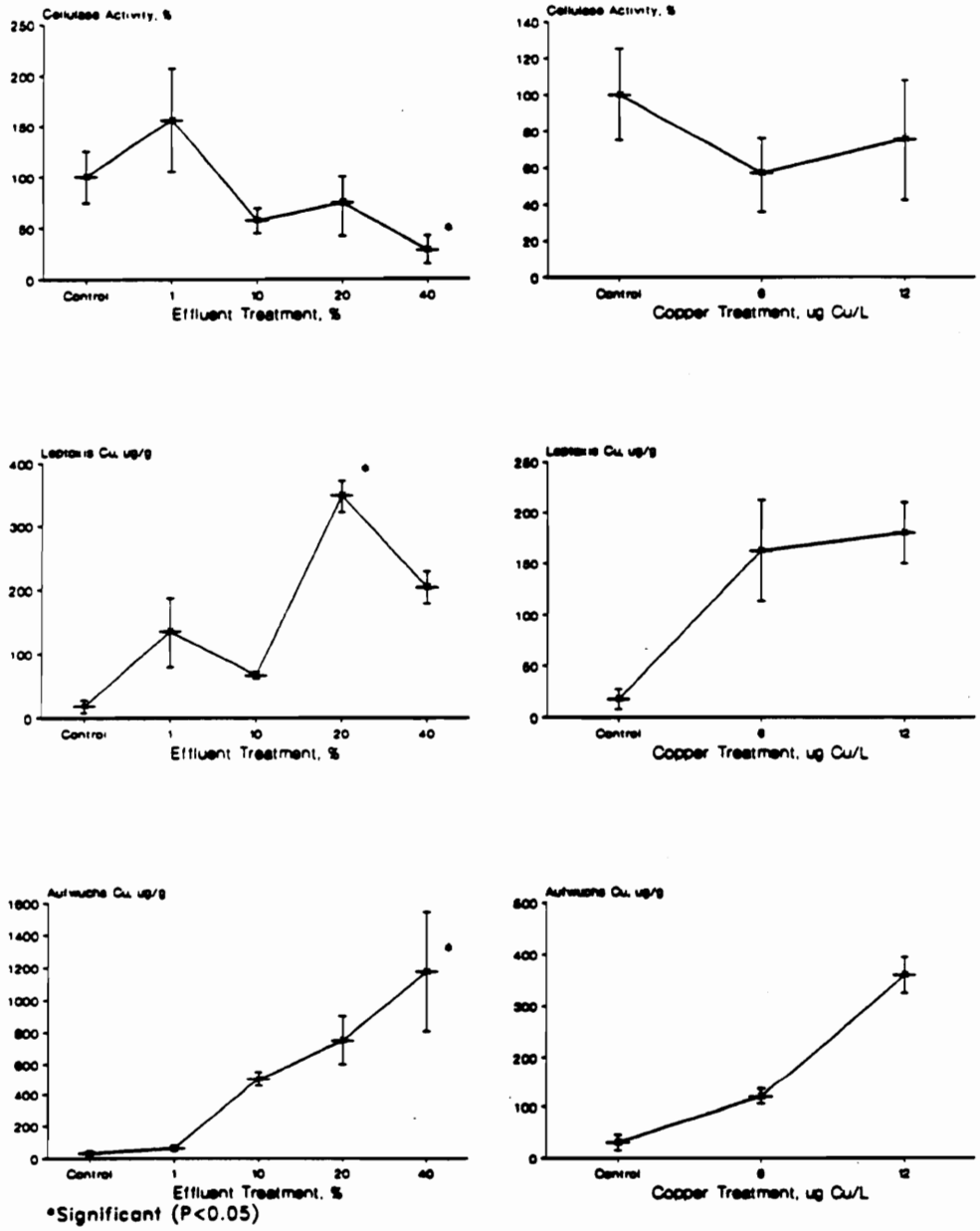


Figure 12. Periphyton bioconcentration, *Leptoxis* bioconcentration, and cellulolytic activity (SE) from 20-day study using artificial streams, 1988, (*Significance was tested at $P<0.05$ and experimental values were compared with their respective controls).

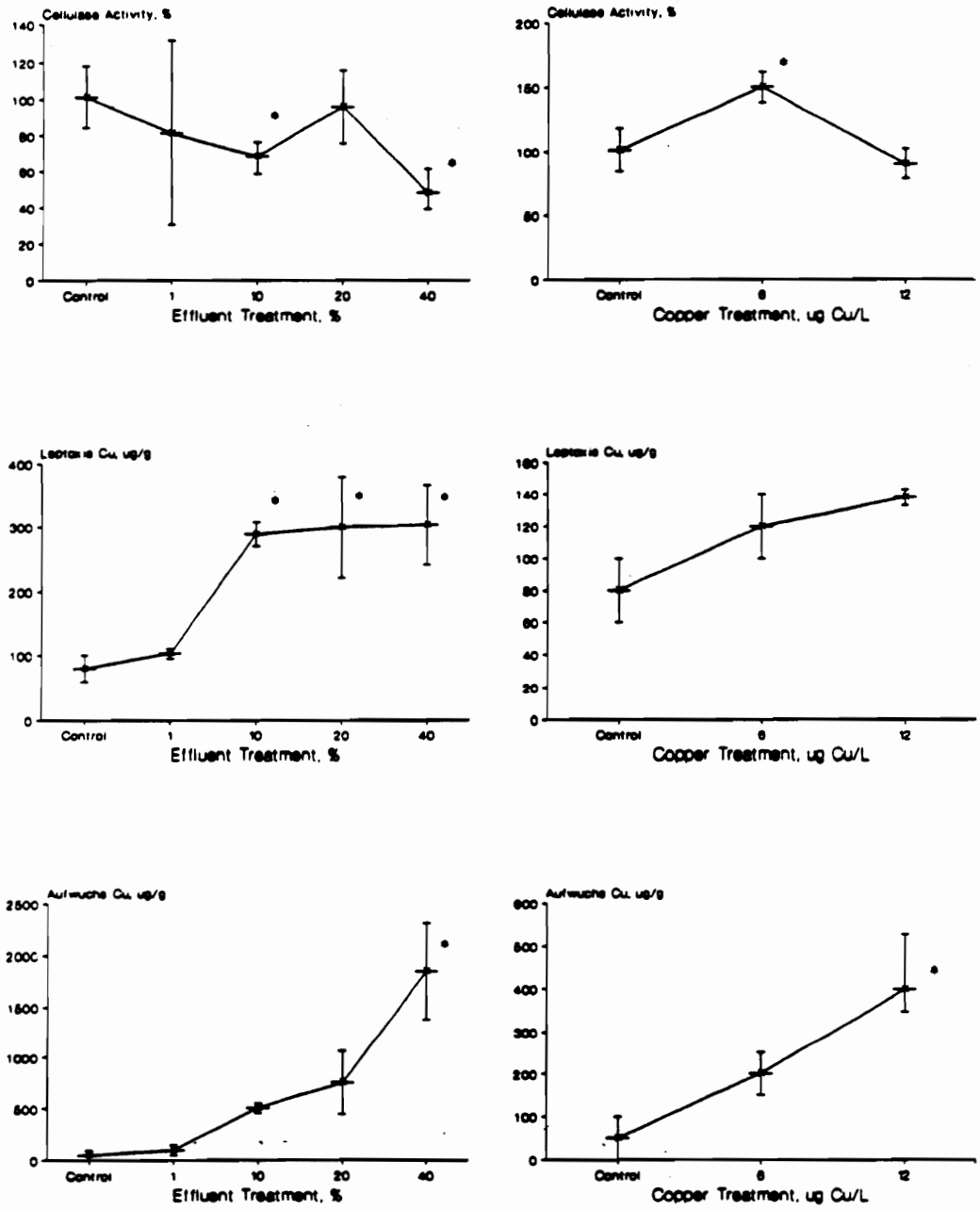


Figure 13. Periphyton bioconcentration, *Leptoxis* bioconcentration, and cellulolytic activity (SE) from 30-day study using artificial streams, 1988, (*Significance was tested at $P<0.05$ and experimental values were compared with their respective controls).

Table 12. Duncan's Multiple Range Test (SAS, 1985) of aperture width gain and total length gain of *Leptoxis* and *Pleurocera* after 14-d exposures to CRP effluent and copper treatments. Treatments connected by the same line are not significantly different.

Variable	Treatments					Statistics	
	Highest value				Lowest value	AVOVA F value	P value
Leptoxis							
Effluent, %							
Aperture width	5	1	20	0	10	6.24	0.0002
Total length		0		20		11.73	0.0050
Cu, ug/L							
Aperture width	25	6		0	12	6.01	0.001
Shell length		0		25		3.60	0.0787
Pleurocera							
Effluent, %							
Shell length	1	0	5	10	20	7.08	0.0001
Aperture width		0		20*		5.66	0.0286
Cu, ug/L							
Shell length	0	6	12		25	9.45	0.0001
Aperture width		0		25		0.93	0.3466

treatment and 25 ug Cu/L $CuSO_4$ treatment (15±2 ug Cu/L, measured concentration). The LOECfor copper was not clearly defined in these studies. No significant impairment was found in the 1988 $CuSO_4$ dosings. In the 1990 study, two 17 ug Cu/Lreplicates produced very different results (Tables 13 and 14); one (with measured concentration of 18.5±1.9 ug Cu/L) produced significant impairment (43% of control enzyme activity) and the other (with measured concentration of 20.0±3.9 ug Cu/L) did not (84% activity). Bioconcentration in *Leptoxis* was higher in the 17 ug/Ltreatments (209-307 ug Cu/g) than the 35 ug treatments (189-209 ug Cu/g; Appendix B, Table B6). The LOECwas nebulous and designated as 17-35 ug/L. The NOECwas 12 ug Cu/L,since neither replicate was significantly impaired. In long-term exposures, enzyme activity was significantly impaired within 114 days in streams treated with 17 ug Cu/L, 47 ug Zn/Land a combination of the two (on day 54, all treatments showed significant impairment) (Table 15). In all treatments except 8 ug/L, enzyme activity decreased as survival decreased (Fig. 14-19).

Bioconcentration

Bioconcentration of copper by *Leptoxis* was significant within 14 days in exposures as low as 5% effluent (12±1 ug Cu/L)and 7±1 ug Cu/L$CuSO_4$ (Fig. 11). In both 20- and 30-day studies, bioconcentration was significant in concentrations as low as 20% effluent (52±10 and 39±7 ug Cu/L, respectively) (Figs. 12 and 13). Periphyton copper bioconcentration was

Table 13. Duncan's Multiple Range Test (SAS, 1983) of cellulolytic enzyme activity and copper bioconcentration in *Leptoxis* from 30-day artificial stream exposure to copper sulfate at the CRP (six snails per treatment). Treatments connected by the same line are not significantly different ($P<0.05$; $n=6$; August 31-September 30, 1990).

Variable	Treatments		Statistics	
	Highest value	Lowest value	AVOVA F value	P value
Cellulase Activity	Cont. 17(A) 12(B) 12(A) 17(B) 35(A) 75(A) [a]35(B)		2.79	0.0159
Cu bioconcentration	17(B) 17(A) 35(A) 35(B) 12(B) 12(A) Cont.		2.01	0.1434

[a]n = 2
NOEC = 12
LOEC = 17-35

Table 14. Means (SD) of cellulolytic enzyme activity and copper bioconcentration in *Leptoxis* from 30-day artificial stream exposures to copper sulfate at the Clinch River Plant August 31-September 30, 1990).

Treatment/Rep	Cellulase Control Product Index, % (n=6)	Cu Bioconcentration, ug/g (n=3)
Control	100 (59)	88 (16)
12/A	48 (39)	133 (91)
12/B	52 (20)	188 (78)
17/A	84 (54)	271 (62)
17/B	43 (22)	307 (179)
35/A	42[a](10)	209 (23)
35/B	20[a](7)	189 (39)
75/A	-[b]	-[b]
75/B	-[b]	-[b]

[a] n=3
[b] 100% mortality

Table 15. Cellulolytic enzyme activity, copper and zinc bioconcentration in *Leptoxis* from 114-day exposures to copper and zinc in artificial streams at the CRP (SD) (n=6; June 13-October 15, 1989).

Stream		Sampling Day								Metals,ug/L	
No.	Treatment	0	40	54	66	78	90	102	114	Cu	Zn
A. Cellulolytic enzyme activity, % control product index											
22	Control	100 (35)	100 (88)	100 (31)	100 (68)	100 (49)	100 (114)	100 (88)	100 (78)	3 (2)	27 (15)
19	8 ug Cu/L	-	263 (332)	45* (29)	51 (56)	206 (143)	111 (86)	337* (201)	163 (111)	6 (3)	-
15	17 ug Cu/L	-	159 (62)	30* (13)	42 (25)	24* (12)	99 (60)	16 (12)	19* (15)	9 (4)	-
20	24 ug Zn/L	-	118 (108)	39* (13)	64 (49)	51 (40)	157 (83)	-	72 (40)	-	33 (18)
16	47 ug Zn/L	-	231 (135)	39* (25)	25* (10)	76 (42)	70 (78)	130 (136)	32* (24)	-	29 (21)
21	17 ug Cu + 47 ug Zn/L	-	93 (92)	5* (3)	57 (27)	46* (31)	40 (6)	-	-	9 (3)	24 (15)
B. Cu bioconcentration, ug/g											
22	Control	15 (5)	50 (10)	44 (20)	78 (17)	44 (12)	133 (109)	60 (20)	54 (10)		
19	8 ug Cu/L	-	93 (18)	122* (17)	97 (9)	28 (25)	62 (15)	33 (20)	64 (9)		
15	17 ug Cu/L	-	150* (82)	146* (14)	206* (48)	62 (16)	81 (26)	21 (23)	90* (18)		
20	24 ug Zn/L	-	55 (15)	71 (14)	155 (186)	16 (11)	56 (13)	-	96 (32)		
16	47 ug Zn/L	-	56 (9)	66 (19)	83 (24)	34 (14)	491 (1160)	90 (48)	61 (34)		
21	17 ug Cu + 47 ug Zn/L	-	91 (18)	168* (58)	207* (78)	71 (71)	118 16)	-	-		
C. Zn bioconcentration, ug/L											
22	Control	30 (12)	25 (26)	59 (26)	89 (38)	89 (17)	139 (111)	84 (35)	79 (15)		
19	8 ug Cu/L	-	95 (110)	45 (23)	220 (76)	91 (65)	116 (54)	82 (63)	113 (33)		
15	17 ug Cu/L	-	78 (143)	75 (61)	361 (141)	1430 (2620)	171 (132)	138 (32)	436 (680)		
20	24 ug Zn/L	-	26 (21)	86 (34)	417 (645)	77 (27)	120 (37)	-	132* (46)		
16	47 ug Zn/L	-	58* (34)	81 (29)	420 (188)	317 (276)	99 (31)	68 (26)	146* (41)		
21	17 ug Cu + 47 ug Zn/L	-	23 (6)	96 (41)	93 (25)	349 (486)	164 (73)	-	-		

*Sign. ($P<0.05$) different from cont. (Duncan's Mult. Range Test, SAS, 1985).

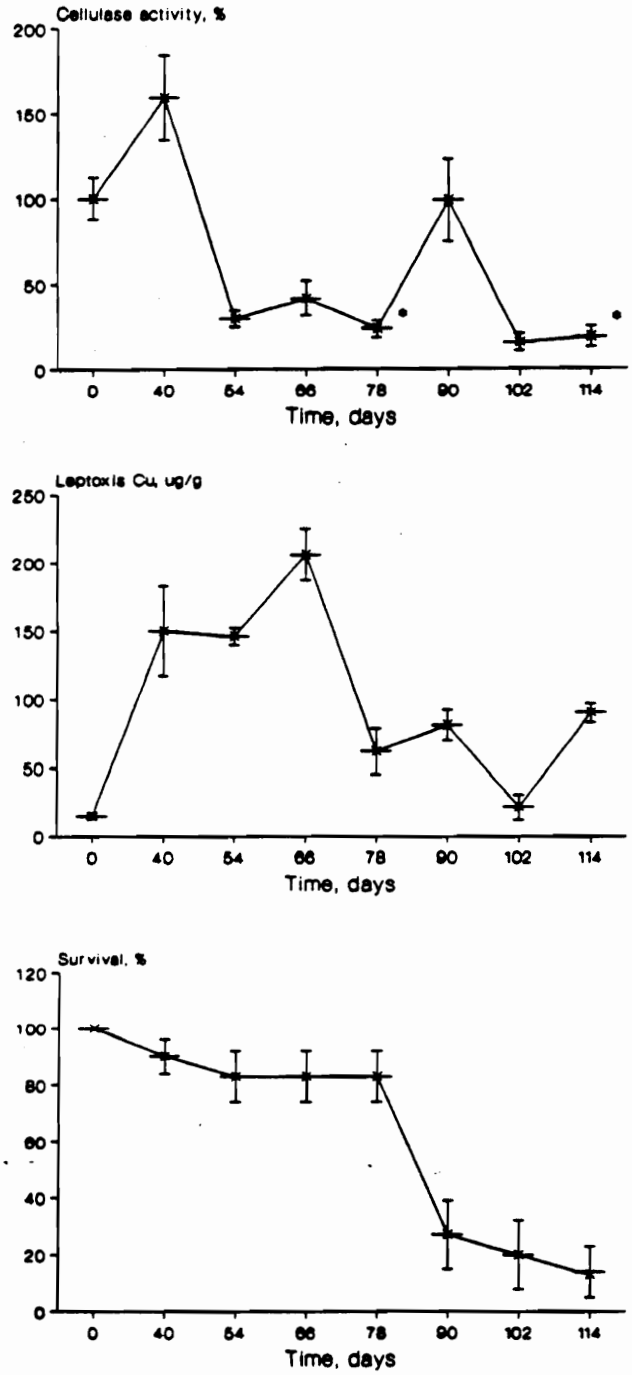


Figure 14. Cellulolytic enzyme activity and survival in *Leptoxis* exposed for 114 days to 17 ug Cu/Ltreatments in artificial stream tests at the CRP, 1989.

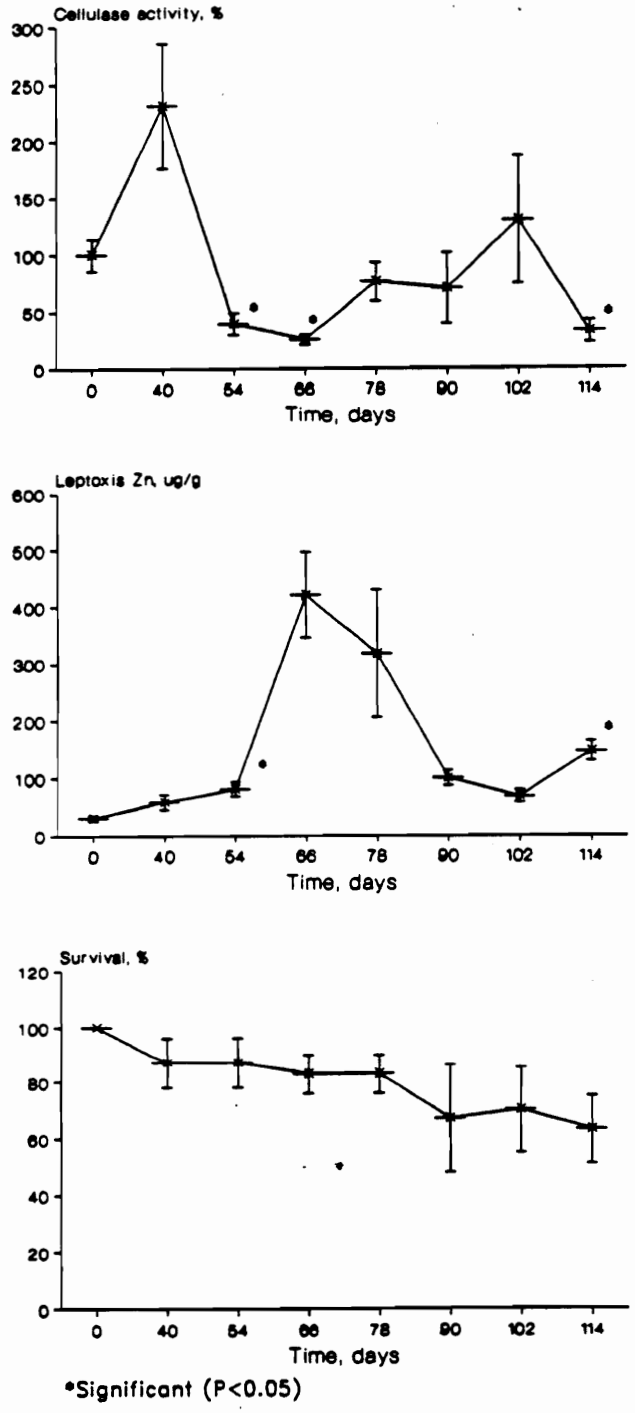


Figure 15. Cellulolytic enzyme activity and survival in *Leptoxis* exposed for 114 days to 24 ug Zn/Ltreatments in artificial stream tests at the CRP, 1989.

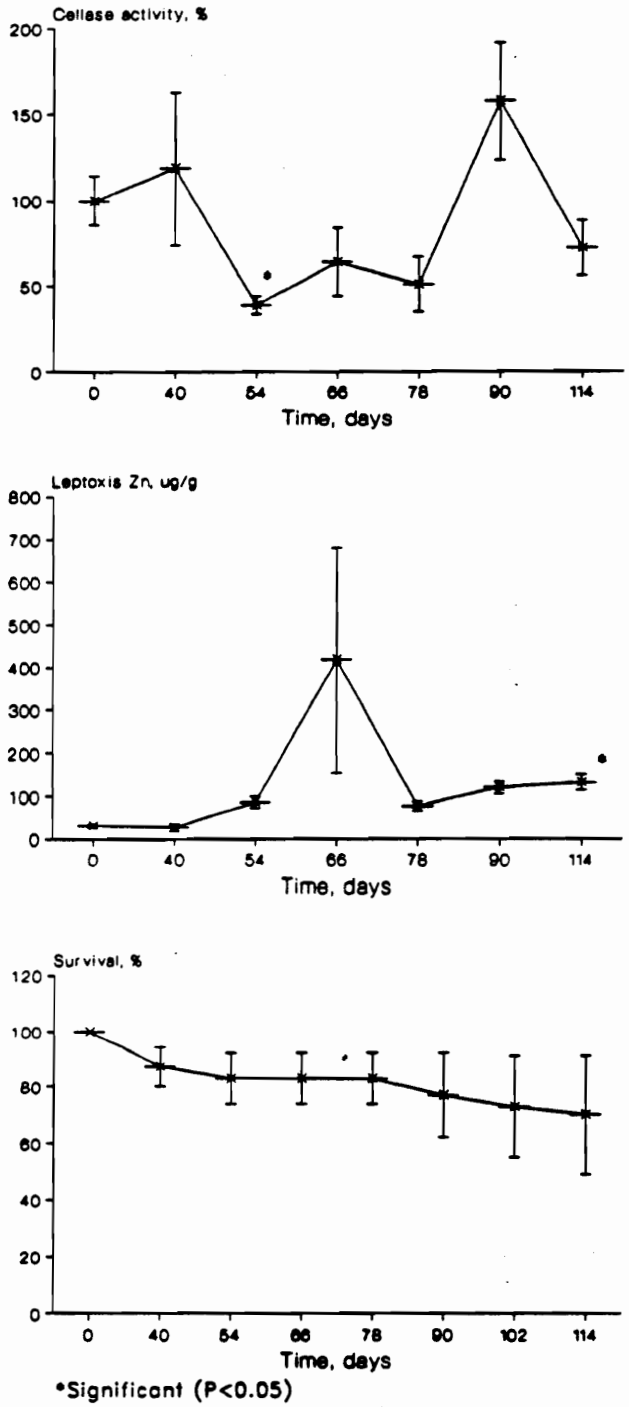


Figure 16. Cellulolytic enzyme activity and survival in *Leptoxis* exposed for 114 days to 47 ug Zn/Ltreatments in artificial stream tests at the CRP, 1989.

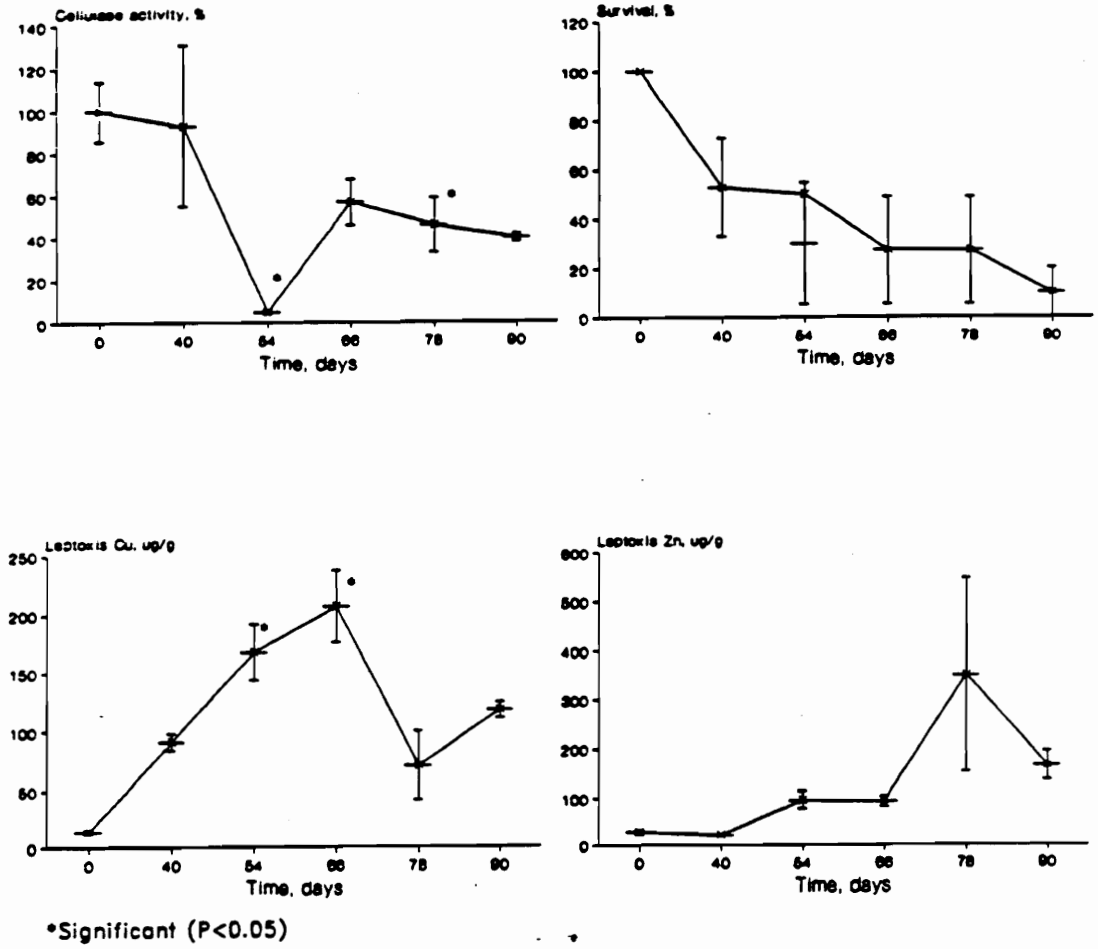


Figure 17. Cellulolytic enzyme activity and survival in *Leptoxis* exposed for 114 days to 17 ug Cu + 47 ug Zn/Ltreatments in artificial stream tests at the CRP, 1989.

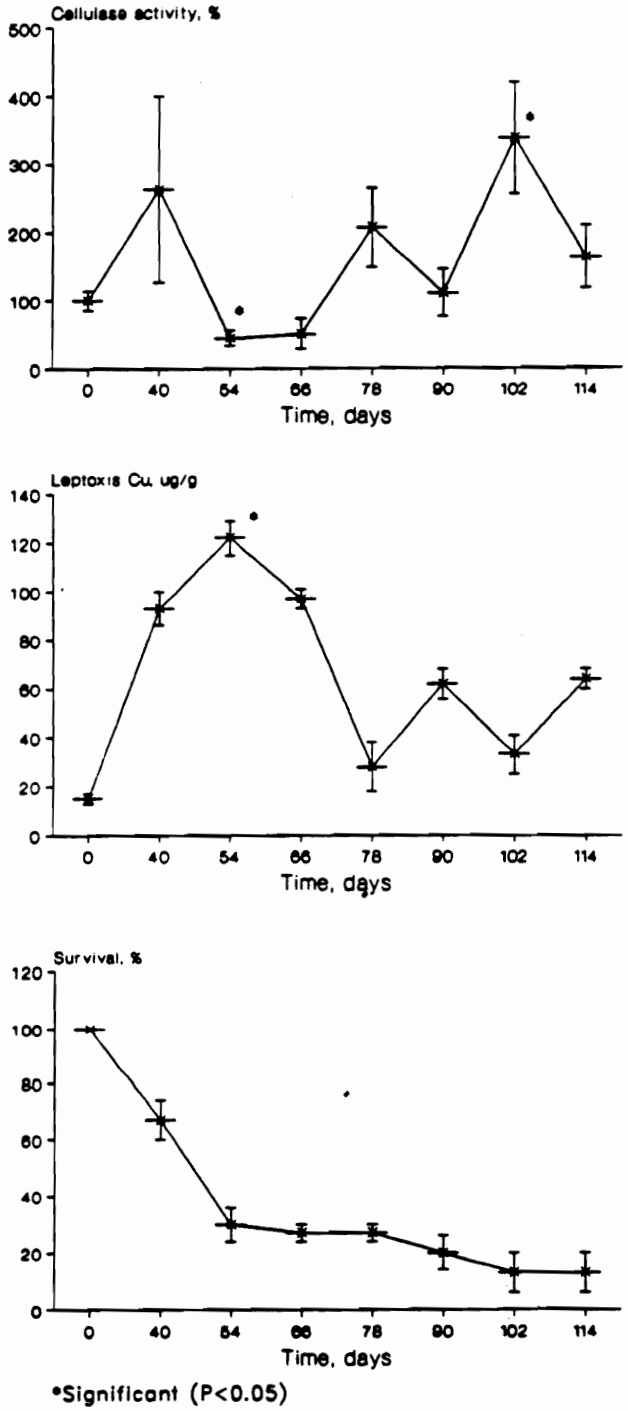


Figure 18. Cellulolytic enzyme activity and survival in *Leptoxis* exposed for 114 days to 8 ug Cu/Ltreatments in artificial stream tests at the CRP, 1989.

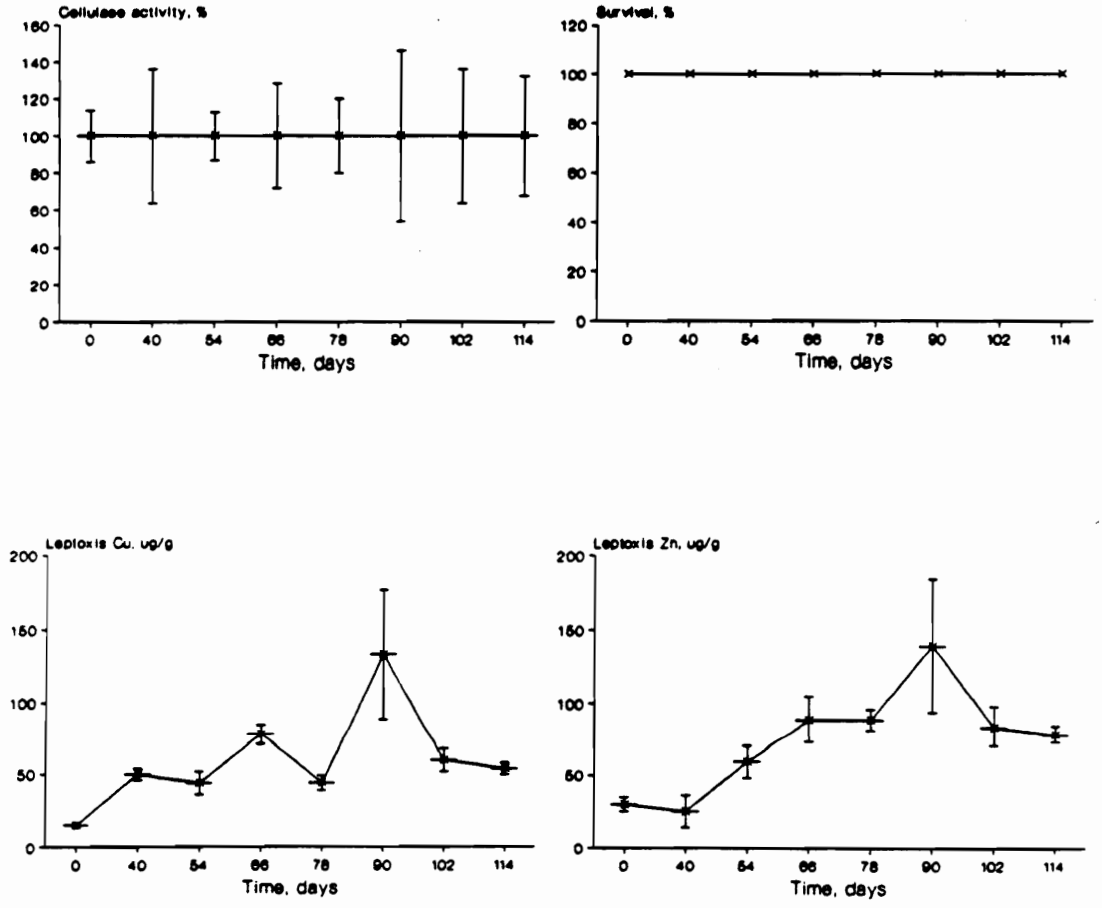


Figure 19. Cellulolytic enzyme activity and survival in *Leptoxis* exposed for 114 days to control treatments in artificial stream tests at the CRP, 1989.

significant during 14-day exposures to 5% effluent (12±1 ug Cu/L)and $CuSO_4$ dosings, containing 13±2 ug Cu/L;20-day exposures to 40% effluent (52±2 ug Cu/L)and $CuSO_4$ dosings containing 10±2 ug Cu/L;and 30-day exposures to 40% effluent (58±8 ug Cu/L)and $CuSO_4$ dosings containing 26±6 ug Cu/L.

Dose-dependent patterns were seen from effluent exposures in all three tests. As effluent exposure was increased, water column copper concentrations increased, and periphyton copper bioconcentration increased. Periphyton had significant ($P<0.05$) copper bioconcentration (compared with controls, Tukey's t-test, SAS, 1985) from 40% effluent in 14- and 20-day exposures and from all treatments >5% effluent in 30-day exposures. Dose-dependent patterns of periphyton copper bioconcentration were also seen in $CuSO_4$ exposures, and were similar to effluent exposures with similar copper concentrations.

Leptoxis copper bioconcentration appeared to be dose-dependent in effluent exposures containing up to approximately 20 ug Cu/L, in 14-, 20- and 30-day studies (Fig. 11-13). In effluent exposures containing higher copper concentrations, however, *Leptoxis* apparently became saturated or began to depurate the metal, as seen by a leveling of the bioconcentration curve in 30-day exposures and actual decrease in 14- and 20-day exposures. In 30-day exposures a pattern seemed to emerge (not as clear in the 14- and 20-day exposures) associated with the leveling of *Leptoxis* copper bioconcentration. As copper bioconcentration reached a peak, cellulolytic enzyme activity was

significantly decreased. At this point, a further increase in dosed copper concentration caused some change in snails, suggesting a decrease in metabolic rate, and possible depuration. The pattern was not as clear in the 14-, 20- and 30-day $CuSO_4$ exposures, possible due to insufficient time for patterns to be seen at these concentrations.

The pattern described above (dose- and time-dependent bioconcentration, followed by levelling or plunging) became apparent when copper bioconcentration and cellulolytic enzyme activity were measured at 12-day intervals in long-term (114-day) $CuSO_4$ exposures. However, at 17 ug Cu/L (8.8±3.7 ug Cu/L measured concentration), exposures, *Leptoxis* copper bioconcentration increased until around day-60, then decreased until approximately day-100, when it began to increase again (Fig. 14). Simultaneously, cellulolytic enzyme activity was initially stimulated, and then decreased, until around day-50, indicating a possible reduction in metabolism. At this time, depuration began, and cellulolytic enzyme activity was temporarily increased before crashing again around day-100, as bioconcentration increased. An accompanying reduction in survival began at day-80 and by day-114, only 100% survival was found. Similar patterns were seen in 114-day zinc exposures of 24 ug Zn/L(33±18 ug Zn/L, measured concentration; Fig. 15) and 47 ug Zn/L(29±21 ug Zn/L, measured concentration; Fig. 16), although survival was not reduced as much as in the 17 ug Cu/L exposures. Snails exposed to a combination of 17 ug Cu and 47 ug Zn/L(9.4±2.7 ug Cu +

24±15 ug Zn/L; Fig. 17) showed similar patterns and survival was reduced more than in separate treatments (only three snails remained on day-90 and were used for enzyme and metal analyses). The lowest $CuSO_4$ treatment tested (8 ug Cu/L, 6.3±3.8 ug Cu/L, measured; Fig. 18) had similar patterns but enzyme changes were not as distinct and overall cellulolytic enzyme activity was not reduced. Control snails bioconcentrated both metals during day-60 through day-100, probably due to increased metals in the Clinch River water apparently washed in by flooding (Fig. 19). This was during the time when depuration occurred in treated streams; increased metals concentrations may have caused the zinc spike in *Leptoxis* bioconcentration on day-80 in the combination treatment. Bioconcentration in snail tissue from long-term exposures was measured in the pellet of homogenized tissue (see Methods). A comparison of the amounts of copper and zinc present in the pellet with the total amounts present in both pellet and supernatant that 33-76% of the two metals were measured using only the digested pellet (Table 16).

Correlations between water column copper concentrations and periphyton bioconcentration were significant (r=0.9593-0.9973; Appendix D, Table D1) for effluent exposures and for 14-day $CuSO_4$ exposures. Periphyton copper also correlated with cellulase activity after 30-day effluent exposures (r=0.8890). Correlation coefficients for cellulase activity and *Leptoxis* copper bioconcentration, and cellulase activity and water column copper concentration in the 30-day effluent-dosing study were -0.8149 and -0.8262, respectively.

Table 16. Copper and zinc concentrations in pellet and supernatant of *Leptoxis* tissue from 40- and 114-day exposures to copper sulfate and zinc sulfate in artificial streams at the CRP (SD) (n=6; June 13-October 15, 1989).

Treatment	Day 40 Pellet	Day 40 Super-natant	Day 40 % in Pellet	Day 114 Pellet	Day 114 Super-natant	Day 114 % in Pellet
Cu measurements, ug/g						
Control	154(10)	57(9)	49	50(10)	31(24)	62
8 ug Cu/L	64(9)	61(16)	51	93(18)	30(35)	76
17 ug Cu/L	90(18)	83(22)	52	150(82)	77(24)	66
24 ug Zn/L	96(32)	68(24)	59	55(15)	25(18)	69
47 ug Zn/L	61(34)	67(40)	48	56(9)	35(9)	62
17 ug Cu + 47 ug Zn/L	--	--	--	91(18)	29(23)	76
Zn measurements, ug/g						
Control	79(15)	76(20)	51	25(26)	42(23)	37
8 ug Cu/L	113(33)	104(26)	52	95(110)	44(47)	68
17 ug Cu/L	436(680)	182(122)	71	78(143)	139(34)	34
24 ug Zn/L	132(46)	167(89)	44	26(21)	56(26)	46
47 ug Zn/L	146(41)	218(92)	40	58(31)	86(14)	40
17 ug Cu + 47 ug Zn/L	--	--	--	23(6)	46(42)	33

Coefficients for cellulase activity and *Leptoxis* copper for 14- and 20-day Cu-dosing studies were -0.7822 and -0.7880, respectively. Periphyton copper and *Leptoxis* copper correlated well (r=0.9318) in 30-day $CuSO_4$ exposures.

In-river validation

Snails caged at the first three left-bank stations downstream of CRP discharges (Stations 8, 11, 12; Table 17) were dead when retrieved on day-40. At the next two downstream stations, enzyme activity was reduced (not significantly) at Stations 12B and 13 (copper concentrations were 20±3 and 10±4 ug Cu/L, respectively). Caged snails at Station 12B had significant copper bioconcentration (243±9 ug/L). Snails from Station 13 had somewhat higher copper bioconcentration than those caged at upstream stations but the difference was not significant ($P<0.05$, Duncan's Multiple Range Test, SAS, 1985). Stations further downstream (Stations 14A, 15A, and 16) did not have significant cellulolytic enzyme activity impairment or bioconcentration (compared to upstream stations).

DISCUSSION

Toxicity

Pulmonates tested were more sensitive to copper, zinc and whole effluent than prosobranchs tested. One exception was a 27 ug Cu/L96-hour

Table 17. Cellulolytic enzyme activity and copper bioconcentration in *Leptoxis* from in-river validation study at selected sites (SD) (n=; Dates, 1989).

Station	Cellulase (% control product index)	*Leptoxis* bioconcentration (ug Cu/g)	Water column Cu (ug /L)
1A	66(10) [a]	--	1(0)
1B	100(19)	60(17)	1(0)
4A	71(12)	95(4)	3(2)
6	135(25)	69(24)	1(0)
8[b]	--	--	89(16)
10	109(20)	53(17)	2(0)
11[b]	--	--	53(12)
12[b]	--	--	42(4)
12B	57(8)	243(19)*	20(4)
13	47(9)	110(11)	10(3)
14A	99(12)	96(20)	10(5)
15A	101(14)	158(10)	6(1)
16	106(18)	111(14)	--

[a]Standard error in parenthesis
[b]100% mortality occurred at Station 8, 11, and 12
*Significantly ($P \leq 0.05$) different from Station 1B (control).

LC_{50} value found for *Leptoxis*, under flow-through test conditions. This value is thought to be aberrant, since 96-hour LC_{50} values found were generally >100 ug Cu/L for *Leptoxis*. Since that test received a constant flow of untreated Clinch River water, some change in the river water, such as upstream runoff, may have caused the mortality.

Similarly, according to the EPA Copper Criteria Document (USEPA, 1984), pulmonates are more sensitive to copper in acute exposures (LC_{50} ranging from 39 to 108 ug Cu/L) than prosobranchs (LC_{50} ranged from 210 to 1700 ug Cu/L), but the two have comparable sensitivities in chronic exposures. Though similar in magnitude, the copper LC_{50} values found in this research for both subclasses were lower than those reported by the EPA, especially among prosobranchs. This could be due to differences among species within each subclass, since no species tested were also used in the EPA document. There was an even greater difference between zinc LC_{50} values found in this study, for both pulmonates and prosobranchs, and those reviewed in the EPA Zinc Criteria Document (USEPA, 1980). Again, pulmonates were more sensitive by one to two orders of magnitude in this research, as well as those studies in the EPA document. The values found in this research were much lower for both subclasses (ranging from 59-114 ug Zn/L for pulmonates; and 850-14,700 ug Zn/L for prosobranchs) than those reviewed in the EPA document (ranging from 303-7100 ug/L for pulmonates and 14,000 to 20,200 ug/L for a prosobranch, the only one reported). This again, may be due to differences among species

tested.

Paulson *et al.* (1983) found acute LC_{50} concentrations of 390 and 590 ug Cu/Lin *Goniobasis*, a genus closely related to *Leptoxis*, at a water hardness of 154 mg as $CaCO_3$/L. Studies conducted with pulmonates (Wurtz & Bridges, 1961), at a water hardness of 100 mg as $CaCO_3$/L, produced LC_{50}s closer to those found in this study, 108 ug Cu/Lfor *Gyraulus* and 69 ug Cu/Lfor *Physa*.

Differences in sensitivity may be due to a number of factors. First, prosobranchs may close their operculum during acute exposures, avoiding much of the toxicity in the water column (Arthur and Leonard, 1970). Second, physiological differences among snail species may affect toxicity (Wurtz, 1962). Third, since pulmonates do not have gills, they do not experience gill damage and uptake trough gill epithelium. Hobson *et al.* (1979) reported that copper uptake is facilitated by factors increasing gill exchange. The first factor, the operculum, decreases short-term sensitivity in prosobranchs. The second factor, differences in physiology, is very complex and may alter sensitivities among species, not limited to major categories (e.g., orders). The third factor, presence of gills, increases sensitivity in prosobranchs. Although pulmonates may be more sensitive to acute exposures, in chronic exposures, prosobranchs may be more sensitive due to the presence of gills. Since physiological differences exist among species, these differences may be very significant, but complex, making it difficult to predict the influence of each. In this research, these predictions held true - pulmonates were more sensitive in acute

exposures. No chronic studies, however, were conducted using pulmonates, so no data is presented for comparison.

In toxicity studies, effluent strength varied from test to test. Results of acute tests showed that measured copper in effluent-dosed studies was a better predictor of toxicity than actual effluent concentration. Measured copper concentration appears to be a much better indicator of effluent toxicity than measured zinc, since copper was more toxic at lower concentrations and was present in effluent in much higher concentrations than zinc. Rogers *et al.* (1980) found that when copper and zinc were combined, copper exerted most of the toxicity to *Corbicula fluminea* (the Asiatic clam). However, this depends upon the concentrations tested. In this research, too much variability occurred to show whether effects of the two metals were additive or synergistic. At concentrations of greater than 1400 ug Zn/L, zinc contributed greatly to the toxicity, reducing the copper LC_{50} by approximately an order of magnitude for *Leptoxis* and *Pleurocera*. Since these zinc concentrations were on the same order as zinc LC_{50} for the snails (1210-10,600 ug zn/L for *Leptoxis* and 3590-14,700 ug Zn/L for *Pleurocera*), this is not surprising, the copper probably contributed very little to the toxicity. At zinc concentrations of less than 1000 ug Zn/L it was not clear if zinc had an effect on the toxicity, though at 730 ug Zn/L, it appeared to lower the copper LC_{50} concentration, especially for *Pleurocera*. In copper-dosed zinc LC_{50} studies, 12 ug Cu/L appeared to reduce the zinc LC_{50} for both snails, though results were not always clear in these

studies due to the concentration ranges. To obtain a better pattern of the contribution of each metal to the toxicity, more information is needed using a variety of ranges. It could not be concluded from these studies that effects of the two metals were anything other than additive.

The toxic zone (Fig. 20) was defined by long-term (40-day) rather than acute (96-hr) toxicity. The area of river occupying an acutely toxic (95% effluent from 96-hr LC_{50} determinations) zone is limited to the immediate mixing zone. Since effluent is released at the left bank only, the area where effluent is channeled by river flow before mixing with river water constitutes this zone. The area encompassed by this zone is dependent upon river flow and was shown to extend only a few meters, even under low-flow conditions. However, when *Leptoxis* was exposed to in-river conditions for 40 days, 100% mortality occurred as far downstream as Station 12 (0.2 km downstream from 004 discharge). Therefore, a toxic zone was delineated as the left side of the river from 004 discharge to Station 12 (Fig. 20).

Sublethal effects

Downstream, where effluent has been diluted by river water, snails may experience sublethal, but longer term, chronic, effects. Type and extent of impairments may depend upon the type of exposure to metals. In the upper reaches of this zone where metals are carried by the water and bioconcentrated in periphyton, gill tissue damage (reported in fish, Heath, 1987; Skidmore,

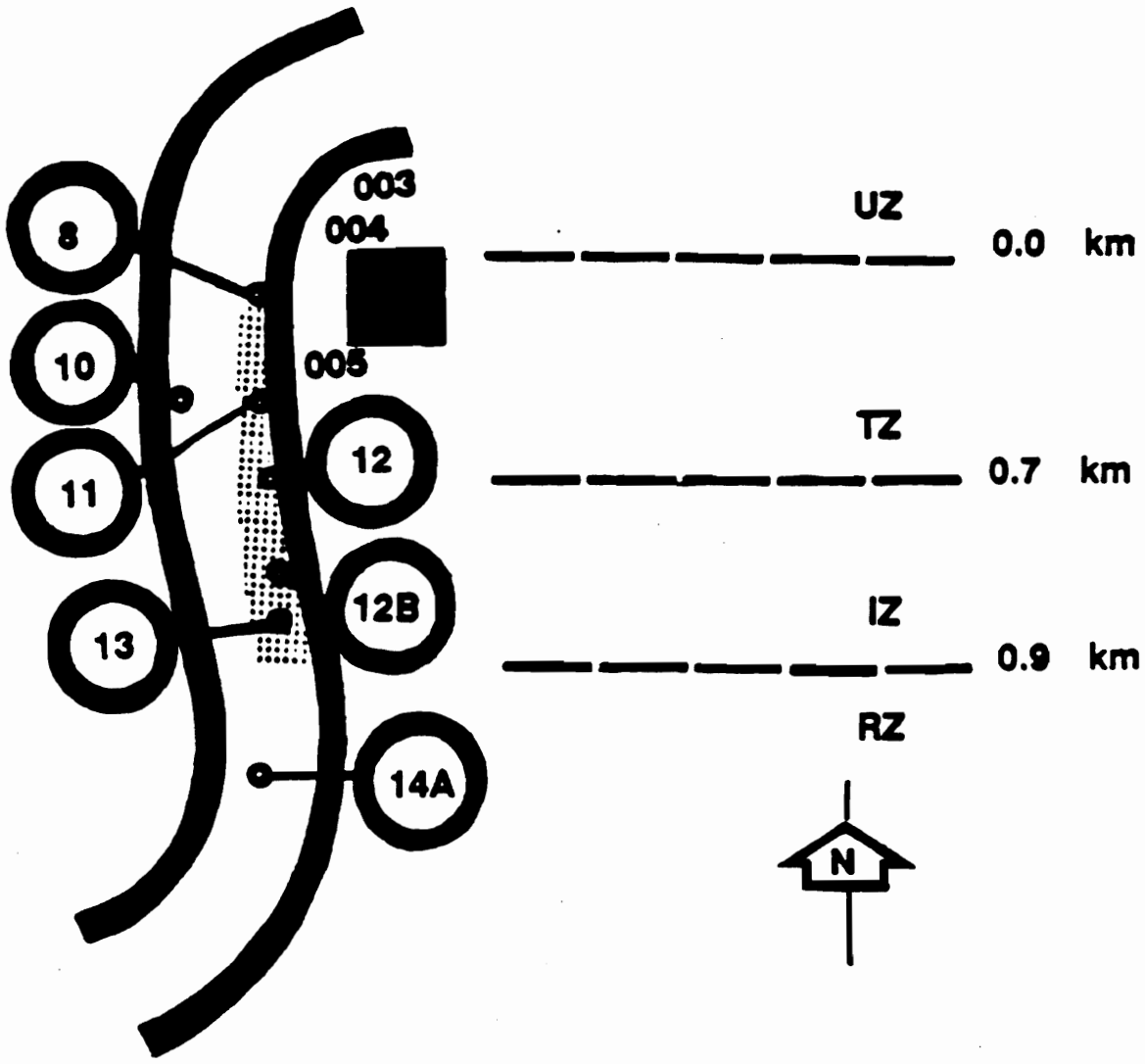


Figure 20. Schematic representation of the Clinch River study area with delineation of estimated zones of effect - upstream zone (UZ), toxic zone (TZ), impairment zone IZ, and recovery zone (RZ).

1970) may occur in addition to bioaccumulation of metals in snail body tissue from water- or food-borne uptake. In the lower reaches of this zone, metals may be accumulated in periphyton from long-term, low-level, and episodic exposures, though elevated concentrations of water-borne metals may not always be measured. In both situations, snails may be weakened by altered food quality. Therefore, the area may be avoided or, when present, snails may have sublethal effects from water-borne and food-borne metals exposures and food-quality degradation.

Cellulolytic enzyme activity impairment

Leptoxis cellulolytic enzyme activity was impaired from 20- and 30-day artificial stream exposures to whole effluent containing 52 and 20 ug Cu/L, respectively. In a 14-day study, no significant impairment was seen in effluent concentrations of up to 20% (the highest concentration tested). The measured copper concentration was 28 ug Cu/L in the 20% effluent treatment. The effluent used in the 20-day study was slightly more concentrated (229 ug Cu/L, versus 176-191 ug Cu/Lused in the 14- and 30-day studies). This variability in effluent copper concentration makes it difficult to determine effects concentrations. In these studies, the lowest-observed effects concentration (LOEC) was 10% effluent. The next lowest test concentration was 1% effluent, so this was the no-observed effect concentration (NOEC). Cellulolytic enzyme activity appeared to be a good indicator of generalized metal stress in

these effluent exposures.

Cellulolytic enzyme activity was also reduced (though not significantly) by 14- and 20-day $CuSO_4$ exposures. Since the highest measured copper concentrations (up to 15 and 10 ug Cu/L, respectively) were lower than the copper concentrations measured in effluent concentrations causing significant cellulolytic enzyme activity impairment, concentrations may have been too low to produce significant impairment. This also emphasizes the importance of copper in the toxicity of this effluent. The highest $CuSO_4$ concentration tested in the 30-day study (25 uproduced a significant increase in cellulolytic enzyme activity. A possible explanation for this increase is found later in this text (see Patterns of bioconcentration and impairment).

The $CuSO_4$ LOECwas not determined from these three studies, due to lack of significant impairment. However, in later studies the LOECfrom 30-day studies with *Leptoxis* was estimated. Since only one replicate (measured concentration, 19 ug Cu/L) in the 17 ug Cu/L (nominal treatment produced significant cellulolytic enzyme impairment and the other (measured concentration, 20 ug Cu/L)did not produce significant impairment, the NOEC may be defined as 17-35 ug Cu/L; 35 ug Cu/L was the next higher concentration tested and produced significant impairment. The LOEC for $CuSO_4$ was 12 ug Cu/L. In longer-term (114-day) studies, cellulolytic enzyme activity decreased as survival decreased in all $CuSO_4$ treatments greater than 8 ug Cu/L(nominal concentration). Although cellulolytic enzyme activity was

also impaired by $ZnSO_4$ treatments, survival was not affected as much. This indicates a possible weakness in assessing toxicity from single impairment criteria (or measurement).

Growth impairment was not used as extensively in this research as cellulolytic enzyme activity to measure impairment. In these studies, total shell length was a more sensitive measurement of growth impairment than aperture width. Impairment was seen in *Leptoxis* from 14-day exposures to 20% effluent (containing 20 ug Cu/L) and 25 ug Cu/L ($CuSO_4$ exposures; 15 ug Cu/L, measured concentration). Growth impairment (as measured by total shell length) was found to be more sensitive than cellulolytic enzyme activity in 14-day exposures (the only study in which the two measurements were compared). This could be due to the higher variability in cellulolytic enzyme activity measurements. The two measurements may have different applications, since cellulolytic enzyme activity impairment may be caused by or lead to decreased metabolism, and thus, decreased growth. When answers are needed in a shorter time, theoretically, measuring cellulolytic enzyme activity should give an indication of stress much sooner than growth impairment. Farris (1986) and Farris *et al.* (1989) linked cellulolytic enzyme activity impairment to growth impairment (or actual decrease in visceral mass) in *Corbicula* (Asiatic clams) exposed to zinc and Cu. These impairments were largely attributed to avoidance (via valve closure) of the toxicant, resulting in changes in metabolic budgeting that resulted in less energy for growth.

Patterns of bioconcentration and impairment

Aufwuchs significantly bioconcentrated copper in treatments as low as 5% effluent (12 ug Cu/L) in 14 days. *Leptoxis* copper bioconcentration appeared to be dose-dependent in effluent exposures containing up to approximately 20 ug Cu/L (in 14-, 20-, and 30-day studies). In effluent treatments containing copper concentrations greater than 20 ug Cu/La levelling or decrease in bioconcentration occurred. The 14-, 20-, and 30-day $CuSO_4$ studies did not show patterns as clearly as the effluent treatments. However, in long-term (114-day) $CuSO_4$ studies, patterns were more distinct. For example, in the 17 ug Cu/Ltreatment, bioconcentration of copper increased in *Leptoxis* over time until approximately day-60. At the same time, cellulolytic enzyme activity decreased.

Leptoxis copper bioconcentration appeared to be dose-dependent in effluent exposures containing up to approximately 20 ug Cu/L, in 14-, 20- and 30-day studies (Fig. 19-11). In effluent exposures containing higher copper concentrations, however, *Leptoxis* apparently became saturated or began to depurate the metal, as seen by a leveling of the bioconcentration curve in 30-day exposures and actual decrease in 14- and 20-day exposures. In 30-day exposures a pattern seems to emerge (not as clear in the 14- and 20-day exposures) associated with the leveling of *Leptoxis* copper bioconcentration. As copper bioconcentration reaches its peak, cellulolytic enzyme activity is significantly decreased. At this point, a further increase in dosed copper

concentration causes some change in snails, suggesting a decrease in metabolic rate, and possible depuration. The pattern was not as clear in the 14-, 20- and 30-day $CuSO_4$ exposures, possible due to insufficient time for patterns to be seen at these concentrations.

This pattern became apparent when copper bioconcentration and cellulolytic enzyme activity were measured at 12-day intervals in long-term (114-day) $CuSO_4$ exposures. However, at 17 ug Cu/L (8.8±3.7 ug Cu/L measured concentration), exposures, *Leptoxis* copper bioconcentration increased until around day-60, then decreased until approximately day-100, when it began to increase again (Fig. 14). Simultaneously, cellulolytic enzyme activity was initially stimulated, and then decreased, until around day-50, indicating a possible reduction in metabolism. At this time, depuration began, and cellulolytic enzyme activity was temporarily increased before crashing again around day-100, as bioconcentration increased. An accompanying reduction in survival began at day-80 and by day-114, only 100% survival was found. Similar patterns were seen in 114-day zinc exposures of 24 ug Zn/L(33±18 ug Zn/L, measured concentration; Fig. 15) and 47 ug Zn/L(29±21 ug Zn/L, measured concentration; Fig. 16), although survival was not reduced as much as in the 17 ug Cu/Lexposures. Snails exposed to a combination of 17 ug Cu and 47 ug Zn/L(9.4±2.7 ug Cu + 24±15 ug Zn/L;Fig. 17) showed similar patterns and survival was reduced more than in separate treatments (only three snails remained on day-90 and were used for enzyme and metal analyses). The

lowest $CuSO_4$ treatment tested (8 ug Cu/L, 6.3±3.8 ug Cu/L, measured; Fig. 20) had similar patterns but enzyme changes were not as distinct and overall cellulolytic enzyme activity was not reduced. Control snails bioconcentrated both metals during day-60 through day-100, probably due to increased metals in the Clinch River water from flooding (Fig. 19). This was during the time when depuration occurred in treated streams and may have caused the zinc spike in *Leptoxis* bioconcentration on day-80 in the combination treatment.

Impairment zone

The impairment zone (Fig. 20) was defined as the area within which functional impairments to the population occurred. It included habitat that would have been suitable without effluent impact and where structural and/or functional impairments were measured. The riffle/shoal area beginning at Station 12 and extending to Station 13 was included in this zone. Though suitable habitat existed with respect to influential variables, substrate, flow rate and algal biomass the left bank was not inhabited.

In-river validation

Concentrations of Cu in the study area caused effects similar to those from artificial stream studies, although impairments were not significant in the river exposures due to high variability. Snails (*Leptoxis*) caged at stations with measured water column copper concentration of 42-89 ug Cu/L had 100%

mortality within 40 days. These concentrations are below the LC_{50} values determined from acute studies, (generally between 100 and 150 ug Cu/L). This may be due to the longer exposure time, or possible fluctuations in copper concentration. According to Haber's Rule, exposures to larger amounts of toxicant over a shorter period should have a similar effect as exposures to smaller amounts of toxicant over a longer period (Haber, 1924). Snails caged at Station 12B had reduced cellulolytic enzyme activity and significant bioconcentration of Cu, while those at Station 13 had a similar reduction in enzyme activity but no significant increase in copper bioconcentration.

The occurrence of this type of avoidance might help to explain the lack of copper bioconcentration in *Leptoxis* at Station 13. If *Leptoxis* avoided conditions via closure of the operculum, bioconcentration would be reduced. After a 40-day exposure, this seems unlikely. It appears that entry of copper at Station 12B was through gill lamellae, since enzyme activity and assimilation of food components in snails were reduced at both Stations 12B and 13.

Recovery

Biological integrity includes both the structural and functional aspects of natural ecosystems (Cairns, 1977). Both downstream and temporal recovery of functional aspects of snail populations were discussed in the previous chapter. Functional, as well as structural, recovery occurred at Station 15A in 1988. Since deep pools and bends characterize the area between Station 13

and 15A, little suitable habitat was found and a recovery gradient (from structural to functional recovery) could not be evaluated. The first appreciable amount of suitable habitat showing downstream recovery of *Leptoxis* density and functional variables (cellulolytic enzyme activity and copper bioconcentration in *Leptoxis* and periphyton) was the riffle/shoal area at Station 15A (1.2 km below 004 discharge). If recovery had been based solely upon lack of significant impairment or bioconcentration, Station 13, where habitat did not support snail populations, would have been included in the recovery zone. Any evaluation of affected zones below effluent outfalls, therefore, must include an assessment of habitat degradation, which may also be sensitive to actual population function.

Site-specific application of results

The 1991 preliminary permit set copper limits at 0-3 ug/L. Landscape runoff can cause these concentrations to rise to 9 ug/L(unpublished data). The federally established copper limit for the Clinch River (17 ug/L) was not protective of *Leptoxis* in long-term studies, but 12 ug/L was shown to be. Since the limit of 3 ug Cu/L was unrealistically low, a limit of 12 ug/L was recommended to protect snail species. Most of the information presented in this text was included in a final report compiling several studies, including annual benthic macroinvertebrate surveys, 21-day *Daphnia magna* chronic toxicity tests, mussel population surveys and other measurements. The

information was presented to the Virginia State Water Control Board in 1992. As a result, a site specific standard of 12 ug Cu/L was developed into the water quality standards, for the 10 mile stretch of river between the CRP and St. Paul, Virginia.

LITERATURE CITED

Academy of Natural Sciences. 1960. The sensitivity of aquatic life to certain chemicals commonly found in industrial wastes. Philadelphia, PA.

Adams, C.C.. 1899. The variations and ecological distribution of the snails of the genus *Io*. National Academy of Sciences. 12:16-18.

Aldridge, D.W.. 1982. Reproductive Tactics in Relation to Life-cycle Bioenergetics in Three Natural Populations of the Freshwater Snail, *Leptoxis carinata*.Ecology. 63(1):196-208.

American Public Health Association (APHA), American Water Works Association, and Water Pollution Control Federation. 1986. Standard methods for the examination of water and wastewater - 16th edition. Washington, DC. pp. 76-136.

Anderson, N.H. and K.W. Cummins. 1979. Influences of diet on the life histories of aquatic insects. J. Fish. Res. Bd Can.. 36:335-42.

Angermeier, P.L. and I.J. Schlosser. 1987. Assessing Biotic Integrity of the Fish Community in a Small Illinois Stream. North American Journal of Fisheries Management. 7:331-338.

Anonymous. (Unpublished). Fish-kill on Clinch River Below Steam-electric Power Plant of Appalachian Power Company, Carbo, Virginia, June 10-14, 1967. Tennessee Valley Authority. 29 pp..

Appalachian Power Company, submitters. 1985. 28-Day Hester-Dendy Artificial

Substrate Survey of Benthic Macroinvertebrates in the Clinch River near Clinch River Plant,August 15 - September 12, 1985. In Partial Fulfillment of Special Condition 9.a. NPDES Permit No. VA0001015 Clinch River Plant. December 6, 1985.

Arthur, J.W. and E.N. Leonard. 1970. Effects of Copper of *Gammarus pseudolimnaeus*, *Physa integra* and *Campeloma decisum* in Soft Water.. J. Fish. Res. Bd. Canada. 27:1277-1283.

Barbour, M.T. and J.L. Plafkin. 1988. The search for the appropriate biomonitoring tool. Presented at The Role of Benthos in Impact Assessment. Technical Information Workshop, Tuscaloosa, Ala., North American Benthological Society.

Belanger, S.E., J.L. Farris and D.S. Cherry. 1989. Effects of diet, water hardness, and population source on acute and chronic copper toxicity to *Ceriodaphnia dubia*. Environ. Contam. Toxicol. 18:601-611.

Belanger, S.E., J.L. Farris, D.S. Cherry and J. Cairns, Jr. 1990. Validation of *Corbicula fluminea* growth reductions induced by copper in artificial streams and river systems. Can. J. Fish.. 47(5):904-914.

Betzer S.B. and P.P. Yevich. 1975. Copper toxicity in *Busycon canaliculatum* L.. Biol. Bull. 148:16-25.

Beveridge, T.J.. 1984. In: M.T. Klug and C.A. Reddy (eds.), Current Perspectives in Microbial Ecology, American Society for Microbiology, Washington D.C. pp. 601-607.

Bickel, D. 1968. *Goniobasis curreyana lyoni*, a pleurocerid snail of west-central Kentucky. Nautilus 82:13-18.

Bowen, H.J.M.. 1985. In: D. Hutzinger (ed.), The handbook of Environmental Chemistry, Vol. 1, Part D: The natural environment and the biogeochemical cycles, Springer-Verlag, New York. 26 pp..

Boycott A.E.. 1936. The habitats of freshwater mollusca in Britain. J. Anim. Ecol.. 5:116-186.

Branson, B. A. 1961. Recent Gastropoda of Oklahoma, part 2: distribution, ecology and taxonomy of freshwater species, with description of , *Helisoma travertina*, sp. nov. Olka. State Univ. Pub. 58:1-72.

Brooks, R.R. and M.G. Rumsby. 1965. The biogeochemistry of traceelement uptake by some New Zealand bivalves. Limnol. Oceanog.. 10:521-8.

Bryan G.W.. 1973. The occurrence and seasonal variation of tracemetals in the scallops *Pecten maximus* (L.) and *Chlamys opercularis* (L.). J. Mar.Biol. Assos. of the United Kingdom. 53:145-166.

Bryan G.W.. 1976. Some Aspects of Heavy Metal Tolerance in Aquatic Organisms. In: A.P.M Lockwood (ed.). Effects of Pollutants on Aquatic Organisms. Society for Experimental Biology- Seminar Series. Cambridge University Press. pp. 7-34.

Cairns, J., Jr. 1983. The case for simultaneous toxicity testing at different levels of biological organization. In: W.E.Bishop, R.D. Cardwell & B.B. Heidolph (eds.) Aquatic toxicology and hazard assessment. Philadelphia,

PA, USA. pp. 111-127.

Cairns, J., Jr. 1977. Quantification of biological integrity. In: R.K. Ballentine and L.J. Guarraia (eds.). The integrity of water.Environmental Protection Agency, Office of Water and Hazardous Materials, Stock Number 055-001-01068-1, Washington, D.C.

Cairns, J., Jr. and D. Orvos. 1989. Ecological consequence assessment: predicting effects of hazardous substances upon aquatic ecosystems using ecological engineering. In: W.J. Mitsch and S.E. Jorgensen (eds.). Ecological engineering: an introduction to ecotechnology. John Wiley and Sons, Inc.. pp. 409-442.

Cairns, J., Jr. and A. Scheier. 1968. A comparison of the toxicity of some common industrial waste components tested individually and combined. Prog. Fish-Cult. 30:3-8.

Cairns, J., Jr., A.L. Buikema, Jr., A.G. Heath and B.C. Parker. 1978. Effects of temperature on aquatic organism sensitivity to selected chemicals. Bulletin 106. Virginia Water Resources Research Center, Blacksburg, Virginia. 88 pp.

Cairns, J., Jr., J.S. Crossman, K.L. Dickson, and E.E. Herricks. 1971. The recovery of damaged streams. Am. Soc. Biol. Bull.. 18(3):79-106.

Cairns, J., Jr., D.I. Messenger, and W.F. Calhoun. 1976. Invertebrate response to thermal shock following exposure to acutely sublethal concentrations of chemicals. Arch. Hydrobiol.. 77:164-75.

Cairns, J., Jr. and B. R. Niederlender. 1989. Problems associated with selecting the most sensitive species for toxicity testing. Hydrobiologia. 153:87-94.

Cherry, D.S., R.K. Guthrie, E.M. Davis and R.J. Harvey. 1984. Coal Ash Basin Effects (Particulates, Metals, Acidic pH) upon Aquatic Biota: An Eight-Year Evaluation. Water Resources Bulletin. 20:535-544.

Cherry D.S., J.H. Rodgers, R.L. Graney and J. Cairns, Jr.. 1980. Dynamics and Control of the Asiatic Clam in the New River, Virginia. Virginia Water Resources Research Center.VPI-VWRRC-Bull 123. 72 pp..

Cherry, D.S., J.H. Van Hassel, P.H. Rebbe and J. Cairns, Jr.. 1987. Factors influencing acute toxicity of coal ash to rainbow trout (*Salmo gairdneri*) and bluegill sunfish(*Lepomis macrochirus*). Wat. Res. B.23(2):293-306.

Clark, A.H.. 1981. The Freshwater Molluscs of Canada. National Museums of Canada. Ottawa.

Clarke, A.H. and C.O. Berg. 1959. The freshwater mussels of Central New York. Cornell Univ. Agr. Exp. Sta. Mem..367:1-79.

Clements, W.H., D. S. Cherry and J. Cairns, Jr.. 1988. Impact of heavy metals on insect communities in streams: a comparison of observational and experimental results. Can J. Fish. Aquat. Sci. 45:2017-2025.

Couthtrey, P.J. and M.H. Martin. 1977. The uptake of lead, zinc, cadmium, and copper by the pulmonate mollusc, *Helix aspersa* Muller, and its relevance to the monitoring of heavy metal contamination of the environment. Oecologia. 27:65-74.

Cole, G.A.. 1983. Textbook of limnology - third edition. St. Louis, Miss., The C. V. Mosby Company. 426 pp..

Courtois, L.A. and R.D. Myerhoff. 1975. Effects of copper exposures on water balance. Bull. Environ. Contam. Toxicol. 14(2):221-224.

Crossman, J.S.. 1973. The Biological recovery of the Clinch river following pollutional stress. Doctoral Dissertation. Biology Dept., VPI&SU, Blacksburg, Virginia. 173 pp..

Cuker, B.E. 1983. Grazing and nutrient interactions in controlling the activity and composition of the epilithic algal community of an arctic lake. Limnol. Oceanogr. 28:133-141.

Cummins, K. W. 1962. An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. Am. Midl. Nat. 67:477-503.

Dazo, B.C.. 1965. The morphology and natural history of *Pleurocera acuta* and *Goniobasis livescens* (Gastropoda: Cerithiacea: Pleuroceridae). Malacologia. 3(1):1-90.

De With, N.D., T.P. Kok and R.C. Van Der Schors. 1986. Relationships between Apparent Net H+ Excretion and Na+, Ca2+ and Cl- Uptake in the Pulmonate Freshwater Snail *Lymnaea stagnalis*. Comp. Biochem. Physiol. A. 87:671-675.

Diamond, J. M. 1982. Stream geomorphology and benthic habitat predictability as determinants of the population dynamics and life history of the snail

Juga plicifera. J. Freshwater. Ecol. 1:577-588.

Dillon, R. T. and E. F. Benfield. 1982. Distribution of pulmonate snails in the New River of Virginia and North Carolina, USA - Interactions between alkalinity and stream drainage area.Freshwater Biology. 12(2):179-186.

Dussart, G. B. J. 1987. Effects of water flow on the detachment of some aquatic pulmonate gastropods. Am. Malacol. Bull. 5:65-72.

Farris, J.L.. 1986. Cellulolytic responses to heavy metal accumulation in *Corbicula fluminea* and *Mudalia dilatata*. Doctoral Dissertation, Biology Dept., VPI&SU, Blacksburg, Virginia. 164 pp..

Farris, J.L., S.E. Belanger, D.S. Cherry, and J. Cairns, Jr. 1989. Cellulolytic activity as a novel approach to assess long-term zinc stress to *Corbicula*. Water Res..23(10):1275-1283.

Farris, J.L., J.H. Van Hassel, S.E. Belanger, D.S. Cherry, & J. Cairns, Jr.. 1988. Application of cellulolytic activity of Asiatic clams (*Corbicula* sp.) to in-stream monitoring of power plant effluents. Environ. Toxicol. Chem.. 7(9):701-713.

Finney, D.J.. 1971. Statistical methods in biological assay, 3rd ed.. London, England, Cambridge University Press, 333 pp..

Flemming, C.A. and J.T. Trevors. 1988. Copper retention and toxicity in a freshwater sediment. Water, Air, and Soil Pollution. 40:419-432.

Flemming, C.A. and J.T. Trevors. 1989. Copper toxicity and chemistry in the environment: A review. Water, Air and Soil Pollution. 44:143-158.

Florence T.M. and G.E. Batley. 1980. Chemical speciation in natural waters. CRC Reviews Anal. Chem.. 9:219-296.

Foin, T.C.. 1971. The distribution pattern of the freshwater prosobranch gastropod *Oxytrema proxima* (Say). J. Elisha Mitchell Sci. Soc.. 87:1-10.

Forstner, U. and G.T.W. Wittmann. 1979. Metal pollution in the aquatic environment, Springer-Verlag, Berlin. 386 pp..

Fuller, R.L., J.L. Roelofs, and T.J. Fry. 1986. The importance of algae to stream invertebrates. J. North Am. Benthol. Soc.. 5:290-296.

Fuller, R.L., T.J. Fry, and J.A. Roelofs. 1988. Influence of different food types on the growth of *Simulium vittatum* (Diptera) and *Hydropsyche betteni*(Trichoptera). J. North Am. Benthol. Soc.. 7(3):197-204.

Goodrich, C. 1924. The genus *Gyrotoma*. Misc. Pub. Mus. Zool. Univ. Mich. 12 and Ala. Mus. Nat. Hist. Pap. 7:1-32.

Goodrich, C. 1922. The Anculosae of the Alabama River drainage. Misc. Pub. Mus. Zool. Univ Mich. 7:1-57.

Gordon, M.E. 1987. Habitat utilization, population dynamics and production of three gastropods (Prosobranchia: Pleuroceridae) from the North Fork of the White River, Missouri. Doctoral Dissertation. Biology Dept., University of Arkansas. 124 pp..

Goodrich, C. 1940. The Pleuroceridae of the Ohio River drainage system. Occ. Pap. Mus. Zool., Univ. Mich., No. 417:1-21.

Gosz, J.R. 1980. The influence of reduced stream flows on water quality.

Pages 3-48. In: W.O. Spofford, Jr., A.L, Parker, and A.V. Kneese, eds. Energy development in the southwest, Volume 2. Resources for the Future, Washington, D.C.

Graney, R.L.. 1980. Heavy Metal Dynamics in the Asiatic Clam *Corbicula fluminea*. Master of Science Thesis. Biology Dept., VPI&SU, Blacksburg, Virginia. 203 pp.

Gregg, B.C.. 1974. The Effects of Chlorine and Heat on Selected Stream Invertevrates. Doctoral Dissertation.Biology Dept., VPI&SU, Blacksburg, Virginia.

Guth, D.J., H.D. Blankesporr and J. Cairns, Jr.. 1977. Potentiation of Zinc Stress Caused by Parasitic Infection of Snails. Hydrobiologia. 55:225-229.

Haber, F. 1924. Zur Geschichte des Gaskrieges. In Funf Vortrage aus den Jahren 1920-1923. pp. 76-92. Springer, Berlin.

Hall, S.W., K.L. Dickenson, F.Y. Saleh, J.H. Rogers, D. Wilcox and A. Entazami. 1986. Effects of Suspended Solids on the Acute Toxicity of Zinc to *Daphnia magna* and *Pimephales promelas*. Water Resources Bulletin. 22:913-920.

Harman, W. N. 1972. Benthic substrates: their effect on fresh-water Mollusca. Ecology. 53:271-277.

Hart, D.D. 1985. Grazing insects mediate algal interactions in stream benthic community. Oikos 44:40-46.

Hawkins, C. P., M. L. Murphy, and N. H. Anderson. 1982. Effects of canopy, substrate composition, and gradient on the structure of macroinvertebrate communities in Cascade Range streams of Oregon. Ecology. 63:1840-1856.

Heath, A.G.. 1984. Changes in tissue adenylates and water content of bluegill, *Lepomis macrochirus*, exposed to copper. J. Fish. Biol.. 24(3):299-309.

Heath, A.G.. 1987. Water Pollution and Fish Physiology. CRC Press, Inc., Boca Raton, Florida. 245 pp..

Hobson, P.V., U. Borgmann, and H. Shear. 1979. Toxicity of copper to aquatic biota. In: J.O. Nriagu (ed.), Copper in the environment. Part II: Health effects. John Wiley and Sons. Toronto. pp. 307-372.

Holeton, G.F. and D.J. Randall. 1967. The effects of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. Exp.Biol.. 46:317-327.

Houp, K.H.. 1970. Population dynamics of *Pleurocera acuta* in a Central Kentucky limestone stream. Am. Midl. Nat.. 83:81-8.

Hunter, W.R.. 1957. Mollusca from limestone in Skye, with special reference to *Pyramidula rupestris* (Draparnaud) J. Conch.. 24:171-3.

Hynes, H.B.N.. 1970. The ecology of running waters. Toronto, University of Toronto Press. 559 pp..

Isaia, J. and A. Masoni. 1976. The effects of calcium and magnesium on water and ionic permeabilities in the sea water adapted eel, *Anguilla anguilla* L.

J.comp. Physiol.. 109:221-233.

Jackson, T.A.. 1978. Biogeochemistry of heavy metals in polluted lakes and streams at Flon, Canada, and a proposed method for limiting heavy metal pollution of natural waters. Environ. Geol. 2(3):173-189.

Jenne, E.A. and S.N. Luoma. 1977. Forms of trace metals in Soils, Sediments, and Associated Waters: An Overview of Their Determination and Biological Availability. Technical Inform.Center. pp. 110-143.

Jernakoff, P. 1983. Factors affecting the recruitment of algae in a midshore region dominated by barnacles. Journal of Experimental Marine Biology and Ecology. 67:17-31.

Jimenez, B.D., C.P. Cirmo and J.F. McCarthy. 1987. Effects of feeding and temperature on uptake, elimination and metabolism of benzo(a)pyrene in the bluegill sunfish (*Lepomis macrochirus*). Aquat. Tox.. 10(1):41-57.

Jones, W. C., and B. A. Branson. 1964. The radula, genital system, and external morphology in *Mudalia potosiensis* (Lea) 1841 (Gastropoda: Prosobranchiata; Pleuroceridae) with life history notes. Trans. Am. Microsc. Soc. 83:41-62.

Karr, J.R. and K.R. Dudley. 1981. Ecological perspective on water quality goals. Environmental Management. 5:55-68.

Karr, J.R., K.D. Fausch, P. L. Angermier, P. R. Yant, and I. J. Schlosser. 1986. Assessing biological integrity in running waters: a method and its rationale. Ill.Nat. Hist. Surv. Spec. Publ. 5. 28 pp.

Kimball, K.D. and S.A. Levin. 1985. Limitations of laboratory bioassays: the need for ecosystem-level testing.Bioscience. 35:165-71.

Kosalwat, P. and A.W. Knight. 1987. Acute Toxicity of Aqueous and Substrate-Bound Copper to the Midge, *Chironomus decorus*. Arch. Environ. Contam. Toxicol..16:275-282.

Krecker, F. H. 1924. Conditions under which *Goniobasis livescens* occurs in the island region of Lake Erie. Ohio J. Sci. 24:299-310.

Krieger, K. A., and W. D. Burbanck. 1976. Distribution and dispersal mechanisms of *Oxytrema* (= *Goniobasis*) *suturalis* Haleman (Gastropoda: Pleuroceridae) in the Yellow River, Georgia, USA. Am. Midl. Nat. 95:49-63.

Lamberti, G.A. and J.W. Moore. 1984. Aquatic insects as primary consumers. In: Resh, V.H. & Rosenberg, D.M.(eds.). The Ecology of Aquatic Insects. Praeger Publishers, New York. pp. 164-195.

Lamberti, G.A. and V. H. Resh. 1983. Stream periphyton and insect herbivores: an experimental study of grazing by a caddisfly population. Ecology. 64(5):1124-1135.

Leakie, J.O. and J.A. Davis. 1979. Aqueous environmental chemistry of copper. In: J.O. Nriagu (ed.),Copper in the environment. Part I:Ecological Cycling. John Wiley and Sons, Inc.. New York, pp. 89-121.

Lee, G.F.. 1973. Chemical Aspects of Bioassay Techniques for Establishing Water Quality Criteria. Wat. Res.. 7:1525-1546.

Leland, H.V.. 1983. Ultrastructural changes in the hepatocytes of juvenile rainbow trout and mature brown trout exposed to copper or zinc. Environ. Toxicol. Chem. 2:353-368.

Les, A. and R.W. Walker. 1983. Toxicity and Binding of Copper,Zinc and Cadmium by the Blue-green Alga, *Chroococcus paris*. Water, Air and Soil Pollution. 23:129-139.

Macan, T.T.. 1949. Ecology of freshwater mollusca in the English Lake District. J. Anim. Ecol.. 19:124-46.

Mancini, E. R. 1978. The biology of *Goniobasis semicarinata* (Say) (Gastropoda: Pleuroceridae) in the Mosquito Creek drainage system, southern Indiana. Ph.D. dissertation, University of Louisville, Louisville. 92 pp.

MacLulich, J. H. 1986. Colonization of bare rock surfaces by microflora in a rocky intertidal habitat. Marine Ecology Progress Series. 32:91-96.

McAuliffe, J.R. 1984. Resource depression by a stream herbivore: effects on distributions and abundances of other grazers. Oikos. 42:327-333.

McCormick, P.V. and R.J. Stevenson. 1991. Grazer control of nutrient availability in the periphyton. Oecologia. 86:287-291.

Miller-Way, C. and C.M. Way. 1989. The Life History of *Leptoxis dilatata* (Conrad) (Prosobranchia:Pleuroceridae) from the Laurel Fork River, West Virginia.122:193-198.

Moore, I. J. 1964. Effects of water currents on fresh-water snails *Stagnicola*

palustris and *Physa propinqua*. Ecology. 45:558-564.

Moore, J.W. and S. Ramamoorthy. 1984. Heavy Metals in Natural Waters - Applied Monitoring and Impact Assessment. pp.77-204.

Morrison, J.P.E.. 1954. The relationships of Old and New World Melanians. Proc. U.S. Nat. Mus.. 103:357-94.

Mount D.I., A.E. Steen, and J.T. Norberg-King, ed. 1985. Validity of effluent and ambient toxicity testing for predicting biological impact on Five Mile Creek, Birmingham, Alabama.Duluth, MN, EPA 600/8-85/015.

Nebeker, A.B., A. Stinchfield, C. Savonen and G.A. Chapman. 1986. Effects of copper, nickel and zinc on three species of Oregon freshwater snails. Environ. Toxicol. Chem. 5:807-811.

Nikinmaa, M.. 1983. Adrenergic regulation of hemoglobin oxygen affinity in rainbow trout red cells. J. Comp. Physiol..152:67-72.

Nowell, A. R. M., and P. A. Jumars. 1984. Flow environments of aquatic benthos. Ann. Rev. Ecol. Syst. 15:303-328.

O'Hara, J.. 1971. Alterations in oxygen consumption by bluegills exposed to sublethal treatment with copper. Water Res.. 5:321-327.

Owen, G.. 1955. Observations on the stomach and digestive diverticula Eulamellibranchia. Quar. J. Microscopical Sci.. 96:517-537.

Patrick, R.. 1978. Effects of trace metals in the aquatic ecosystem. Am. Sci.. 66:185-190.

Paulson, P.C., J.R. Pratt and J. Cairns, Jr.. 1983. Relationship of Alkaline

Stress and Acute Copper Toxicity in the Snail *Goniobasis livescens* (Menke). Bull. Environ.Contam. Toxicol.. 77:164-175.

Pennack, R.W. 1989. Freshwater Invertebrates of the United States: protozoa to mollusca - 3rd ed.. John Wiley and Sons,Inc. USA.

Perkin-Elmer. 1982. Analytical Methods for Atomic Absorption Spectrophotometry. Perkin-Elmer Corporation, Norwalk, Connecticut, USA.

Phillips, D.J.H. 1976. The Common Mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals.Marine Biology. 38:59-69.

Potts, W.T. and W.R. Fleming. 1971. The effect of environmental on sodium balance in *Fundulus kansae*. J. Exp. Biol. 55:63-76.

Ramamoorthy, S. and D.J. Kushner. 1975. Heavy metal binding sites in river water. Nature. 256:399-401.

Ramamoorthy, S. and B.R. Rust. 1978. Heavy metal exchange processes in sediment-water systems. Environ. Geol.. 2(3):165-172.

Rand, G.M. and S.R. Petrocelli. 1985. Introduction. In: Rand, G.M. and S.R. Petrocelli. Fundamentals of Aquatic Toxicology. Hemisphere Publishing Company, New York, NY, USA. 30 pp.

Rehwoldt, R., L. Lasko, C. Shaw, and E. Wirhowski. 1973. The acute toxicity of some heavy metal ions toward benthic organisms. Bull. Environ. Contam. Toxicol.. 10:291-294.

Rietsma, C.S., I. Valiela and R. Buchsbaum. 1988. Detrital chemistry, growth, and food choice in the salt-marsh snail (*Melampus bidentatus*). Ecology. 69(1):261-266.

Rodgers, J.H., Jr., D.S. Cherry, R.L. Graney, K.L. Dickson and J. Cairns, Jr.. 1980. Comparison of heavy metal interactions in acute and artificial stream bioassay techniques for the Asiatic clam (*Corbicula fluminea*). In: Aquatic toxicology. Eaton, J.G., P.R. Parrish and A.C. Hendricks (Eds.). ASTM STP 707.American Society for Testing and Materials,Philadelphia, PA. pp. 266-280.

Rollo, C.D. and M.D. Hawryluk. 1988. Compensatory scope and resource allocation in two species of aquatic snails. Ecology. 69(1)146-156.

Ross, M.J. and G.R. Ulrich. 1980. Temperature and substrate influences on habitat selection in two Pleurocerid snails (*Goniobasis*). Am. Midl. Nat.. 103(2):209-217.

Schnitzer, M. and H. Kerndorff. 1981. Reactions of fulvic acid with metal ions. Water, Air and Soil Pollution. 15:97-108.

Segar, D.A., J.D. Collins and J.P. Riley. 1971. Distribution of major and some minor elements in marine animals. 2. Molluscs. J. Marine Bi.. 51(1):131.

Sellers C.M., Jr., A.G. Heath, and M.L. Bass. 1975. the effect of sublethal concentrations of copper and zinc on ventilatory activity, blood oxygen and pH in rainbow trout(*Salmo gairdneri*). Water Res.. 9:401-408.

Skidmore, J.F.. 1970. Respiration and osmoregulation in rainbow trout with

gills damaged by zinc sulphate. J. Exp. Biol.52:481-494.

Smith, F.M. and D.R. Jones. 1982. The effect of changes in blood oxygen carrying capacity on ventilation volume in the rainbow trout (*Salmo gairdneri*). J. Exp.Biol.. 97:325-334.

Smith, L.S.. 1982. Introduction to Fish Physiology. T.F.H. Publications, Inc. Neptune, NF, USA. 352 pp.

Sprague, J.B.. 1964. Avoidance of copper-zinc solutions by young salmon in the laboratory. J. Water Pollut. Control Fed.. 36:990-1004.

Statistical Analysis System (SAS). 1985. SAS User's Guide: Statistics, version 5 edition. Cary, NC.

Stauber, J.L. and T.M. Florence. 1987. Mechanism of toxicity of ionic copper and copper complexes to algae. Mar. Biol..94:511-9.

Steinman, A.D., S.V. Gregory, G.A. Lamberti, L.R. Ashkenas. 1987. Effects of herbivore type and density on the taxonomic structure and physiognomy of algal assemblages in laboratory streams. J. N. Am. Benthol. Soc. 6:175-188.

Steinman, A.D., C.D. McIntire, S.V. Gregory, G.A. Lamberti. 1989. Effects of irradiance and grazing on lotic algal assemblages. J. Phycol. 25:478-485.

Stiff, M.F.. 1971. Water Res.. 12:899.

Sumner, W.T., C.D. McIntire. 1982. Grazer-periphyton interactions in laboratory streams. Arch. Hydrobiol. 93:135-157.

Swift, M.J., O.W. Heal and J.H. Anderson. 1979. Decomposition processes in terrestrial ecosystems. Blackwell Scientific Publication. Oxford, England. 372 pp..

Sylva, R.N. 1976. The environmental chemistry of copper (II) in aquatic systems. Water Res. 10(9):789-792.

Takatsuki S.. 1934. On the nature and functions of the amoebocytes of *Ostrea edulis*. Quar. J.Microscopical Sci.. 76:379-431.

Thomas, J.D., D.I. Nwanko and P.R. Sterry. 1985. The feeding strategies of juvenile and adult *Biomphalaria glabrata* (Say) under simulated natural conditions and their relevance to ecological theory and snail control. Proc. R.Soc. Lond. B.. 226:177-209.

Thruston, R.V., R.C. Russo, C.M. Fetterolf, Jr., T.A. Edsall and Y.M. Barber, Jr., eds.: 1979. A review of the EPAredbook: quality criteria for water. American Fisheries Society, Water Quality Section. Bethesda, Maryland.

Tooby, T.E.. 1978. A scheme for the evaluation of hazards to non-target aquatic organisms from the use of chemicals. Proceedings of the Fifth Symposium of the European Weed Research Society. pp. 287-294.

Turner, J.D., C.M. Wood and D. Clark. 1983. Lactate and proton dynamics in the rainbow trout (*Salmo gairdneri* Richardson) exposed to hypoxia. J. Fish. Biol.. 6:379-387.

Tuchman, N. C. and R. J. Stevenson. 1991. Effects of selective grazing by snails on benthic algal succession. J. N. Am. Benthol. Soc. 10(4):430-

443.

Underwood, A. J. and P. Jernakoff. 1981. Effects of interactions between algae and grazing gastropods on the structure of a low-shore intertidal algal community. Oecologia. 48:221-233.

United States Environmental Protection Agency (USEPA).1980. Ambient water quality criteria for zinc. Washington, D.C. PB81-117897. 207 pp..

United States Environmental Protection Agency (USEPA).1984. Ambient water quality criteria for copper. Washington, D.C. PB85-227023. 142 pp..

United States Environmental Protection Agency (USEPA). 1985. Technical support document for water quality-based toxics control. Washington, D.C. EPA-440/4-85-032. 74 pp..

United States Environmental Protection Agency (USEPA). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms.Washington, D.C. EPA 600/4-89/001. 162 pp..

Valdes, R.A., L.A. Hartwell, J.R. Maxwell, and M.M. Dell. 1982. Metal bioconcentration factors with digestion procedures. Pharmac. Biochem. Behav.. 16:915-917.

Van Hassel, J.H.. 1989. Clinch River in-house biological monitoring - 1982-1988 final report, February 24, 1989. American Electric Power Company, Colombus, OH, USA. pp. 1-6.

Van Hassel, J.H. and A.E. Gaulke. 1986. Site-specific water quality criteria

from instream monitoring data. Environ. Toxicol. Chem.. 5(5):417-426.

Watson, T.A. and F.W.H. Beamish. 1980. Effects of zinc on branchial ATPase activity *in vivo* in rainbow trout, *Salmo gairdneri*. Comp.Biochem. Physiol.. 66C:77-82.

Weber, C.I.. 1981. Evaluation of the effects of effluents on aquatic life in receiving waters - an overview. In: J.M. Bates and C.I. Weber (eds.). Ecological assessments of effluent impacts on communities of indigenous aquatic organisms. American Society for Testing and Materials. Philadelphia, PA, USA. pp. 3-13.

Weser, U., L.M. Schubotz, and M. Younes. 1979. In: J.O. Nriagu (ed.), Copper in the environment. Part II: Health effects. John Wiley and Sons. Toronto. pp. 197-240.

Williams, D. D. 1981. Migrations and distributions of stream benthos. Pages 155-207. In: M. A. Loch and D. D. Williams, eds. Perspectives in running water ecology. Plenum Press, New York.

Wood, C.M., D.G. McDonald, and B.R. McMahon. 1982. The influence of experimental anaemia on blood acid-base regulation *in vivo* and *in vitro* in the starry flounder (*Platichthys stellatus*) and the rainbow trout (*Salmo gairdneri*). J. Exp. Biol.. 96:221-237.

Wurtz C.B.. 1962. Zinc effects of fresh-water molluscs. Nautilus. 76:53-61.

Wurtz C.B. and C.H. Bridges. 1961. Preliminary results from macroinvertebrate bioassays. Proc. Pa. Acad. Sci. 35:51-56.

Yonge, C.M.. 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. J.Mar. Biol. Assoc. of the United Kingdom. 14:295-386.

Young, M.L.. 1977. The roles of food and direct uptake from water in the accumulation of zinc and iron in the tissues of the dogwhelk, *Nucella lapillus* (L.). J. Exp. Mar.Biol. Ecol.. 30:315-25.

Zakharov, I.S. and P.M. Balaban. 1987. Neural mechanisms of age-dependent changes in avoidance behavior of the snail *Helix lucorum*. Neuroscience. 23(2):721-729.

APPENDIX A: HABITAT MEASUREMENTS

Table A1. Results of Study I. Measurements made at eight locations within one river-mile upstream of discharge. Sites 1, 2, 3, and 8 were chosen to represent good snail habitat, while Sites 4-7 were chosen to represent less desirable snail habitat from observations of snail population density. Ranges reported represent results of three replicate measurements.

Parameter	Site 1	2	3	4	5	6	7	8
L. praerosa, snails/sq. ft.	5-20	2-14	0-1	0	0-1	0	0-1	60-100
P. unciale, snails/sq. ft.	1-6	1-9	1-3	0	0-1	0	0-5	0-4
Depth, in.	5-13	12-16	4-11	29	16-18	17-18	17	6-8
Surface Velocity, cm/sec	15-91	45-72	51-64	14	10-12	0.2-2	7-20	98-126
Bed Velocity, cm/sec	7-103	18-44	44-57	3	0.4-2	0.4-0.5	3-14	98-126
Substrate Type	GCB	GCSAS	GBSAS	GCSAS	GCSAS	GCSAS	GCSAS	GC
Shading	NS	NS	SH	NS	SH	NS	SH	NS
% Algal Cover	10-20	10-50	20-75	0-10	20-40	10	5	10-15
AFDW, mg/cm^2	48	11	77	8	0.2-11	13-19	4-9	7-8
Silt, mg/cm^2	17	3	14	2	2-172	388-643	40-80	10-37
Chl. a, mg/cm^2	<0.01-0.12	<0.01	<0.01	<0.01	<0.01-1.22	<0.01-0.48	0.01-0.44	<0.01-0.81
Phe. a, mg/cm^2	<0.01	0.19	<0.01	<0.01	<0.01-0.73	0.16-1.18	0.17-0.92	<0.01-0.81
D.O. mg/L,	7.8	7.8	7.8	7.8	8.6	8.5	8.4	8.4
pH	8.1	8.2	8.2	8.2	8.2	8.3	8.3	8.3
Alk., mg/L	119	122	124	122	140	150	154	153
Hardness, mg/L	155	154	146	157	153	142	140	142

G-gravel C-cobble
B-bedrock SA-sand
S-silt NS-not shaded
SH-shaded Chl. a-Chlorophyll a
Phe. a-Pheophytin a D.O.-dissolved oxygen
Alk.-Alkalinity.

Table A2(a). Means (SD) of snail density and habitat variable measurements in Study II - Station 1A. Three replicate measurements were made at the left bank, middle and right bank of pool and riffle transects.

	Riffle			Pool		
	Left	Mid.	Right	Left	Mid.	Right
L. praerosa	4(2)	3(1)	11(7)	0(0)	1(1)	0(0)
P. unciale	4(4)	0(0)	0(1)	0(0)	1(1)	1(1)
Depth, in.	12.0	11.5	8.0	19.0	23.0	21.0
	9.0	11.0	9.0	19.3	24.0	21.0
	10.0	12.0	8.5	19.7	23.8	22.1
Surf. Vel.,	-	87	-	14	36	16
cm/sec	-	235	-	17	35	14
	-	110	-	10	28	12
Bed Vel.,	66	81	110	5	30	2
cm/sec	67	101	126	1	18	3
	74	89	117	1	20	2
Substrate	GCSA	GCSA	GCSA	SAS	SAS	SAS
% Algal	5	5	5	0	0	0
Cover	5	5	5	0	0	0
	5	5	5	0	0	0
AFDW,	3.0	0.6	0.5	3.6	9.9	0.7
mg/cm^2	2.3	0.2	0.2	0.7	1.1	0.4
	2.2	0.7	1.8	0.7	1.4	1.0
Silt,	16.7	1.9	2.0	49.7	74.2	3.6
mg/cm^2	12.4	0.7	1.9	3.7	16.4	1.8
	5.9	5.8	3.8	5.0	14.0	2.4
Chl. Ext.,	0.131	0.231	0.053	0.067	0.288	0.107
mg/cm^2	0.187	0.052	0.199	0.087	0.192	0.049
	0.132	0.247	0.255	0.097	0.127	0.088
pH	8.04	8.05	8.09	8.05	8.12	8.09
	8.04	8.12	8.07	8.05	-	-
	8.05	8.09	8.07	8.05	-	-
Alk., mg/L	87.9	87.6	88.4	87.7	87.8	87.6
	87.4	86.5	88.0	90.7	-	-
	87.4	87.7	83.6	83.3	-	-
Hardness,	114	144	148	149	144	156
mg/L	150	156	152	156	-	-
	250	144	160	142	-	-

G-gravel C-cobble Surf. Vel.-Surface Velocity
B-bedrock SA-sand Vel.-Velocity
S-silt ND-not detectable
Alk.-alkalinity Chl. Ext.-chlorophyll A extract reading at 664 nm

Table A2(b). Means (SD) of snail density and habitat variable measurements in Study II - Station 1B. Three replicate measurements were made at the left bank, middle and right bank of pool and riffle transects.

	Riffle			Pool		
	Left	Mid.	Right	Left	Mid.	Right
L. praerosa	12(2)	43(36)	17(10)	0(0)	0(0)	0(0)
P. unciale	1(1)	5(5)	1(1)	0(1)	0(0)	0(0)
Depth, in.	12.0	7.0	6.0	24.5	24.8	19.5
	12.0	7.0	6.0	25.0	24.8	21.3
	12.3	6.5	6.2	25.2	25.0	22.4
Surf. Vel.,	-	-	-	14	0	0
cm/sec	-	-	-	16	0	0
	-	-	-	32	0	0
Bed Vel.,	42	64	23	11	0	5
cm/sec	39	54	35	13	0	5
	38	45	51	6	0	5
Substrate	GCSA	GCSA	GC	GCSAS	GCSAS	GCSAS
% Algal	5	5	5	0	0	0
Cover	5	5	5	0	0	0
	5	5	5	0	0	0
AFDW,	0.0	3.1	0.4	0.1	0.0	0.0
mg/cm^2	0.2	3.1	0.0	0.0	0.0	0.6
	0.0	5.7	0.0	0.4	0.4	0.2
Silt,	0.8	78.7	2.3	1.4	0.8	0.7
mg/cm^2	2.5	27.7	1.1	1.0	1.3	6.6
	0.7	3.7	0.6	0.0	0.9	0.4
Chl. Ext.,	0.051	0.214	0.013	0.001	0.017	0.001
mg/cm^2	0.057	0.289	0.028	0.001	0.012	0.004
	0.001	0.217	0.006	0.001	0.044	0.001
pH	8.19	8.23	8.32	8.12	8.16	8.19
Alk., mg/L	88.7	86.3	89.7	88.6	88.8	86.9
Hardness,	144	156	142	158	76	152
mg/L						

G-gravel C-cobble Surf. Vel.-Surface Velocity
B-bedrock SA-sand Vel.-Velocity
S-silt ND-not detectable
Alk.-alkalinity Chl. Ext.-chlorophyll A extract reading at 664 nm

Table A2(c). Means (SD) of snail density and habitat variable measurements in Study II- Station 2. Three replicate measurements were made at the left bank, middle and right bank of pool and riffle transects.

	Riffle			Pool		
	Left	Mid.	Right	Left	Mid.	Right
L. praerosa	0(0)	1(2)	6(5)	0(0)	0(0)	0(0)
P. unciale	0(0)	10(8)	2(2)	0(0)	0(0)	0(0)
Depth, in.	10.5	15.0	6.8	33.3	38.0	28.1
	9.2	14.8	6.0	33.8	38.5	27.2
	9.2	17.6	6.6	31.8	39.8	26.8
Surf. Vel.,	-	-	-	ND	ND	ND
cm/sec	-	-	-	ND	ND	ND
	-	-	-	ND	ND	ND
Bed Vel.,	37	49	43	ND	ND	ND
cm/sec	53	47	47	ND	ND	ND
	44	37	48	ND	ND	ND
Substrate	GCBSAS	GC	GCSA	SAS	SAS	SAS
% Algal	0	40	10	0	0	0
Cover	0	40	20	0	0	0
	0	40	20	0	0	0
AFDW,	0.8	1.1	1.1	6.2	7.2	7.8
mg/cm^2	1.9	1.3	0.7	6.2	0.7	9.0
	0.8	1.4	1.1	1.0	7.3	12.8
Silt,	7.2	5.9	8.8	42.4	102.7	113.4
mg/cm^2	12.7	6.6	3.2	105.7	11.9	180.9
	3.2	15.1	4.4	11.2	71.1	20.1
Chl. Ext.,	0.001	0.119	0.143	0.023	0.043	0.001
mg/cm^2	0.002	0.035	0.060	0.004	0.046	0.001
	0.001	0.123	0.009	0.047	0.029	0.001
pH	8.30	8.25	8.33	8.35	8.28	8.25
Alk., mg/L	89.0	90.0	99.3	94.5	89.9	89.9
Hardness,	130	180	150	156	154	170
mg/L						

G-gravel C-cobble Surf. Vel.-Surface Velocity
B-bedrock SA-sand Vel.-Velocity
S-silt ND-not detectable
Alk.-alkalinity Chl. Ext.-chlorophyll A extract reading at 664 nm

Table A2(d). Means (SD) of snail density and habitat variable measurements in Study II - Station 13. Three replicate measurements were made at the left bank, middle and right bank of pool and riffle transects.

	Riffle			Pool		
	Left	Mid.	Right	Left	Mid.	Right
L. praerosa	0(0)	60(44)	269(131)	0(0)	0(0)	0(0)
P. unciale	0(0)	7(3)	1(1)	0(0)	8(3)	5(4)
Depth, in.	10.8	9.0	9.8	10.5	11.7	18.0
	11.0	9.5	11.2	12.2	12.2	18.0
	10.0	7.8	10.5	12.1	13.0	17.8
Surf. Vel.,	-	-	-	-	-	9
cm/sec	-	-	-	-	-	16
	-	-	-	-	-	16
Bed Vel.,	43	33	36	22	17	19
cm/sec	39	30	54	25	14	19
	45	46	55	27	14	18
Substrate	GCBSAS	GCSA	GCSA	GCSAS	GCSAS	GCSAS
% Algal	100	5	5	2	5	0
Cover	100	5	5	0	0	0
	100	15	5	0	0	0
AFDW,	0.9	0.3	2.1	0.0	1.6	1.0
mg/cm^2	0.1	1.2	0.4	0.3	1.1	2.4
	1.4	1.3	2.1	1.8	5.6	1.0
Silt,	1.4	1.4	13.7	1.0	8.9	3.0
mg/cm^2	2.1	7.8	1.5	2.6	5.6	9.4
	5.8	3.4	4.9	29.1	30.7	3.6
Chl. Ext.,	0.047	0.110	0.001	0.032	0.006	0.052
mg/cm^2	0.045	0.049	0.018	0.037	0.111	0.061
	0.087	0.053	0.255	0.027	0.059	0.144
pH	8.47	8.49	8.40	8.56	8.42	8.40
	8.47	8.47	8.40	8.56	8.44	8.40
	8.49	8.47	8.40	8.56	8.42	8.40
Alk., mg/L	92.4	85.0	97.5	92.2	93.2	95.5
	92.6	95.0	95.5	102.2	93.2	96.5
	91.5	95.5	97.4	88.8	94.0	96.4
Hardness,	182	182	154	204	164	156
mg/L	194	168	152	216	162	170
	178	162	156	208	178	154

G-gravel C-cobble Surf. Vel.-Surface Velocity
B-bedrock SA-sand Vel.-Velocity
S-silt ND-not detectable
Alk.-alkalinity Chl. Ext.-chlorophyll A extract reading at 664 nm

Table A2(e). Means (SD) of snail density and habitat variable measurements in Study II - Station 15A. Three replicate measurements were made at the left bank, middle and right bank of pool and riffle transects.

	Riffle			Pool		
	Left	Mid.	Right	Left	Mid.	Right
L. praerosa	31(27)	11(9)	9(6)	25(10)	5(5)	4(2)
P. unciale	35(14)	9(3)	25(5)	41(15)	23(25)	7(6)
Depth, in.	7.8	12.5	5.8	12.0	18.3	19.0
Surf. Vel., cm/sec	-	-	-	11	17	0
Bed Vel., cm/sec	30	45	14	11	10	0
Substrate	GCSA	GCBSAS	GCSAS	GCBSAS	BSAS	GCSAS
% Algal	0	20	5	30	0	0
Cover	5	20	5	20	0	0
	5	50	5	10	15	0
AFDW,	1.3	1.9	0.7	0.1	0.0	0.6
mg/cm^2	0.9	3.6	1.0	10.2	0.3	0.2
	0.4	6.8	0.9	20.3	0.4	0.3
Silt,	5.2	9.0	4.4	0.9	0.8	4.0
mg/cm^2	2.8	8.4	4.2	17.2	1.2	1.3
	1.6	10.6	5.6	1.6	1.6	2.6
Chl. Ext.,	0.202	0.335	0.172	0.003	0.001	0.020
mg/cm^2	0.018	0.242	0.123	0.001	0.017	0.034
	0.035	0.318	0.030	0.001	0.042	0.012
pH	8.31	8.27	8.22	8.31	8.29	8.22
Alk., mg/L	130	130	132	130	130	131
Hardness, mg/L	169	179	179	181	177	185

G-gravel C-cobble Surf. Vel.-Surface Velocity
B-bedrock SA-sand Vel.-Velocity
S-silt ND-not detectable
Alk.-alkalinity Chl. Ext.-chlorophyll A extract reading at 664 nm

Table A2(f). Means (SD) of snail density and habitat variable measurements in Study II - Station 15. Three replicate measurements were made at the left bank, middle and right bank of pool and riffle transects.

	Riffle			Pool		
	Left	Mid.	Right	Left	Mid.	Right
L. praerosa	20(8)	21(27)	7(5)	0(0)	0(0)	0(0)
P. unciale	10(7)	4(2)	4(1)	0(0)	0(1)	0(1)
Depth, in.	9.0	12.0	9.2	22.0	32.2	18.2
Surf. Vel., cm/sec	-	-	-	0	11	23
Bed Vel., cm/sec	15	36	47	0	11	20
Substrate	GCSA	GCBSAS	GCSA	GCSAS	GCSAS	SAS
% Algal	5	5	5	0	0	0
Cover	10	5	5	0	0	0
	5	5	5	0	0	0
AFDW,	1.8	12.9	0.4	0.7	1.0	1.4
mg/cm^2	4.2	62.4	6.0	1.2	1.2	0.6
	1.9	7.2	0.4	0.1	0.8	0.5
Silt,	9.7	21.0	1.8	4.7	1.8	4.1
mg/cm^2	32.1	149.4	95.9	11.1	47.4	2.6
	9.3	7.1	1.1	7.0	20.4	1.2
Chl. Ext.,	0.130	0.187	0.043	0.037	0.028	0.045
mg/cm^2	0.131	0.337	0.061	0.001	0.060	0.032
	0.230	0.223	0.027	0.098	0.068	0.043
pH	8.17	8.22	8.25	8.27	8.22	8.25
Alk., mg/L	131	132	131	130	130	130
Hardness, mg/L	180	201	182	187	180	199

G-gravel C-cobble Surf. Vel.-Surface Velocity
B-bedrock SA-sand Vel.-Velocity
S-silt ND-not detectable -
Alk.-alkalinity Chl. Ext.-chlorophyll A extract reading at 664 nm

Table A2(g). Means (SD) of snail density and habitat variable measurements in Study II - Station 16. Three replicate measurements were made at the left bank, middle and right bank of pool and riffle transects.

	Riffle			Pool		
	Left	Mid.	Right	Left	Mid.	Right
L. praerosa	20(7)	27(12)	3(1)	1(1)	1(1)	0(1)
P. unciale	0(0)	1(0)	2(2)	1(1)	0(1)	0(0)
Depth, in.	7.0	11.0	16.2	17.0	27.0	24.2
Surf. Vel., cm/sec	-	-	-	0	14	9
Bed Vel., cm/sec	113	94	36	0	15	15
Substrate	GCSA	GCSA	GCSAS	GCBSAS	GCSAS	GCSAS
% Algal	5	5	5	0	0	0
Cover	5	5	5	0	0	0
	5	5	5	0	0	0
AFDW,	1.2	2.4	1.8	0.9	0.1	0.9
mg/cm^2	3.5	2.0	1.1	1.6	1.0	2.5
	2.5	1.6	1.9	1.0	0.9	0.8
Silt,	2.6	9.0	2.2	2.0	0.8	1.4
mg/cm^2	12.7	2.7	1.2	6.0	1.3	1.5
	6.7	3.7	1.3	9.0	2.3	2.9
Chl. Ext.,	0.052	0.143	0.107	0.059	0.017	0.037
mg/cm^2	0.155	0.270	0.111	0.088	0.043	0.012
	0.088	0.113	0.068	0.103	0.035	0.032
pH	8.22	8.22	8.22	8.20	8.22	8.17
Alk., mg/L	124	130	130	130	130	129
Hardness, mg/L	172	175	185	186	179	195

G-gravel C-cobble Surf. Vel.-Surface Velocity
B-bedrock SA-sand Vel.-Velocity
S-silt ND-not detectable
Alk.-alkalinity Chl. Ext.-chlorophyll A extract reading at 664 nm

APPENDIX B: SNAIL DENSITY

Table B1. Evaluation of *Leptoxis*, *Pleurocera* and *Ferrissia* density, taxa richness and total snail density from pool transects during Summer, 1988 using Duncan's Multiple Range Test. Connected values are not significantly different.

Variable	Sampling Station (Highest value)						(Lowest value)	Statistics AVOVA F value	P value
	Left, Middle and Right Substations Combined								
Leptoxis	15A	16	2	1A	1B	15	13	18.35	0.0001
Pleurocera	15A	16	1B	2	1A	15	13	23.97	0.0001
Ferrissia	15A	16	2	1A	1B	15	13	2.33	0.1015
No. of Taxa	15A	16	1B	2	1A	15	13	12.06	0.0001
No. of Snails	15A	16	1B	2	1A	15	13	39.60	0.0001
	Left Bank of River								
Leptoxis	15A	16	2	1A	1B	15	13	18.35	0.0001
Pleurocera	15A	16	1B	2	1A	15	13	23.97	0.0001
Ferrissia	15A	16	2	1A	1B	15	13	2.23	0.1015
No. of Taxa	15A	16	1B	2	1A	15	13	12.06	0.0001
No. of Snails	15A	16	1B	2	1A	15	13	39.60	0.0001
	Middle of River								
Leptoxis	15A	1A	16	2	1B	15	13	2.40	0.0833
Pleurocera	15A	13	1A	15	16	1B	2	2.41	0.0818
Ferrissia	16	13	1B	2	15A	15	1A	2.36	0.0869
No. of Taxa	13	15A	16	1A	15	1B	2	5.81	0.0032
No. of Snails	15A	16	13	1A	15	1B	2	2.07	0.1224
	Right Bank of River								
Leptoxis	15A	16	2	1A	1B	15	13	20.71	0.0001
Pleurocera	15A	13	1A	15	1B	2	16	3.41	0.0275
Ferrissia	13	15A	15	1A	16	1B	2	13.31	0.0001
No. of Taxa	15A	13	1A	15	16	1B	2	14.87	0.0001
No. of Snails	13	15A	15	1A	16	1B	2	15.17	0.0001

Table B2. Evaluation of *Leptoxis*, *Pleurocera* and *Ferrissia* density, taxa richness, and total snail density from surber sampling across riffle transects during Summer, 1988 using Duncan's Multiple Range Test. Connected values re not significantly different.

Variable	Sampling Station							Statistics	
	Highest value						Lowest value	AVOVA F value	P value
	Left, Middle and Right Substations Combined								
Leptoxis	13	1B	15A	16	15	1A	2	3.97	0.0022
Pleurocera	15A	15	2	13	1B	1A	16	12.93	0.0001
Ferrissia	15A	15	2	13	16	1A	1B	3.45	0.0057
No. of Taxa	15	15A	16	1B	1A	2	13	3.32	0.0073
No. of Snails	13	15A	15	1B	16	2	1A	3.66	0.0239
	Left Bank of River								
Leptoxis	15A	16	15	1B	1A	2	13	3.56	0.0237
Pleurocera	15A	15	1A	1B	13	2	16	2.87	0.0490
Ferrissia	15A	15	1A	16	2	13	1B	6.17	0.0224
No. of Taxa	15A	15	1A	16	1B	2	13	27.33	0.0001
No. of Snails	15A	15	16	1A	1B	2	13	9.09	0.0004
	Middle of River								
Leptoxis	13	1B	16	15	15A	1A	2	2.40	0.0836
Pleurocera	2	15A	13	1B	15	16	1A	2.71	0.0584
Ferrissia	15	15A	13	16	2	1A	1B	4.82	0.0072
No. of Taxa	13	15	16	15A	1B	2	1A	5.47	0.0042
No. of Snails	13	1B	15	15A	16	2	1A	2.47	0.0766
	Right Bank of River								
Leptoxis	13	1B	1A	15A	15	2	16	9.19	0.0003
Pleurocera	15A	15	2	16	1B	13	1A	33.94	0.0001
Ferrissia	2	15A	15	13	16	1B	1A	3.69	0.0107
No. of Taxa	15A	2	15	16	1B	13	1A	1.56	0.2318
No. of Snails	13	15A	2	15	16	1B	1A	11.94	0.0001

Table B3. Mean (SD) density of *Leptoxis*, *Pleurocera*, and *Ferrissia* per 0.1 m^2, abundance, and richness from left bank riffles at each station (n=3; October, 1988).

	Station						
Variable	1A	1B	2	13	15A	15	16
Leptoxis density	2	12	0	0	10	13	146
	(1)	(5)	(0)	(0)	(2)	(11)	(33)
Pleurocera density	6	1	6	0	4	5	0
	(6)	(1)	(3)	(0)	(2)	(2)	(0)
Ferrissia density	1	0	1	0	2	6	0
	(1)	(0)	(1)	(0)	(3)	(4)	(0)
No. of Snails,	3	4	2	0	5	8	49
(abundance)	(4)	(6)	(3)	(0)	(4)	(7)	(75)
No. of Taxa,	2	2	1	0	3	3	1
(richness)	(1)	(1)	(1)	(0)	(1)	(0)	(0)

APPENDIX C: TEST CONDITIONS

Table C1. Water chemistry means (SD) from dosed (Glen Lyn) and undosed (ESL) streams in the feeding study (July 3-August 8, 1989).

Stream Number	Treatment, ug/L	Cu, ug/L	Zn, ug/L	pH	Conductivity, umho/cm	Alkalinity, mg $CaCO_3$/L	Hardness, mg $CaCO_3$/L
Dosed Streams							
1	Control	4(0)	44(24)	7.66 (0.01)	108(6)	38(3)	30(11)
2	8.6 Cu	7(2)	-	7.65 (0.01)	107(6)	38(4)	21(8)
3	17.2 Cu	8(6)	-	7.70 (0.03)	106(7)	38(4)	34(10)
4	47 Zn	-	56(31)	7.68 (0.01)	106(7)	38(3)	38(10)
5	94 Zn	-	66(43)	7.71 (0.04)	106(7)	37(3)	32(1)
6	17 Cu +94 Zn	5(2)	56(32)	7.73 (0.01)	106(6)	38(4)	26(4)
Undosed Streams							
1	-	4(2)	5(4)	7.60 (0.11)	126(16)	36(6)	30(2)
2	-	2(1)	-	7.62 (0.13)	118(15)	35(6)	27(6)
3	-	2(1)	-	7.65 (0.14)	120(12)	36(4)	26(4)
4	-	-	4(3)	7.64 (0.16)	120(11)	38(11)	26(6)
5	-	-	4(5)	7.68 (0.12)	120(11)	36(2)	24(4)
6	-	1(0)	5(3)	7.64 (0.18)	120(11)	37(1)	25(6)

Table C2. Duncan's analysis of cellulolytic enzyme activity (n=6), copper and zinc bioconcentration (n=3) in *Leptoxis* and *Mudalia* after 30-day exposures to copper and zinc treatments (in ug/L) from waterborne (dosed) and food-borne (undosed) exposures, Study I. Treatments connected by the same line were not significantly different (P<0.05; July 3 - August 8, 1989).

Variable	Treatment						Statistics	
	Highest value					Lowest value	ANOVA F value	P value
Dosed streams - NR3								
Leptoxis								
Cellulase	Con[1]	Cmb[2]	9Cu	94Zn	47Cu	17Cu	0.12	0.9861
Cu biocon.	Cmb	94Zn	9Cu	47Zn	17Cu	Con	9.88	0.0001
Zn biocon.	Con	Cmb	94Zn	47Zn	9Cu	17Cu	2.77	0.0358
Mudalia								
Cellulase	Con	17Cu	9Cu	94Zn	Cmb		3.18	0.0387
Cu biocon.	Cmb	17Cu	9Cu	Con	94Zn		1.39	0.2783
Zn biocon.	Cmb	94Zn	9Cu	Con	17Cu		7.28	0.0011
Undosed streams - NR3A								
Leptoxis								
Cellulase	17Cu	9Cu	47Zn	Con	Cmb	94Zn	4.63	0.0030
Cu biocon.	Con	9Cu	Cmb	47Zn	94Zn	17Cu	6.55	0.0003
Zn biocon.	Con	9Cu	94	47Zn	Comb	17Cu	7.86	0.0001
Mudalia								
Cellulase	47Zn	94Zn	Con	9Cu	17Cu		1.40	0.2818
Cu biocon.	9Cu	17Cu	Con	47Zn	94Zn		3.00	0.0525
Zn biocon.	94Zn	17Cu	47Zn	Con	9Cu		1.25	0.3332

[1] Control

[2] Combination treatment - 9 ug Cu + 47 ug Zn/L

Table C3. Metal concentrations from soak solutions and test water from 30-day feeding study using precolonized (with aufwuchs) ceramic tiles (Study II, August 16 - September 15, 1990).

Chamber Number	Target soak ug/L	Measured concentration (before soak), ug/L (n=1)		Measured concentration (after soak), ug/L (n=1)	
		Cu	Zn	Cu	Zn
1	Control	8	4	-	-
2	50 Cu	95	-	-	-
3	100 Cu	149	-	-	-
4	500	585	-	436	-
5	100 Zn	-	80	-	-
6	500 Zn	-	465	-	-
7	2000 Zn	-	1736	-	401
8	50 Cu + 100 Zn	102	105	-	-
9	50 Cu + 2000 Zn	87	1574	39	231

Testing Stream	Mean measured concentration (SD) ug/L (n=13)	
	Cu	Zn
1	10(3)	7(5)
2	10(2)	7(12)
3	8(3)	3(5)

Table C4. Water chemistry from 96-h Cu and CRP effluent toxicity tests (September-October 1988).

Study	Target exposure	Measured Cu conc. (ug/L)	pH	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	Conductivity (umhos/cm)
Flow-through CRP artificial stream tests						
	Control	6	8.11	145	125	317
	Effluent (%)					
	10	16	8.20	207	119	463
	20	40	8.11	310	104	635
	40	22	8.09	190	116	436
	60	99	7.86	483	67	1010
	80	116	7.81	530	59	1096
	100	148	7.56	755	25	1436
	Cu (ug/L)					
	10	104	8.38	150	146	314
	50	41	8.36	165	152	308
	100	56	8.35	150	151	304
	400	120	8.35	160	153	305
	600	128	8.31	156	147	307
	1000	414	8.35	575	152	312
Static tests at VPI&SU						
	Control	18	8.34	155	133	333
	Effluent (%)					
	10	17	8.34	180	129	407
	20	37	8.20	230	118	498
	40	48	8.13	315	102	650
	60	74	8.03	410	84	811
	80	77	7.89	550	62	964
	100	105	7.66	590	103	1095
	Cu (ug/L)					
	10	15	8.27	151	137	341
	50	6	8.34	159	146	344
	100	67	8.36	182	150	356
	400	133	8.23	179	151	361
	600	131	8.43	188	163	377
	1000	311	8.04	193	168	386

[a] Clinch River Plant, Carbo, Virginia.

Table C5. Water chemistry from artificial stream copper and CRP effluent impairment tests (June-August 1988).

Study	Targeted exposure	Measured Cu conc. (ug/L)	pH	Hardness (mg $CaCO_3$ /L)	Alkalinity (mg $CaCO_3$ /L)	Conductivity (umhos cm)
14-day exposures						
	Control	4(1)[a]	8.14(0.08)	147(3)	121(1)	251(7)
	Effluent (%)					
	1	5(1)	8.18(0.06)	142(2)	120(1)	262(8)
	5	12(1)	8.20(0.04)	173(2)	117(2)	304(6)
	10	20(1)	8.14(0.06)	188(7)	112(1)	343(7)
	20	28(2)	8.13(0.04)	236(9)	107(1)	411(5)
	100	176(16)	5.92(1.72)	683(64)	327(4)	799(39)
	Cu (ug/L)					
	6	7(1)	8.20(0.05)	140(0)	120(1)	251(8)
	12	13(1)	8.16(0.06)	140(6)	121(2)	247(10)
	25	15(1)	8.18(0.05)	148(8)	120(1)	252(8)
20-day exposures						
	Control	8(4)	8.32(0.01)	116(12)	118(1)	272(10)
	Effluent (%)					
	1	14(5)	8.36(0.01)	121(13)	118(1)	287(11)
	10	21(4)	8.31(0.02)	149(15)	116(2)	358(13)
	20	52(10)	8.28(0.02)	118(18)	108(3)	442(17)
	40	52(2)	8.14(0.03)	231(24)	99(1)	528(13)
	100	229(22)	7.64(0.02)	603(120)	28(2)	1261(125)
	Cu (ug/L)					
	6	6(1)	8.32(0.02)	110(12)	120(1)	272(10)
	12	10(2)	8.33(0.01)	114(11)	118(1)	282(12)
30-day exposures						
	Control	8(2)	8.18(0.03)	109(11)	117(1)	273(8)
	Effluent (%)					
	1	14(3)	8.26(0.03)	112(11)	120(1)	279(10)
	10	20(3)	8.17(0.03)	132(14)	112(1)	339(14)
	20	39(7)	8.19(0.02)	136(18)	109(1)	384(17)
	40	58(8)	8.14(0.03)	193(21)	98(2)	471(24)
	100	191(33)	7.72(0.16)	404(150)	52(22)	894(251)
	Cu (ug/L)					
	6	12(1)	8.19(0.03)	108(12)	118(1)	435(165)
	12	26(6)	8.24(0.03)	112(11)	116(1)	278(9)

[a] Standard error in parenthesis.

Table C6. Water chemistry measurements, means (SE) from 30-d artificial stream exposures to copper sulfate at the Clinch River Plant (August 31-September 30, 1990).

Treatment	Cu, ug/L, (n=11)	pH	(n=6) Conductivity, umhos/cm	Alkalinity, mg/L	Hardness, mg/L
Control	3.5(0.3)	8.5(0.0)	326(8)	138(3)	170(4)
Control B	3.1(0.4)	8.5(0.0)	325(9)	139(2)	170(4)
12A	13.4(1.7)	8.5(0.0)	325(9)	140(3)	174(4)
12B	14.0(1.8)	8.5(0.1)	323(10)	140(3)	170(4)
12C	7.8(1.5)	8.5(0.0)	326(9)	139(3)	170(4)
17A	20.0(3.9)	8.5(0.0)	326(9)	140(2)	173(4)
17B	18.5(1.9)	8.5(0.0)	326(9)	138(3)	170(4)
35A	31.5(1.4)	8.5(0.0)	327(9)	138(3)	173(4)
35B	26.2(1.4)	8.5(0.0)	328(8)	138(3)	167(4)
75A	54.6(10.1)	8.5(0.0)	326(9)	138(2)	170(4)
75B	56.4(6.9)	8.5(0.0)	326(9)	139(2)	173(4)

Table C7. Means (SD) of water chemistry measurements from 114-day artificial stream study at the CRP (n=9; June 13-October 15, 1989).

Treatment, ug/L	pH	Conductivity umhos/cm	Alkalinity, mg $CaCO_3$/L	Hardness, mg $CaCO_3$/L
Control	8.38 (0.09)	279(32)	132(12)	158(14)
8 Cu	8.39 (0.09)	281(29)	132(13)	152(16)
17 Cu	8.37 (0.06)	278(30)	132(13)	154(25)
24 Zn	8.07 (0.24)	821(436)	83(38)	488(262)
47 Zn	8.21 (0.29)	455(358)	112(45)	256(209)
17 Cu + 472 Zn	8.38 (0.20)	283(30)	132(11)	156(16)

APPENDIX D: CORRELATION

Table D1. Pearson Correlation Coefficients for measurements of water column Cu concentration (Wat Cu), *Leptoxis* Cu burden (*Lep* Cu), *Leptoxis* cellulolytic activity (cell) and periphyton Cu concentration (Peri Cu), from artificial stream studies conducted at the CRP Summer, 1988.

Study	Wat Cu vs Cell	Snail Cu vs Cell	Peri Cu vs Cell	Wat Cu vs *Lep* Cu	Wat Cu vs Peri Cu	Peri Cu vs *Lep* Cu
14-d CRP Effluent Treatment (n=5)	-0.5742	-0.4336	-0.5825	0.5280	0.9973*	0.4698
20-d CRP Effluent Treatment (n=5)	-0.7552	-0.2793	-0.8578	0.4022	0.9575*	0.5350
30-d CRP Effluent Treatment (n=5)	-0.8262	-0.8149	-0.8890*	0.7692	0.9593*	0.7468
Combined data from CRP Effluent Studies (n=15)	-0.4996	-0.2210	0.9455	0.6755	0.9455	0.6520
14-d Cu Treatment (n=4)	-0.2464	-0.7822	-0.1421	0.7614	0.9623*	0.6192
20-d Cu Treatment (n=3)	-0.5443	-0.7880	-0.3450	0.0876	0.5996	0.8497
30-d Cu Treatment (n=3)	-0.1594	0.3846	0.0234	0.8500	0.9832	0.9318
Combined data from Cu Studies (n=10)	0.2126	0.0452	0.0161	0.3278	0.7646	0.4663
40-d In-river Caged Study (n=8)	-0.3473	-0.0119	-	0.6252	-	-

* $P<0.05$

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Environmental Chemistry
Water Pollution Control
Environmental Health Practices
Env. Health Field Experience
Air Pollution Principles
Animal Populations
Developmental Biology
General Ecology
Cell Biology
Organic Chemistry
Hazard Evaluation
Topics in Aquatic Ecology
Biochemistry
Limnology
Toxicology
Environmental Microbiology
Water Pollution
Env. Health Administration
Institutional Health
Biostat. and Epidemiology
Animal Ecology
Undergraduate Research
Vertebrate Zoology
Invertebrate Zoology
Genetics

PROFESSIONAL EMPLOYMENT:

Environmental Protection Specialist, Environmental Protection Agency, Washington, D.C. - June, 1992-present.

Provide technical support for water quality impact analyses in National Pollutant Discharge Elimination System (NPDES)permitting for industrial permits, and serve as technical liaison with the Office of Science and Technology.

Participate in workgroups, including: organize activities of Pesticides Data Subtask Group; review criteria documents; and assist activities of biocriteria workgroup and waste treatment industry workgroup.

Provide technical support for NPDES permits in which biocides are the pollutant of concern.

Respond to technical questions, including NPDES Permit review and comments, concentrating on issues of national importance or significant impact. Provide communication and coordination with other Divisions, Offices, Agencies, and interested parties.

Provide technical advice and audit expertise in performing Permit Quality Reviews at the state and regional level.

Manage extramural projects (contracts).

Senior Research Biologist, Wildlife International Ltd., Easton, Maryland - 1991-1992.

Functioned as a Study Director for freshwater and marine studies in a GLPlab using FIFRA,TSCA, OECDand EECguidelines. Responsible for developing protocols, coordinating studies with clients, supervising studies, and writing reports.

Responsible for supervision of two biologists (and any other personnel involved in studies).

Manipulated design of studies to maximize solubility and solve other problems specific to each compound; work closely with chemists and sponsors to determine design.

Graduate Research Assistant, Biology Department, VPI&SU, Blacksburg, Virginia - 1988-1991.

Responsible for set-up and maintenance of acute and chronic toxicity tests using molluscs, fish and daphnids. In addition to measurement of water chemistry parameters, effects measured included reproductive, growth and cellulolytic enzyme activity impairment. Effects were related to bioconcentration in organisms and food source.

Other responsibilities included assistance in research design, data analysis, field surveys, laboratory maintenance, quality control and training of staff.

Graduate Teaching Assistant, Biology Department, VPI&SU, Blacksburg, Virginia - Principles of Biology Lab, 1987-1988.

Duties included lecture and exam preparation, laboratory supervision and assignment of grades.

Chemist, Tri State Analytical Laboratory, Kingsport, Tennessee, 1986-1987.
Responsible for analyzing drinking water, industrial and domestic wastewater, process waters, toxic wastes and other materials, and interpreting results for clients.

Initiated and maintained a Toxicity Test Program.

Supervised technicians and trained new chemists.

Laboratory tests included: EPTOX (water extraction, oil oxidation and metals analysis), Hg, As, Se (atomic absorption analyzer - sodium borohydride and cold vapor methods), other metals (digestion and atomic absorption analysis), organic compounds (gas chromatograph), total organic carbon (TOC analyzer), total and fecal coliform (membrane filtration method), oil and grease (partition-gravimetric method), chlorides (argentometric method, potentiometric method), acute toxicity (24-hr static, non-renewal toxicity texts using fathead minnow, *Pimephales promelas*), fluoride, ammonia, and nitrate nitrogen (specific ion probe/meter), total kjeldahl nitrogen (digestion and titration), phenols (distillation and chloroform extraction method), sulfates (gavimetric method with ignition of residue), sulfites (iodometric method), sulfides (pretreatment and iodometric method), solids and moisture (gravimetric methods), dissolved oxygen and biochemical oxygen demand (probe/meter), cyanide (gas distillation and colorimetric analysis), alkalinity and hardness (titration, corrosivity (calculation), total phosphorous (digestion and colorimetric analysis), orthophosphate (filtration and colorimetric analysis), flashpoint (closed-cup method), surfactants (methylene blue method), chemical oxygen demand.

Chemist Assistant, City of Lynchburg, Virginia, 1983-84

Responsible for sample collection and routine wastewater analyses for the City's wastewater treatment plant. Coordinated with state officials, the initiation of a chlorine reduction/fecal coliform study at the treatment plant in response to an effort by the state to reduce chlorine levels in the James River.

Laboratory tests included biochemical oxygen demand, chemical oxygen demand, residual chlorine, fecal coliform and various solids analyses.

Laboratory Technician, City of Kingsport, Tennessee, 1981

Operated the City's wastewater treatment plant laboratory; including collection and analysis of samples, maintenance of records, preparation of reagents and maintenance of laboratory stocks.

Laboratory tests included biochemical oxygen demand, alkalinity, volatile acids, fecal coliform and various solids analyses.

Research Assistant, Department of Environmental Health, East Tennessee State University, Johnson City, Tennessee, 1979-80.

Operated two laboratory-scale activated sludge units used in treating coal gasification wastewater; including analysis of wastewater at various stages of treatment and calculation of flow rates, sludge wastage rates and aeration rates for maximization of treatment efficiency.

Determined the biokinetic coefficients of the activated sludge phase of the treatment.

Analyses included dissolved oxygen, biochemical oxygen demand and volatile suspended solids.

Contributed to the writing of a report on the treatability of the wastewater, published by the United States Department of Energy.

Substitute Teacher, Hancock County and Sullivan County School Systems, Tennessee, 1982-83 and 1985.

PUBLICATIONS:

Reed, DK, JL Farris, DS Cherry and J Cairns, Jr. (In preparation) Acute Toxicity, Functional Impairment and Recovery of the Snail, *Leptoxis praerosa* from Copper-Dominated Effluent Exposures. Presented at the 10th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Toronto, Canada - 11/89. Submitted to Environmental Pollution, 1991.

Reed, DK, JL Farris, DS Cherry, EP Smith and J Cairns, Jr. (In preparation) A Comparison of Habitat Preferences between the Snails, *Leptoxis praerosa* and *Pleurocera unciale* in the Clinch River, Virginia. Presented at the 38th Annual Meeting of the North American Benthological Society, Blacksburg, Virginia - 5/90.

Reed, DK, JL Farris, DS Cherry and J Cairns, Jr. (In preparation) Functional Responses in *Leptoxis praerosa* from Long-term (114-D) Exposures to Ambient Cu and Zn Water Quality Criteria Concentrations.

Presented at the 11th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Washington, DC - 11/90.

DK Reed, JL Farris, DS Cherry and J. Cairns, Jr. (In preparation) Effects of Adherent and Water-borne Metals on Cellulolytic Enzyme Activity.

Presented at the 12th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Seattle, Washington - 11/91

Belanger, SE, DH Davidson, JL Farris, DK Reed and DS Cherry (In preparation) Effects of Cationic Surfactant Exposure to a Bivalve Mollusc in Stream Mesocosms.

MEMBERSHIPS:

Society of Environmental Toxicology and Chemistry

North American Benthological Society

Phi Sigma Biological Honor Society

Phi Kappa Phi Educational Honor Society

HONORS AND AWARDS:

Recipient of two departmental instructional fee scholarships

Recipient of two scholastic scholarships

Recipient of High School Salutatorian Award

REFERENCES:

Available upon request.