Effects of Copper on Benthic Communities in Artificial Microcosms

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Due to perceived shortcomings in some aspects of hazard assessment for chemicals administered to aquatic systems, experiments were conducted to determine the effects of copper on various ecological parameters in artificial streams and microcosms. Effects investigated were colonization and growth of periphyton communities in artificial streams, community composition and nutritional content of periphyton in these streams, leaf conditioning and nutritional content in laboratory microcosms, and nutritional suitability of these leaves to a shredding macroinvertebrate.

Main effects observed in periphyton growth experiments were significant reduction in substrate colonization due to copper treatment, which led to significantly lower standing biomass in treated streams. Observed growth rates were generally similar between control streams and copper treated streams. Light treatments did not affect periphyton

responses to copper. Community composition of the periphyton was affected by 2.5 ug/L copper. Periphyton phosphorus and nitrogen contents were not affected by copper treatment.

Conditioning rate of leaves was significantly decreased by 50 ug/L copper treatments in some cases. Leaf phosphate and nitrogen contents were not significantly affected by copper treatment. Significant differences in nutritional suitability of copper-treated leaves to a shredding macroinvertebrate were not detected.

These experiments suggest that aquatic primary producers are more sensitive to copper than heterotrophs. Additionally, adverse effects on periphyton were observed at concentrations well below measures of chronic toxicity to organisms dependent on periphyton as a trophic resource and the chronic criteria for copper. As such, greater emphasis should be placed on the sensitivities of periphyton communities in future copper criteria determination.

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Table of Contents

List of Figures

List of Tables

Chapter 1. Introduction and Literature Review

A. General considerations concerning aquatic ecosystems.

All organisms have physiological needs for water. Developed human societies are dependent on additional uses of water, and all early societies were centered on riverine systems. This occurred, whether intentionally or not, for several reasons. The effort needed to acquire the two liters of water humans require daily for survival was reduced. Also, any wastes which entered the aquatic system were removed from the immediate area. As the human population has grown in the relatively short period since the Industrial Revolution, per capita supplies of potable water have declined. Vallentyne (1972) calculated that per capita requirements of water in developed countries (for industrial and agricultural uses) is now about three orders of magnitude greater than physiological needs. Wetzel (1978) projects that acquisition of fresh water will soon be the foremost environmental resource limitation on humans.

As the human population grows, greater demands are placed on ecosystem resources. Because these resources are

finite, it is essential to determine the limits to which they can be sustainably exploited (*sensu* Foy, 1990). Ecosystems have differing capacities for assimilating anthropogenic stresses (Cairns, 1977). Underestimation of these capacities leads to underutilization and inefficient use of available resources, with losses of economic development opportunity, although the integrity of the ecosystem is assured. Overestimation can lead to biotic impoverishment or possibly diminution of other services in the future at the cost of short term gains in economic or development opportunities.

The goal of environmental protection should be the maintenance and protection of ecosystem structure and function. Ecosystem function is a result of the biological processes of their structural components. Three relationships exist between the structural and functional attributes of aquatic systems (Cairns and Pratt, 1986): 1) the two parameters are so intimately linked that they are inseparable, with any change in one leading to an alteration of the other; 2) the functional redundancy of aquatic communities will result in little change in functional attributes despite alterations of structural components; and 3) the functional capacities of stressed communities may be altered without changes in their structure. In studies conducted in lentic systems, contradictory evidence concerning the sensi-

tivity of structural versus functional attributes is available. Schindler (1987) reviewed studies conducted at the Experimental Lakes Area in northwestern Ontario, and concluded that ecosystem functions (i.e. primary production, nutrient cycling, respiration) were not altered at stress levels (including eutrophication, acidification, or cadmium addition) which induced changes in phytoplankton communities. Alternatively, in estuarine mesocosms, Cooper and Copeland (1973) found that changes in system productivity and respiration were more sensitive indicators of stress than species composition.

An assumption is made by regulatory agencies that by protecting the structural integrity of biological communities, functional attributes of these communities will be maintained (Cairns, 1988). However, these criteria are designed to maintain species diversity by providing protection to the majority of species inhabiting that system. In other words, by ensuring that individual species are protected, species assemblages and communities in those systems will be protected, and hence ecosystem function will be maintained.

The term *stress* has been defined in several ways in relation to disturbed ecosystems. Odum (1985) used stress to describe inputs to a system which resulted in negative effects upon that system, although this definition requires

subjective decisions on the part of the observer. Pratt (1990) defined stress in a more objective manner as a disorganizing influence, or anything which results in a deviation from the nominal state of the system. This definition, which would also include inputs described by Odum (1985) as subsidies, is similar to the definition of perturbation given by Odum *et al.*, 1979).

Regardless of the definition employed, ecosystem parameters are adversely affected by stress (excluding perturbation-dependent systems; Vogl, 1980), and the effects are often related in some manner to the magnitude of the stress. There may be threshold levels of stress below which an ecosystem may not be visibly impacted, although this is not always the case (Woodwell, 1975). Attributes that can be affected include structural parameters such as species diversity and community composition, and such functional parameters as productivity and nutrient cycling (Cairns and McCormick, 1991). Conventional hazard assessment, as stated previously, implicitly assumes that these parameters can be protected based upon evidence from simple toxicity tests (also see Cairns and Pratt, 1989). However, it should be clear that this is an oversimplification, and that ecosystems cannot be reduced and analyzed based on a few simple parameters, as much as it is desirable to do so (Cairns, 1985).

The use of microcosms as ecosystem surrogates involves several assumptions, as outlined by Pratt and Bowers (1990). Source ecosystems are assumed to have functional equivalence mediated by characteristic community components, and the implicit assumption is made that functional processes of the ecosystem susceptible to stress will behave similarly in a microcosm. The value of such systems, however, is emphasized by Wevers *et al.* (1988). It simply is not feasible economically or environmentally to test each of the estimated six million chemicals known using field tests in real ecosystems, much less all of the possible combinations of these chemicals.

B. Hazard assessment protocols for aquatic systems.

Current management of our aquatic resources is conducted according to the Resource Conservation Ethic, which attempts to derive the greatest benefits from a resource for the most people for the longest time (Callicott, 1991). Criteria for the release of hazardous materials into aquatic environments are devised with the ultimate goal of protecting human health and those species of direct or indirect importance to humans (Stephan *et al.*, 1985). These criteria are established in part by determining the toxicity of materials to a range of organisms under laboratory conditions

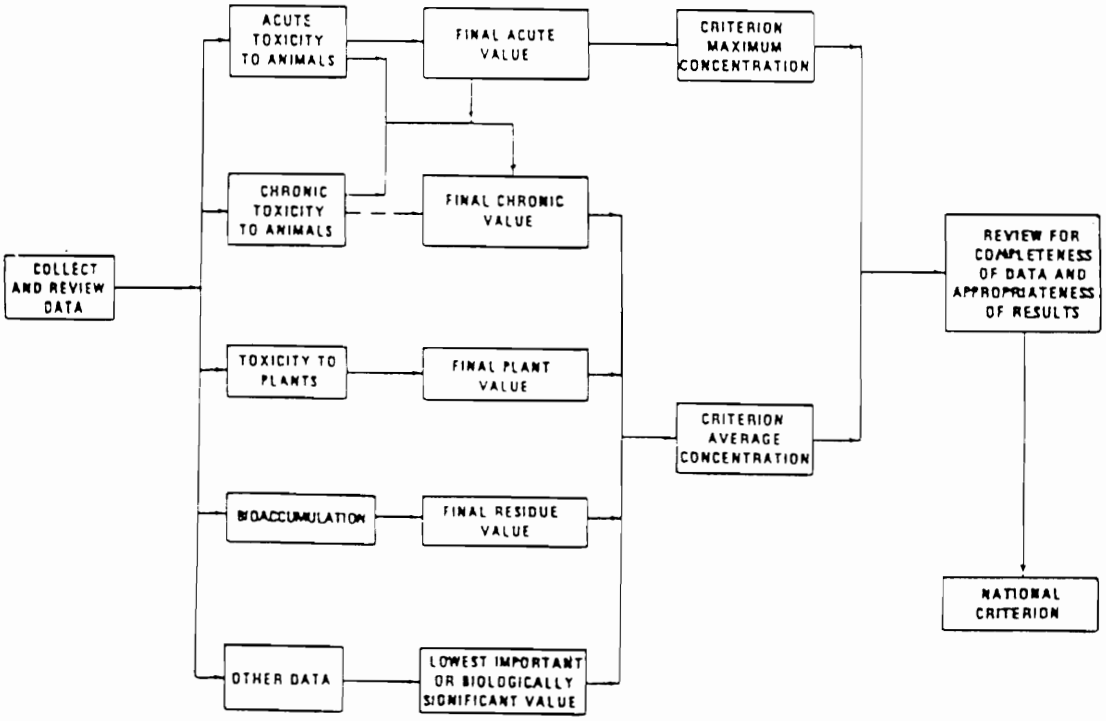

Figure 1.1. Scheme of mechanism for determination of numerical Water Quality Criteria (from Stephan et. al., 1985).

and extrapolating these results to all organisms in the environment. A schematic of the processes leading to the derivation of numerical water quality criteria (Fig. 1.1, from Stephan *et al.*, 1985) illustrates how these values are determined through the use of single species toxicity tests. Results of acute animal toxicity tests are used to set criterion maximum concentration levels. Chronic toxicity tests with animals and plants, bioaccumulation tests, and other relevant data are used to set criterion average concentration levels. The National Criterion is defined in terms of both the criterion maximum concentration and the criterion average concentration. The validity (or lack thereof) has been discussed by Cairns (1988). These tests typically lack environmental realism and therefore may not be accurate predictors of ecosystem responses. Rai *et al.* (1981), in an extensive review of algal responses to heavy metals, strongly criticized the use of single species tests for water criteria determinations because of the range of responses by different algae to a singular toxicant. They suggest that such determinations be made with natural assemblages or communities, and noted that this is done very infrequently. They also criticized the abundance of simple investigations which provide little information concerning the role of environmental factors in field toxicity determinations.

A further criticism is the inherent reliance on direct toxicity to animals. It has not been demonstrated that these estimates are the best predictors of ecosystem response. Indirect mechanisms, such as reductions in trophic production or food quality (except bioaccumulation) due to a toxicant, are not directly considered. It is stated that species of indirect importance should be protected, but the extent of this indirectness which must be considered (i.e., trophic distance in ecological relationships) is not defined. Although plant toxicities are considered, the dependence of higher trophic levels on the maintenance of aquatic primary producers (and also microbial heterotrophs) does not receive proportional consideration, despite the overwhelming significance of both of these groups to aquatic ecosystem energy flow. Wang (1985) proposed an alternate scheme which would give greater consideration to aquatic primary producers and also include effects on microorganisms (Fig. 1.2). Although this hazard assessment system would give greater consideration to aquatic productivity, Wang also proposed that the most sensitive organism be used to set criteria. The untenability of this approach was discussed by Cairns (1983).

Copper is ubiquitously present in both natural and human environments. It is required as a micronutrient by virtually all organisms, but causes deleterious effects at

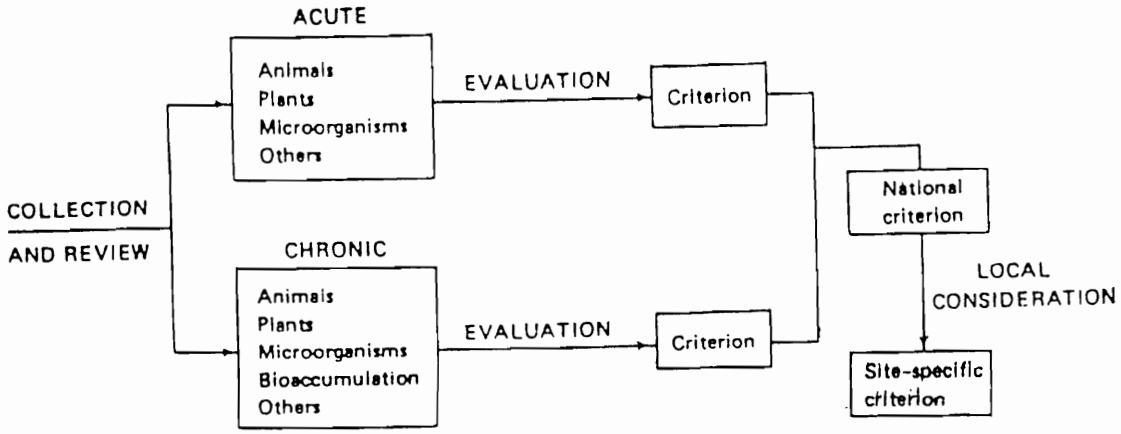

Figure 1.2. Scheme proposed by Wang (1985) as an alternate for use in derivation of national water quality criteria. Note inclusion of microorganisms in the evaluation process.

elevated exposures . These toxic effects are more frequently observed in aquatic organisms than in terrestrial ones due to greater exposure via the surrounding medium (Lewis, 1992). Unpolluted surface waters have concentrations of cupric ion (Cu^{++}), either free or complexed forms, generally ranging from 1 to 10 ug/L (Boyle, 1979), whereas aquatic systems either receiving anthropogenic inputs or disturbed due to human activities have concentrations much higher, sometimes in the mg/L range (Hutchinson, 1979). It must be pointed out, however, that such concentrations can be encountered naturally in regions rich in copper ore. In addition to its uses in the electronics and construction industries, copper is also used commercially as an herbicide, fungicide, and nematodicide. Impacts of these latter uses include toxic effects on non-target organisms in treated systems and export to other systems. As such, attempts to treat the symptoms of one problem (algal blooms due to eutrophication, for example) can lead to further problems.

The United States Environmental Protection Agency reviewed available literature pertaining to the effects of copper on aquatic organisms, and (through the use of Stephan *et al.*, 1985) has set national criteria for allowable concentrations of introduced copper in aquatic systems (USEPA, 1984). Two values are given. The first value, termed the

chronic criteria, is defined as the maximum average four-day concentration which cannot be exceeded more than once every three years. The second value, termed the acute value, is defined as the maximum average one-hour concentration which cannot be exceeded more than once every three years. These values are calculated as

$$C_C = e^{(0.8545[\ln(\text{hardness})] - 1.465)}$$

and

$$C_A = e^{0.9422[\ln(\text{hardness})] - 1.464},$$

where hardness is water hardness in mg/L as $CaCO_3$. It is stated (EPA, 1985) that "freshwater aquatic organisms and their uses should not be affected unacceptably" if these guidelines are met. As mentioned previously, these criteria are set after analysis of data from single-species toxicity tests. The mechanism of this process (Fig. 1.1) and terms used in definitions deserve closer scrutiny.

C. Review of literature concerning environmental toxicity of copper to aquatic autotrophs and heterotrophs.

Numerous studies have been conducted concerning the toxicity of copper to algae. Most studies in lotic systems did not incorporate continuous monitoring of copper concentrations, but relate alterations of biological parameters (i.e., changes in species abundances or standing biomass) to either instantaneous measurements of copper or a

few such measurements. Other studies concern measurements of responses to instantaneous increases in copper concentrations (i.e., changes in rates of photosynthesis due to metal exposure) which may not be suitable predictors of responses to chronic exposures. Laboratory studies are usually conducted *in vitro* with a single species (virtually always phytoplanktonic), and the media used are often not suitable for predicting responses in natural waters. Many studies have been conducted on the toxicity of copper to aquatic macroinvertebrates and fish. In contrast, very few studies have been conducted concerning the responses of lotic microbial heterotrophs to introduced copper. The following sections review literature which are applicable to understanding the responses of algae, microbial heterotrophs, and primary consumers of these groups to copper.

i. Effects of copper on algae.

Rai *et al*. (1981) conducted an extensive review of algal responses to heavy metals, concentrating on environmental studies. They concluded that a number of environmental factors, including pH, water hardness, phosphorus concentration, and trophic conditions greatly affect toxicity thresholds for algae. Reed and Gadd (1989) reviewed the mechanisms of metal tolerance by both eukaryotic and prokaryotic algae. They concluded that

organometallic compounds often had higher toxicity to algae than the free ion, due to increased mobilization of the metal across the cell wall (also see Stauber and Florence, 1987). Among the metals, the order of toxicity was generalized to be Hg > Cu > Cd > Ag > Pb > Zn. This is in contrast to Bringmann and Kuhn (1980), who found the order for *Scenedesmus quadricauda* to be Ag (9.5 ug/L) > Be = Cd (31 ug/L) > Hg (70 ug/L) > Cu (1.1 mg/L) > Pb (3.7 mg/L). Finally, they outlined and discussed four major specific and indirect mechanisms by which algae tolerate heavy metals (also reviewed to a lesser extent by Rai *et al.*, 1981): 1) extracellular binding and precipitation; 2) impermeability and exclusion; 3) internal detoxification; and 4) metal transformations. The final mechanism, which occurs by either reduction of a cation to elemental form (as with mercury, followed by volatization) or methylation (with As, Cd, Hg, Pb, Se, and Sn) is probably confined to prokaryotes, and does not seem to occur with Cu. Extracellular binding and precipitation can occur through production of organic ligands, siderophores, or anionic polysaccharides and mucilages such as carageenan. Internal detoxification was sometimes related to production of metallothionein-like proteins and in other cases to formation of polyphosphate bodies.

Leland and Carter (1985) examined the effects of copper on periphyton production, nitrogen fixation, and processing

of leaf litter in copper-dosed stream channels. Water hardness in this system was calculated to be 57 mg/L as $CaCO_3$ (by JLC from data in Leland and Carter, 1984; formula from APHA, 1992) with slightly alkaline pH. Nutrient levels were generally low (less than 10 ug/l NO_3^--N and soluble phosphate less than 0.2 ug/L PO_4^{3-}-P). The channels were continuously dosed for one year. Autotrophic productivity was reduced by 60-80% in reaches dosed with 2.5 ug/L as compared to controls depending on season, with greater reductions at higher copper concentrations. Heterotrophic production was affected to a lesser extent, with reductions between 30-60% as compared to controls. These communities did not acclimate to the copper stress, with inhibitions persisting throughout the experiment. Production in previously dosed stream reaches rose to match control levels after four weeks following cessation of copper additions, although communities dosed at 10 ug/L required seven weeks to recover to control levels. Chlorophyll-specific productivity rates declined in all treatments, although rates on newly colonized surfaces were consistent among treatments. This was attributed to colonization by copper-resistant species, especially *Achnanthes minutissima*. Nitrogenase levels were not impacted at 2.5 ug/L, although reductions were observed at higher concentrations. Leland and Carter (1984) examined the effects of copper on species composition of periphyton

in these same streams. The numerically most abundant taxa were nine diatoms and the cyanophycean *Lyngbya*. The chlorophytes *Spirogyra* and *Cladophora* were also present. *Lyngbya* was substantially reduced in abundance at 2.5 ug/L Cu^{++}, and the chlorophytes and one diatom reduced in abundance at 5 ug/L. In 10 ug/L treatments, 16 of the 22 species dominant in control reaches were reduced in abundance. Cell abundance (total individuals of all taxa) was not affected by copper treatments. *Achnanthes minutissima*, which was a co-dominant in control reaches, was the primary replacement species. In 5 ug/L reaches, *Ceratoneis arcus*, *Cocconeis placentula*, *Navicula* spp. and *Synedra rumpens* were also more abundant than in controls. Only *A. minutissima* and the cyanophycean *Calothrix* spp. were more abundant at 10 ug/L than in controls. Clark *et al.* (1982) investigated effects of 50 ug/L Cu^{++} on periphyton communities in artificial streams, and found that copper treatment shifted community composition from diatoms (*Melosira*, *Nitzschia*, and *Cymbella*) in control streams to dominance by Cyanophyceae (*Oscillatoria*, *Nostoc*).

Crossey and La Point (1988) compared structural and functional responses of periphyton to mine drainage containing several heavy metals. They determined that structural measurements (biomass, species richness) could be better determinants than functional measurements (gross primary

productivity) because of high variability associated with functional measurements.

Pratt *et al.* (1987) studied the effects of copper on protozoan colonization. They found that colonization rates were not significantly different from control rates at 6.6 ug/L, but were reduced at 12.7 ug/L. Water quality parameters (hardness, alkalinity, and pH) were similar to those in this study.

Januszko (1976) treated experimental carp ponds with a total of 71 ug/l of copper over five dosings in addition to nitrogen and phosphorus. Algal production was approximately twice as high in the copper treated ponds as compared to control ponds which received identical nitrogen and phosphorus treatments. The increased production was due mainly to increases of diatoms (*Stephanodiscus hantzschii* and *Melosira granulata*) and green algae (*Chlorella minutissima*), although these species were also dominant in the control ponds. The author attributed the increase to remediation of copper deficiency in the ponds, although evidence to support this claim was not presented. The author did note that the initial copper dosing of the ponds failed to bring about rapid growth of algae.

Nielsen and Laursen (1976) found that copper decreased the photosynthetic rates of phytoplankton samples from three Danish lakes. The reduction was dose-dependent, and gener-

ally greater during the summer. In a eutrophic lake studied simultaneously, addition of 25 ug/l stimulated photosynthetic rates. The authors attributed this to the high concentration of humic materials dissolved in the water of this lake, assuming that the phytoplankton of this lake were normally limited by copper. These results are similar to those of Januszko (1976).

Bringmann and Kühn (1980) conducted tests comparing the toxicity of 168 different potential water pollutants to three aquatic microorganisms. Organisms tested were a bacterium (*Pseudomonas putida*), a green alga (*Scenedesmus quadricauda*), and a protozoan (*Entosiphon sulcatum*). The endpoint used was observable reduction in cell division. For the metal cations tested (Ni, Ag, Hg, Cu, Be, Cd, and Pb), the alga had sensitivities intermediate to the other organisms for all metals tested. However, these results are misleading, because the organisms were not tested in the same culture medium. Hardnesses calculated (by JLC; formula from APHA, 1992) for the three waters were 81 mg/L as $CaCO_3$ for bacterial tests and 137 mg/L as $CaCO_3$ for protozoan tests, while the alga was tested in a culture medium having calculated hardness of 533 mg/L as $CaCO_3$. In his review, Whitton (1984) stressed how algal uptake of metals was inversely proportional to water hardness, numerous other researchers e.g., Pellegrini *et al.*, 1994) have demonstrated

reductions in metal toxicity associated with increased Ca^{++} concentrations. As such, these results may not be useful for comparative toxicity studies.

Tubbing *et al.* (1994) investigated the toxicity of chelated copper to *Selenastrum capricornutum* and planktonic aquatic bacteria in artificial water and Rhine River water. The artificial water (Dutch Standard Water) had a calculated hardness of 136 mg/L as $CaCO_3$; other water quality parameters for this water and the Rhine water were not reported. The bacteria were not identified. Algal photosynthesis was inhibited 50% by 325 ug/L copper, even when supplemented with equal or double molar concentrations of EDTA. Bacterial reproduction was similarly reduced by 12 ug/L in Rhine water without the addition of EDTA. In both cases, free cupric ions were not detectable by differential pulse anodic stripping voltametry.

Stauber and Florence (1987) tested the toxicity of ionic copper and copper complexes to two marine diatoms (*Nitzschia closterium* and *Asterionella glacialis*) and a freshwater green alga (*Chlorella pyrenoidosa*). Copper ions reduced cell division and photosynthesis in *A. glacialis* and *C. pyrenoidosa* (50% reductions in cell division rates at 100 and 50 ug/L, respectively), while this same reduction in cell division in *N. closterium* (at 175 ug/L) did not affect photosynthesis, respiration, ATP production, electron trans-

port, or membrane ultrastructure. The authors inferred that the mechanism of toxicity was therefore due to suppression of mitosis through disruption of internal glutathione metabolism. Additionally, the copper complexes exhibited greater toxicities than the cupric ions, possibly from enhanced mobilization of the material through the algal plasmalemma. Other researchers have found different mechanisms for copper toxicity. Garman *et al.* (1994) found that nuclear migration during gametophyte development of the giant kelp *Macrocystis pyrifera* was significantly decreased at 20 ug/L Cu^{++}. However, Wong *et al.* (1994) found that chloroplasts were the most sensitive organelle in *Chlorella fusca*.

Sicko-goad and Stoermer (1979) found that exposure of a diatom to copper induced a reduction in the number of polyphosphate bodies formed during luxury uptake, but did not morphologically affect cellular organelles. They interpreted this as meaning that copper was incorporated into the polyphosphate bodies as a means of detoxifying intracellular metals. Twiss and Nalewajko (1992) studied the interactions between phosphorus and copper toxicity in three strains of the green alga *Scenedesmus acutus*. They found that cells with higher phosphorus contents showed less depression of photosynthesis following copper exposure. However, they suggested that polyphosphate played only a passive role in copper detoxification. Verma *et al.* (1993) found that the

toxicity of 190 ug/L Cu^{++} (LC_{50}) to *Nostoc calcicola* was decreased by the addition of 3 mM phosphate (285 mg/L).

Cembella *et al.* (1984) conducted an exhaustive review of the roles of phosphorus in algal ecology. This review tabulates the eukaryotic algae known to produce polyphosphates. Members of nine classes are included, with Bacillariophyceae (five species), Chlorophyceae and Charophyceae (25 species), and Rhodophyceae (six species) being classes with the most representatives.

Rose and Cushing (1970) found that metal uptake by natural periphyton mats occurred at the upper surfaces of the mat, and a diffusion gradient existed within the community. Lower concentrations of metal were found in the bottom portion of the periphyton mat. Rai *et al.* (1981) found that heavy metal toxicity could be density-dependent, due to increased cellular binding sites at higher population densities.

ii. Effects of metals on stream microbial heterotrophs and macroinvertebrate consumers.

Bringmann and Kuhn (1980) found that the bacterium *Pseudomonas putida* was more susceptible to Ni, Ag, Hg, and Cu than the green alga *Scenedesmus quadricauda* or the protozoan *Entosiphon sulcatum* when division rates were studied. Hornor (1984) found that while artificial streams treated by

addition of 22 ug/L Zn^{++} (as compared to reference stream concentrations of 20 ug/L) actually increased the number of detectable bacteria as compared to controls, microcosms treated with 26 mg/L Zn^{++} consumed 31% less O_2 than reference microcosms. It is possible that in the first case bacteria-consuming protozoans were substantially reduced by the treatments, allowing for higher bacterial populations in the treated streams, although the author did not discuss this. However, results from the microcosm experiments suggest that aquatic microorganisms (bacteria and fungi) are far more resistant to heavy metals than other organisms due to the relatively small decrease in respiration observed with these extremely high doses.

Leland and Carter (1985) examined the effects of copper on periphyton production, nitrogen fixation, and processing of leaf litter in copper-dosed stream channels. Heterotrophic production was affected to a lesser extent, with reductions between 30-60% as compared to controls. Leaf litter processing was affected at all copper concentrations tested (minimum nominal dosing, 2.5 ug/L). Microbial respiration on leaf matter and C:N ratio of the leaf material was significantly lower in treated channels than in controls. These communities did not acclimate to the copper stress, with inhibitions persisting throughout the experiment. In further data from this same study, Leland *et al.* (1989)

examined effects on aquatic insects. Of the 37 most abundant taxa, none declined in abundance at continuous exposure to 2.5 ug/L copper. Nineteen of the species declined at 5 ug/L, and 28 declined at 10 ug/L. Population densities of herbivores and detritovores were more susceptible than predators, although annual production of all guilds was depressed. The authors suggested that predator production declined in response to decreased prey abundances.

Doherty *et al.* (1988) found that Asiatic clams (*Corbicula fluminea*) fed a diet of algae exposed to cadmium (algal concentrations not measured) did not exhibit increased mortality or altered siphoning rates, and visceral levels of metallothionein-like proteins doubled as compared to control clams.

iii. Factors affecting the quantity and quality of trophic resources to aquatic consumers: N content, PO_4 content, and Cu bioconcentration.

Steinman and McIntire (1990) reviewed literature concerning the recovery of periphyton communities from heavy metal stress, and concluded that recovery of these communities is crucial for the recovery of other trophic levels because of their importance as a food resource.

The single greatest factor affecting the type and abundance of algae in aquatic systems is the quantity and quali-

ty of light (Lee, 1989). Wootton and Power (1993) experimentally manipulated channels within a natural stream, varying the light intensity and number of trophic levels. They found that increased light resulted in increased algal productivity in units with three trophic levels (primary producers, grazers, and primary predators). Increased grazer biomass was not observed, whereas the biomass of primary predators did increase. When a fourth trophic level was added (another level of predation), grazer biomass increased while algal and primary predator biomass decreased.

Clark *et al.* (1982) investigated effects of 50 ug/L Cu^{++} on periphyton communities in artificial streams. Response variables examined included biomass, organic content, carbohydrate content, protein content, and community composition. Water chemistry parameters (hardness, alkalinity, and pH) were similar to those of the present study. Periphyton dry weight (g/m^2) was usually greater in treated streams than in controls, although AFDW (g/m^2) was lower. This may be attributable to higher diatom densities in these streams, as personal investigations have shown. In copper-treated streams, protein (2% NaCl soluble) on an areal basis did not differ significantly from control streams. Percent protein of the community (AFDW) was usually higher than in controls, although all values were between 3-8%. It must be pointed out that in the present study proteins quan-

tified were those soluble in 10% NaOH; it is possible that a typographical error existed in Clark *et al.*'s manuscript.

Paine and Vadas (1969) examined the calorific values for a number of marine algae, including Chlorophyta, Rhodophyta, Phaeophyta, and one Bacillariophyta. The diatom (*Nitzschia paradoxa*) had a substantially higher ash percent following combustion than all others (excluding calcareous thalli), yet had a higher caloric content (as kilocalories per unit ash-free dry weight). After consideration of the dietary preferences of a number of herbivorous marine invertebrates, they concluded that these preferences were based on availability of the food source rather than on discrimination based on nutritional content.

Corner and Cowey (1968) reviewed data regarding dietary requirements of marine zooplankton. As with many other studies, they emphasized the greater requirement of fixed nitrogen in zooplankton diets, with little emphasis on the requirements for phosphorus. It was pointed out, however, that the majority of phosphorus ingested by zooplankton was egested rapidly. Turnover rates (egested phosphorus versus body content) were often greater than once per day.

Haug *et al.* (1973) studied the chemical composition of phytoplankton communities in a fjord for 14 months. They found that phosphate averaged 0.83% of the dry weight for 25 sample dates, while nitrogen averaged 5.9%.

Cargill *et al.* (1985) found that shredding caddisfly larvae could discriminate between food choices based on lipid content (treatment). These preferences were only evident in last-instar larvae which have a greater need for triglycerides prior to metamorphosis and reproduction. Early instars did not exhibit preferential feeding.

Franke and Hillebrand (1980) cultured various algae at 650 ug/L and 6.5 mg/L copper. The copper bioconcentrations averaged approximately 300 ug/mg and 2000 ug/mg dry weight. Internal distributions of the metal varied between species, although the majority (46-74%) was concentrated within the cell wall. Cytoplasmic and vacuolar copper content accounted for the next greatest distribution in *Oedogonium*, *Draparnaldia*, and *Hormidium*, while *Ulothrix* and *Spirogyra* tended to concentrate it in the chloroplasts, nucleus, and mitochondria. Species showed differential responses to the toxicant.

Mierle and Stokes (1976) resolved the copper uptake by two species of *Scenedesmus* into two phases. The first phase, which occurred at a faster rate, was attributed to copper binding to the cell wall. The second phase, which occurred at a slower rate, was interpreted as transport across the plasmalemma, and was found to be inversely related to the calcium concentration of the culture media. Copper uptake was accompanied by loss of potassium ions, indi-

cating that plasmalemma permeability was affected. A copper tolerant strain did not accumulate copper in short-term exposures, and potassium loss did not occur. The authors suggested that enhanced exclusionary mechanisms in this alga were responsible. Additionally, the authors found that large amounts of copper were bound to cell walls in a non-inhibitory manner when light was excluded. During light treatment, the uptake of intracellular copper was greatly enhanced.

Foster (1977) found that *Chlorella* could exclude copper as a mechanism to reduce toxicity by working with strains from above and below inputs from a copper mine. It was found that tolerant strains grew comparably faster and had lower copper bioconcentrations than non-tolerant strains when cultured in various media amended with copper. It was also determined that this resistance had a genetic basis.

Whitton (1984) reviewed data concerning the relationships between algae and heavy metals in freshwater ecosystems. He found that algae generally concentrate metals at about three orders of magnitude over environmental concentrations, although values of up to 50,000 were not uncommon.

Gachter and Geiger (1979) conducted extensive experiments in limnetic enclosures testing the effects of added metals on uptake by phytoplankton, periphyton, and zooplankton. Concentration factors for the metals (Cu^{++}, Cd^{++}, and

Zn^{++}) varied independently and were not related to season. Algal concentration factors in the treated enclosures were generally between 2500 and 5000, while controls were in the range of 2500 to 60,000. Factors for Cd^{++} and Zn^{++} were approximately equal, although usually three to five times those of Cu^{++}. This implies greater uptake of Cu^{++} as compared to the other metals. They also determined that Cu^{++} uptake was linearly related to the aqueous concentration up to 45 ug/L during 24 hour incubations. Zooplankton concentration factors were about two orders of magnitude lower than algal concentration factors, with controls about twice those from treated systems. Cd^{++} factors for zooplankton were generally double those for the other two metals. Additional examination of all trophic levels found that algae had the highest metal concentrations, with zooplankton, chironomids, and *Sialis* lower, and the lowest concentrations in fish. As such, it was determined that these metals (and Hg and Pb, although data was not presented) do not increase in concentration up trophic levels.

D. Objectives

The present research was designed to address perceived shortcomings in the methods used to calculate national water quality criteria. These perceived shortcomings include the

lack of consideration of potential impacts on trophic resources such as aufwuchs production and detritus processing. If it could be shown that these ecological attributes are more sensitive to stressors than predicted by criteria, it would provide evidence for greater consideration of such functional measurements in criteria derivation.

The objectives of the studies were: 1) Determine the concentration of copper necessary to reduce periphyton production; 2) Determine the concentration of copper necessary to affect algal community composition; 3) Determine if the nutritional quality of periphyton is affected by copper; 4) Determine if copper toxicity is affected by irradiance levels; 5) Determine the relative sensitivity of leaf-processing heterotrophic microorganisms to copper, and determine if an aquatic macroinvertebrate consumer would be affected by any changes in food quality related to copper.

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Chapter 2. Effects of copper on periphyton growth in artificial streams

Introduction

Periphyton communities are complex assemblages of autotrophs and heterotrophs attached to the substrates of aquatic systems (Steinman and McIntire, 1990). These communities are often an important component of lotic food webs, and can provide a significant proportion of the trophic resources available to aquatic consumers (Cummins, 1974; Vannote *et al.*, 1980). Grazer densities are often related to algal densities (e.g., Richards and Minshall, 1988), and increased availability of periphyton as a food resource results in increased aquatic consumer abundance and survival (Mundie *et al.*, 1991). As such, effects on periphyton communities must be determined when evaluating potential environmental effects of a toxic compound. Despite the importance of these communities to the maintenance of higher trophic levels, they are not collectively considered in determination of Water Quality Criteria (Stephan *et al.*, 1985; USEPA, 1985). In the case of copper, autotroph values are not considered in Criteria calculation, due to both conflicting and incomplete data (USEPA, 1985).

Most toxicity tests on algae have been conducted using species that would be ecologically characterized as phytoplanktonic or lentic. In addition, these tests are conducted under static conditions, emulating lentic conditions. Extrapolation of these results to lotic conditions may not be warranted. The importance of water current to lotic algal physiology cannot be underestimated, as it increases diffusion gradients and exchange between periphyton mats and the water column (McIntire, 1966). Uptake rates of phosphate by periphyton are enhanced by current (Schumacher and Whitford, 1965; Lock and John, 1979). If uptake rates are proportional to dissolved concentrations, then it is reasonable to assume that uptake of other dissolved substances, including toxic materials, would be increased in a similar fashion.

An extensive literature review revealed that data concerning the effects of copper on periphyton growth under lotic conditions are largely lacking. The few experiments that have been conducted examined periphyton declines after exposure, but did not calculate growth rates, exclude effects due to grazing macroinvertebrates, or use a wide range of copper concentrations. As such, a significant gap exists in knowledge concerning the effects of copper on lotic periphyton dynamics.

The present experiments were designed to determine ef-

fects of copper on periphyton community growth dynamics and standing stocks in artificial streams, thereby addressing this gap.

Materials and Methods

Three experiments were conducted in artificial stream mesocosms in the Ecosystem Simulation Laboratory at Virginia Tech. Streams were fiberglass ovals 0.93 meters on the long axis, 0.63 meters on the short axis, with channel width of 23 centimeters (Figure 2.1). Standpipes in each stream were adjusted to maintain 32 liters of water in each stream (depth approximately 6 cm). Streams were supplied with 1 L/minute municipal tap water dechlorinated with activated charcoal (system schematic in Fig. 2.2). Input rates were adjusted volumetrically daily. Water hardness, alkalinity, and pH were measured every third day; mean values for all measurements over all experiments were approximately 50 mg/L $CaCO_3$, 38 mg/L $CaCO_3$, and 7.6, respectively (Table 2.1). Total dissolved orthophosphate levels (determined by ascorbic acid method; APHA, 1992) were approximately 0.85 mg/L PO_4^{3-}-P. Nitrate levels (determined by cadmium reduction method; APHA, 1992) were approximately 1.0 mg/L NO_3^--N. These high nutrient levels are a result of using a municipal

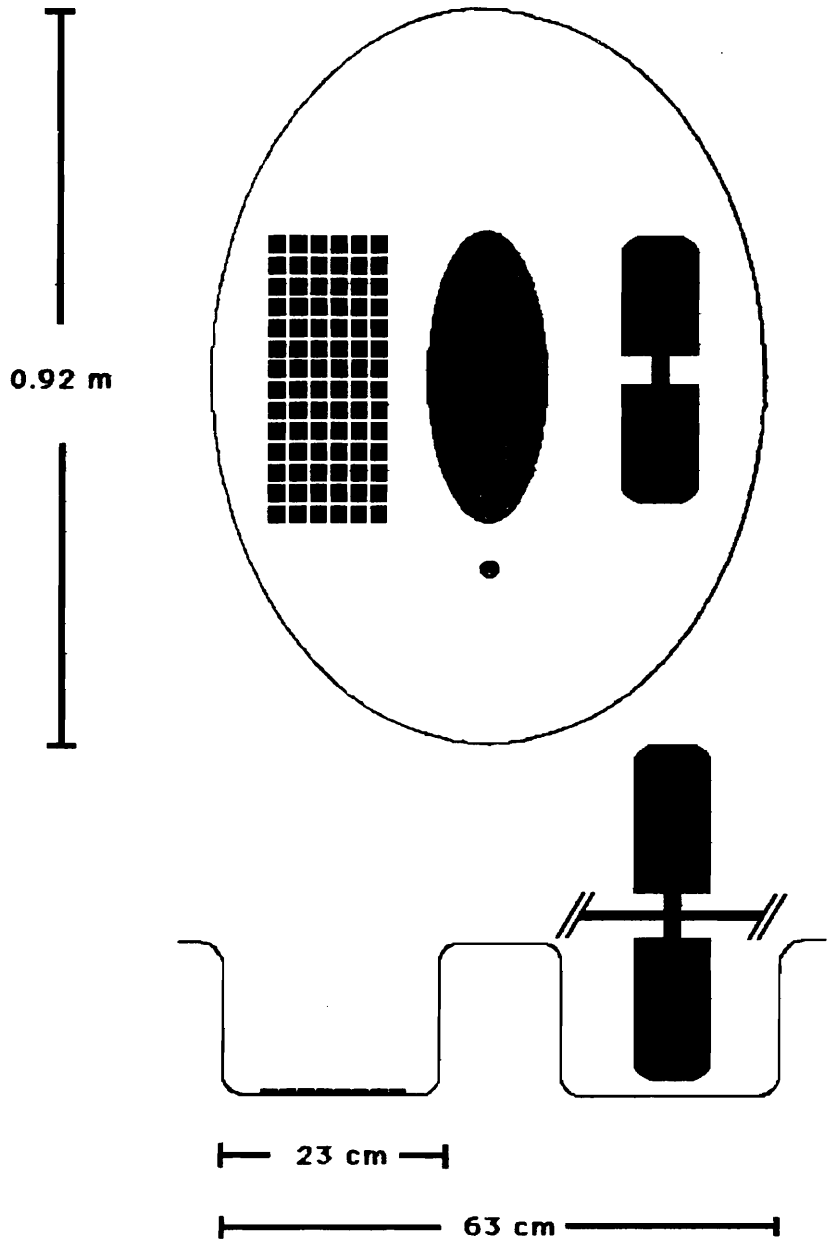

Figure 2.1. Overhead (top) and cross-sectional views (bottom) of an artificial stream used in this study. Shaded oval in upper illustration is the central hub of the stream, while the shaded circle is the location of the standpipe. Substrates (squares) and paddlewheel were located as indicated.

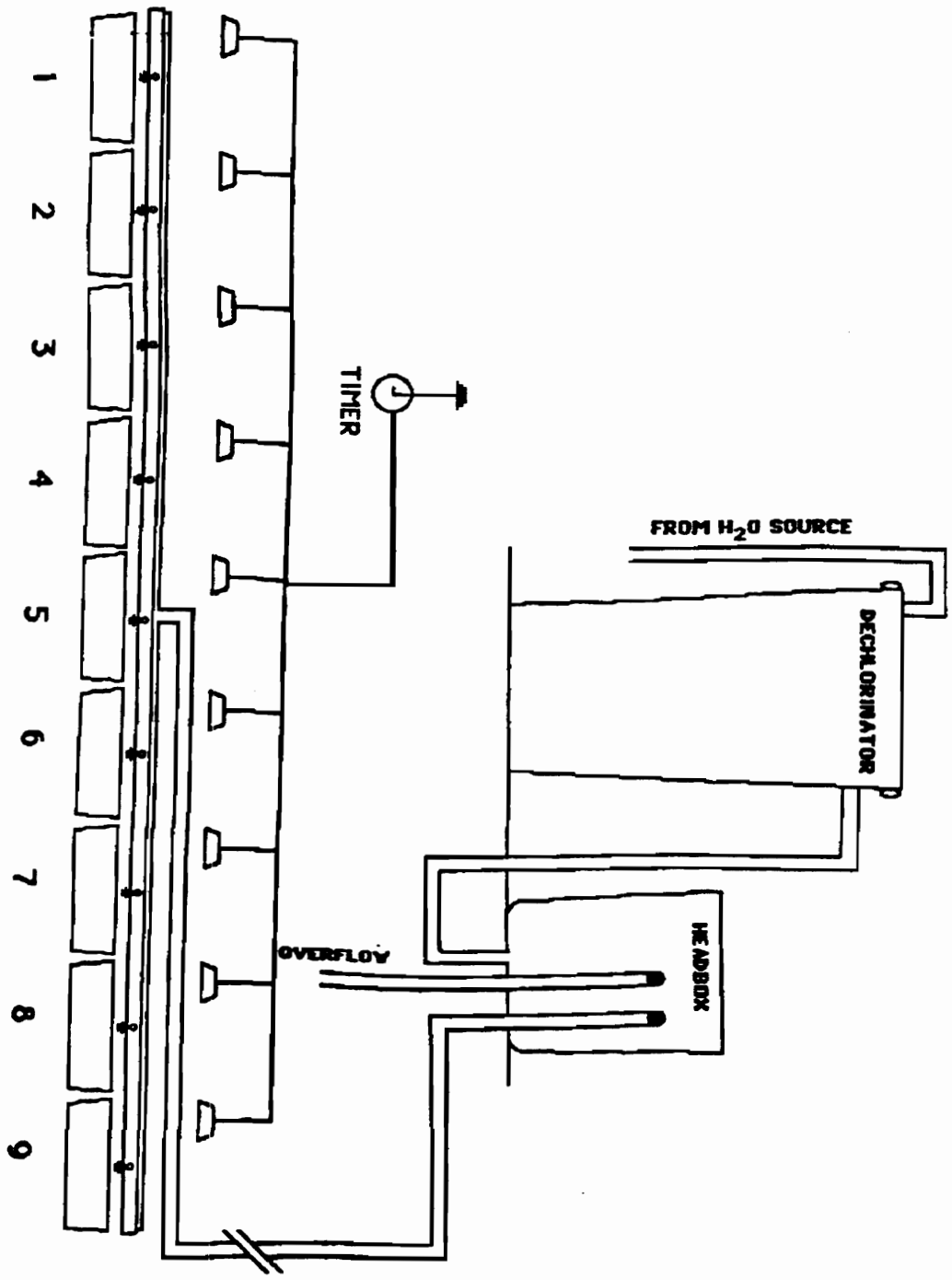

Figure 2.2. Schematic of artificial stream system used for periphyton growth experiments.

water supply which could not be avoided. Residual chlorine concentration was below detection using amperometric titration (< 0.01 mg/L; APHA, 1992). Ammonia concentration was below detection using the phenate method (< 10 ug NH_3-N/L; APHA, 1992). Stream flow was maintained at 15 cm/sec by motor-driven paddlewheels. Light (40 $\mu E/m^2/sec$) was supplied by full-spectrum Vita-Lites® on a 12:12 light-dark regimen.

Cu^{++} (as $CuCl_2$) was supplied to streams by peristaltic pumps. Concentrations of stock solutions were made considering the pump flow rates (ca. 0.75 ml/min) and stream water input rate. Pump rates were measured gravimetrically on a weekly basis by collecting toxicant outflow for 20 min and new stock concentrations were adjusted accordingly. Water column Cu^{++} concentrations samples were measured every fifth day during Experiment 1 and every third day in Experiments 2 and 3 (Table 2.1) from samples taken upstream from paddlewheels.

Unglazed square ceramic tiles (23 cm^2) were placed in the streams prior to dosing and served as sample units (as in McCormick and Stevenson, 1991). Streams were filled to capacity and an aliquot of $CuCl_2$ solution added to bring the stream [Cu^{++}] to nominal concentrations. Stream inputs of water and Cu^{++} were then initiated, followed by addition of periphyton propagules. In all experiments propagules were

Table 2.1. Means and standard deviations of Cu^{++} concentrations, pH, water hardness, alkalinity, and temperature in artificial streams during Experiments 1-3. Water chemistry was measured every fifth day for Experiment 1 (N = 27) and every third day for Experiments 2 and 3 (N = 25 and 20); temperature was measured daily. Chronic criteria are calculated for each mean water hardness using the formula $C_C = \exp(0.8543[\ln(\text{hardness})] - 1.465)$ (USEPA, 1985).

A. Experiment 1

Treatment	[Cu^{++}]	pH	Hardness	Alkalinity	T, °C
Control	0.11 ± 0.054	7.71 ± 0.24	51.1 ± 4.7	44.3 ± 6.6	24 ± 4.3
5 ug/L	5.55 ± 0.64				
10 ug/L	12.96 ± 1.83		Chronic criterion		
50 ug/L	59.26 ± 4.66		6.66 ug/L		
100 ug/L	117.59 ± 10.78				
500 ug/L	537.67 ± 52.97				

B. Experiment 2

Treatment	[Cu^{++}]	pH	Hardness	Alkalinity	T, °C
Control	0.12 ± 0.048	7.52 ± 0.18	50.4 ± 3.6	37.7 ± 3.7	21 ± 3.6
2.5 ug/L	2.82 ± 0.28				
5 ug/L	5.75 ± 0.71		Chronic criterion		
10 ug/L	11.14 ± 1.07		6.58 ug/L		
25 ug/L	26.57 ± 3.60				
50 ug/L	53.46 ± 6.78				
250 ug/L	255.32 ± 25.71				

C. Experiment 3

Treatment	[Cu^{++}]	pH	Hardness	Alkalinity	T, °C
Control	0.11 ± 0.040	7.57 ± 0.19	49.6 ± 3.2	38.3 ± 3.9	22 ± 3.9
2.5 ug/L	2.58 ± 0.48				
5 ug/L	5.43 ± 0.50		Chronic criterion		
10 ug/L	10.77 ± 0.72		6.49 ug/L		
25 ug/L	26.05 ± 1.99				

supplied to the streams by passing system input water through a headbox containing periphyton-covered rocks before it entered the streams (Figure 2.2). Because of low colonization in the first experiment, streams in further experiments were also inoculated with a slurry of periphyton scraped from rocks in the New River in Montgomery County, VA at the start of the experiment.

Biomass was sampled on 8 occasions for Experiment 1, four occasions for Experiment 2, and 6 occasions for Experiment 3. Experiment 1 was considered a range-finding experiment, and biomass was sampled only during periods of high biomass for Experiment 2. Biomass was sampled more regularly during Experiment 3. Periphyton was removed from tiles (N = 3 for Experiments 1 and 2; N = 4 for Experiment 3) by scraping with stainless steel razor blades and suspended in 100 mls of deionized distilled water. The number of substrates sampled was a compromise between decreasing the variance of measurements and sampling effort required. Periphyton suspensions were homogenized using a hand-held mixer. Ash-free dry weight (AFDW) estimates of biomass were determined by filtering a portion of the suspensions on preashed, acid-washed, tared glass-fiber filters. Filters were then dried at 105°C for 24 hours and weighed. Organic matter on the filters was ignited at 500°C for one hour and reweighed. Wetting caused filters to adhere to weighing pans

during the drying process, and weight loss due to dehydration of clays (APHA, 1992) was considered inconsequential in these samples, so rewetting and redrying steps were omitted. AFDW was calculated as dry weight minus ash weight. Substrates were sampled until sloughing of mats from control stream substrates was demonstrated. Chlorophyll *a* and pheophytin *a* contents of periphyton suspensions were determined spectrophotometrically according to APHA (1992).

Polynomial least-squares regression (Kleinbaum and Kupper, 1978) was used to detect trends in biomass responses to Cu^{++} dosing. Regression equations relating biomass levels observed in streams to measured Cu^{++} concentrations were fitted using polynomial (linear, quadratic, and cubic; alone and in combination) and logistic models for each experimental sampling day. The model which best described the response data for a particular experimental sampling day (i.e., yielding the greatest statistical significance and highest r^2) was used to predict Cu^{++} effect concentrations (EC_X) which would reduce control stream biomass by 20% and 50%. EC_{20} was chosen because it approximates the no-observed-effect-concentration in many cases (McCormick *et al., in press*). EC_{50} was used because of the prevalence of 50% effects reports (e.g., LC_{50}) in the literature. Confidence limits for predicted effect concentrations were calculated by determining the predicted Cu^{++} concentrations associated

with the upper and lower 95% confidence limits associated with biomass predictions for the effect concentrations. Growth rate analyses were conducted using dummy variable regression (Ott, 1992) on natural log-transformed data. Data were analyzed using Statistical Analysis Software (SAS Institute, 1990).

Results and Discussion

Water column Cu^{++} concentrations were generally 10% higher than nominal concentrations (Table 2.1). This was a result of decreases in stream inputs through the course of the day from impingement of organic matter within delivery faucets.

The ratio of absorbances of chlorophyll *a* and pheophytin *a* was never less than 1.60, demonstrating that the periphyton was in good physiological condition (APHA, 1992).

i. Effects of Cu^{++} on biomass accumulation

Biomass curves (Fig. 2.3) are plotted as biomass (mg AFDW/cm^2) versus sampling day. Similar growth responses are observed in individual stream plots. Biomass levels rose exponentially through time, eventually reaching a maximum level. At this point, the periphyton mat had begun to

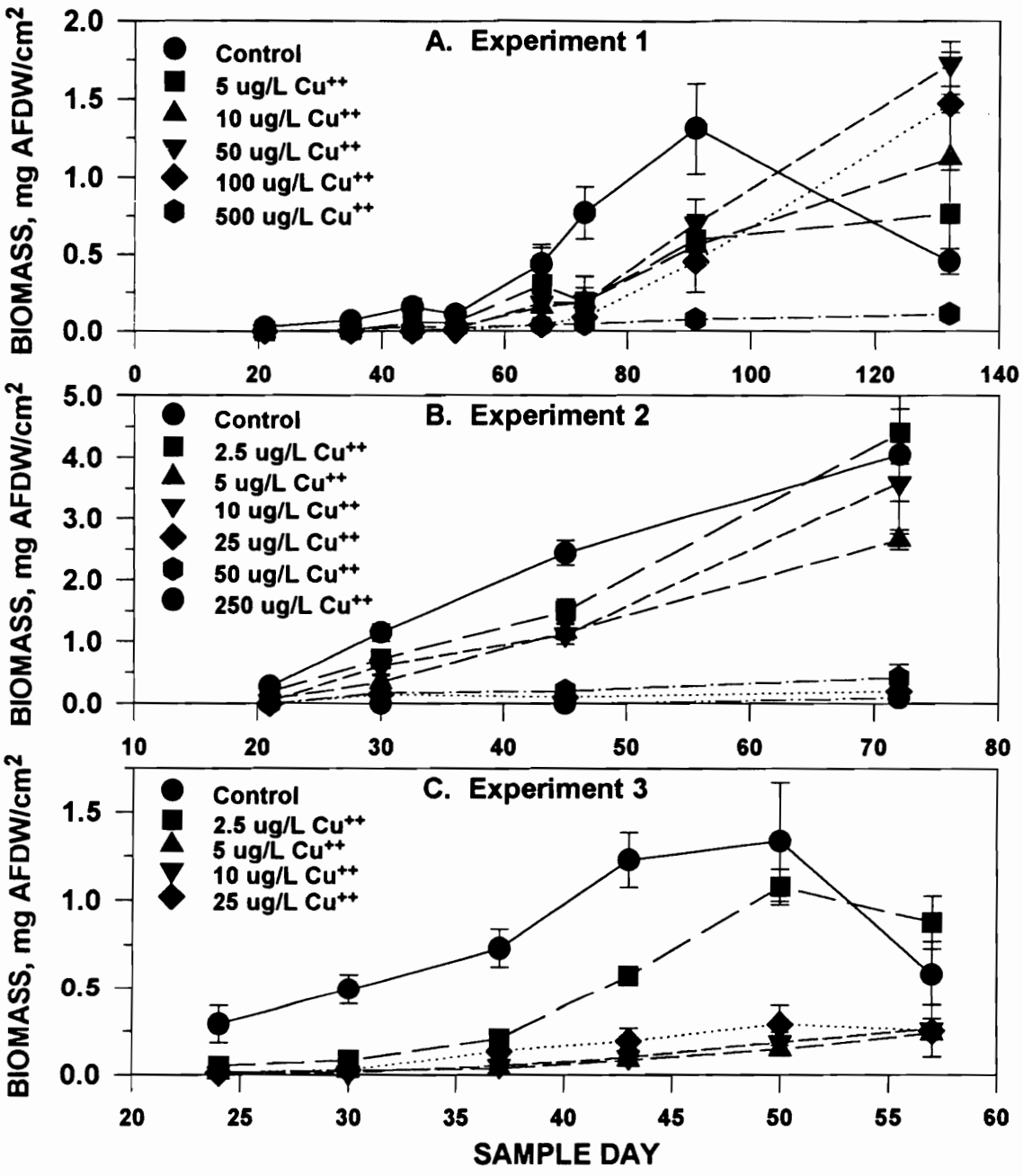

Figure 2.3. Biomass curves for periphyton growth experiments: A) Experiment 1, conducted in 1991; B) Experiment 2, conducted in 1992; and C) Experiment 3, conducted in 1993. Plots are means of three samples from each stream on each sampling day for A and B and four samples for C; error bars represent ± 1 standard deviation. Nominal Cu^{++} concentrations are indicated.

senesce, and portions of the mat sloughed off. Data for sampling days after senescence was demonstrated in control streams were not included in analyses. Data from the Experiment 1 500 ug/L stream were not used because examination of material which accrued on the substrates revealed that it consisted entirely of particulates. These particles were identified as $CuPO_4$ (CRC, 1982), and were not observed in any other stream samples.

Biomass responses to Cu^{++} for each sampling day (Figs. 2.4-2.6) were generally best modelled by a combined linear/quadratic equation (Table 2.2A). A modified logistic equation was used for one sampling day (Experiment 2, Day 21). All regressions were statistically significant ($P < 0.05$), and usually explained more than 80% of the variability observed in the data.

Predicted EC_{20} values were below the lowest Cu^{++} treatment in all cases (Table 2.2B). Lower confidence limits for these predictions were equal to or less than the control stream concentrations for all days. Upper confidence limits were highest for Experiment 1, while usually below 4 ug/L for Experiments 2 and 3. Predicted EC_{50} values were generally below 4 ug/L for all days. The highest predicted EC_{50} (11.25 ug/L, Experiment 1 Day 66) was produced by the regression with the lowest r^2 and highest P-value. Lower confidence limits for predicted EC_{50}s were always less than

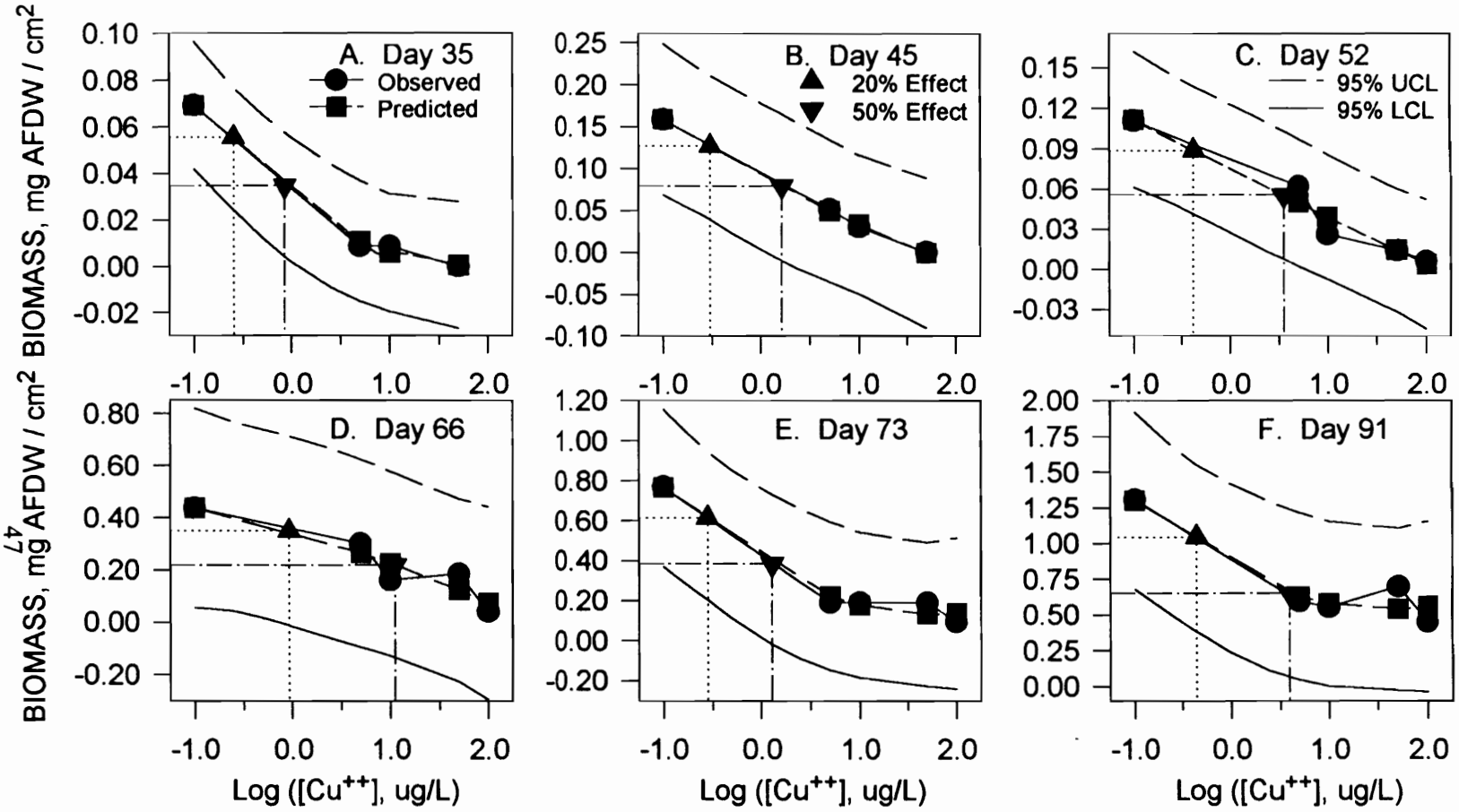

Figure 2.4. Biomass responses to Cu^{++} treatment by sampling day for Experiment 1. Observed and regression-predicted biomass levels are shown. 95% upper (UCL) and lower (UCL) confidence intervals for the regression are plotted. Predicted effect concentrations (EC_{20}, EC_{50}) are indicated by drop lines.

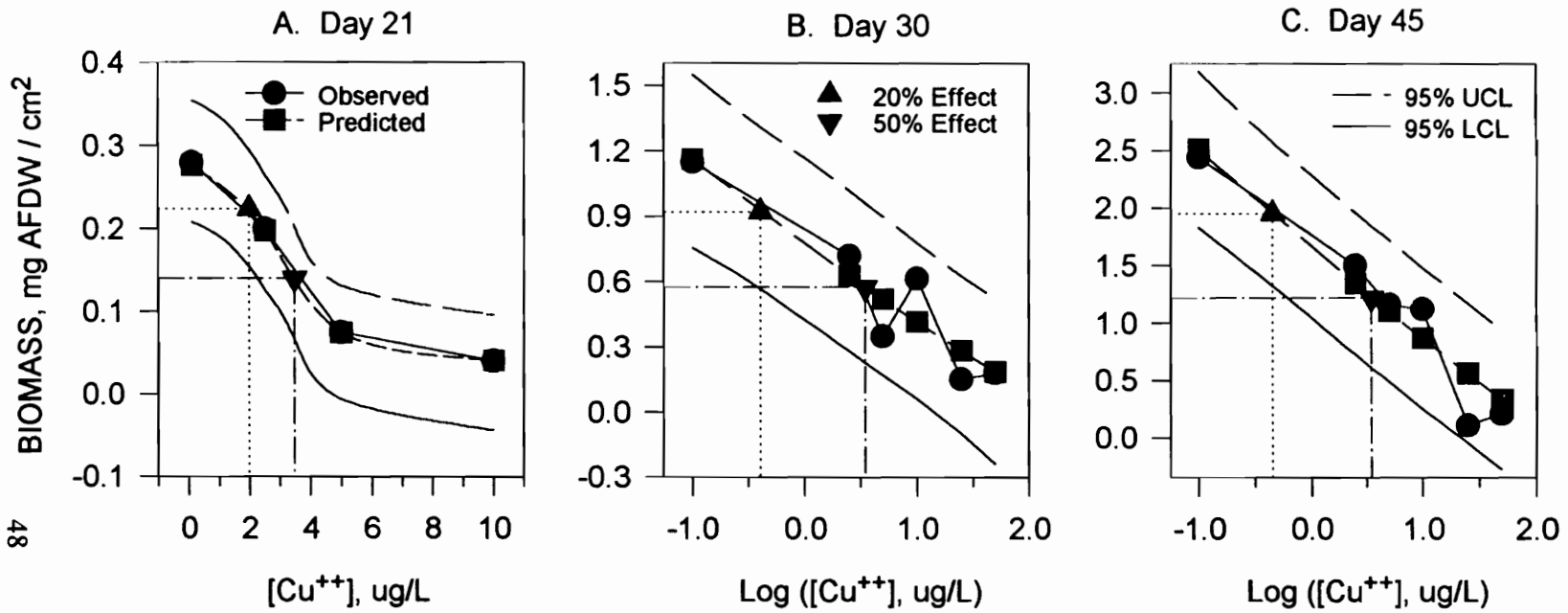

Figure 2.5. Biomass responses to Cu^{++} treatment by sampling day for Experiment 2. Observed and regression-predicted biomass levels are shown. 95% upper (UCL) and lower (UCL) confidence intervals for the regression are plotted. Predicted effect concentrations (EC_{20}, EC_{50}) are indicated by drop lines.

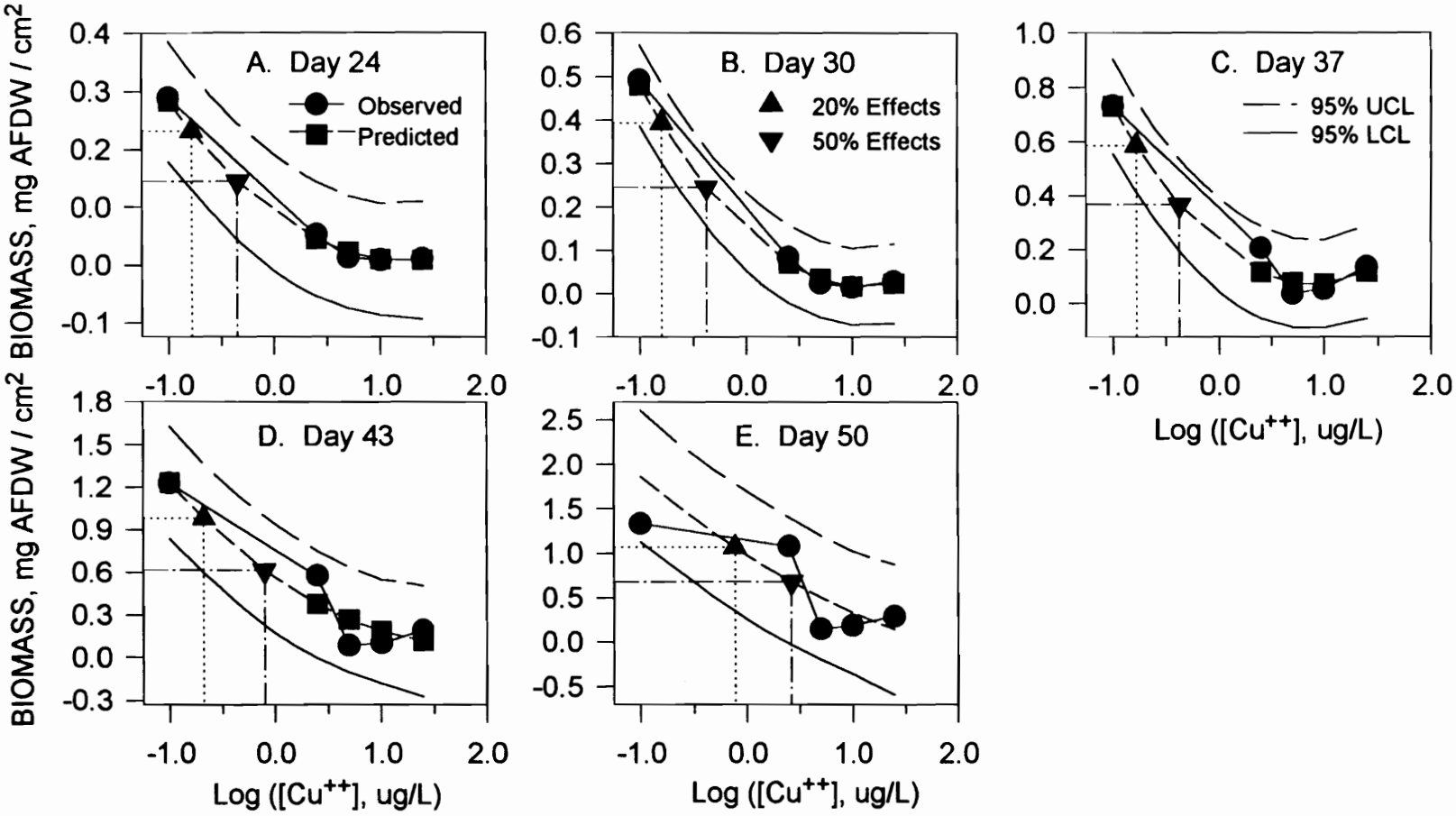

Figure 2.6. Biomass responses to Cu^{++} treatment by sampling day for Experiment 3. Observed and regression-predicted biomass levels are shown. 95% upper (UCL) and lower (UCL) confidence intervals for the regression are plotted. Predicted effect concentrations (EC_{20}, EC_{50}) are indicated by drop lines.

Table 2.2. Regression parameters (A), predicted effects concentrations, and prediction 95% confidence limits for effects concentrations (B) for each sampling day for Experiments 1-3. For C, $Y = \beta_1 X + \beta_2 X^2 + \beta_3$, where X = Log ([$Cu^{++}$], ug/L); for Lo, $Y = \beta_1/(1 + e^{(\beta_2(X-\beta_3))}) + \beta_4$, where X = [$Cu^{++}$]; Y = observed biomass. N = term not used in model; * = concentration outside of regression domain.

A. Regression parameters

EXP	DAY	MODEL	β_1	β_2	β_3	β_4	r^2	P-VALUE
1	35	C	-0.032	0.009	0.029	N	0.903	0.001
1	45	C	-0.063	0.005	0.091	N	0.802	0.001
1	52	C	-0.036	0.0002	0.075	N	0.816	0.001
1	66	C	-0.106	-0.015	0.347	N	0.464	0.024
1	73	C	-0.293	0.084	0.388	N	0.749	0.001
1	91	C	-0.359	0.113	0.826	N	0.633	0.002
2	21	Lo	0.254	0.951	3.050	0.0407	0.929	0.001
2	30	C	-0.373	0.016	0.773	N	0.866	0.001
2	45	C	-0.820	0.022	1.665	N	0.903	0.001
3L	24	C	-0.136	0.056	0.091	N	0.870	0.001
3L	30	C	-0.231	0.103	0.145	N	0.953	0.001
3L	37	C	-0.329	0.185	0.217	N	0.940	0.001
3L	43	C	-0.524	0.147	0.561	N	0.874	0.001
3L	50	C	-0.768	0.121	0.978	N	0.814	0.001

B. Predicted effects concentrations and confidence limits

EXP	DAY	EC_{20}	95% CL	EC_{50}	95% CL
1	35	0.25	(*, 1.41)	0.85	(0.18, 13.49)
1	45	0.30	(*, 7.63)	1.64	(*, *)
1	52	0.42	(*, 8.96)	3.51	(0.17, 77.09)
1	66	0.93	(*, *)	11.25	(*, *)
1	73	0.28	(*, 6.62)	1.29	(0.12, *)
1	91	0.44	(*, *)	3.91	(0.13, *)
2	21	1.98	(*, 3.27)	3.48	(2.46, 5.36)
2	30	0.41	(*, 3.66)	3.49	(0.32, 36.32)
2	45	0.45	(*, 2.61)	3.49	(0.61, 21.58)
3	24	0.17	(*, 0.55)	0.45	(0.14, 2.59)
3	30	0.16	(*, 0.28)	0.42	(0.23, 0.90)
3	37	0.17	(*, 0.33)	0.42	(0.20, 1.17)
3	43	0.21	(*, 0.84)	0.80	(0.20, 6.56)
3	50	0.77	(0.11, *)	2.63	(0.32, *)

2.5 ug/L, while upper confidence limits were often outside the domain of the regression concentrations. The highest upper confidence limits for these predictions were associated with the experiments (2, 3) in which fewer subsamples were taken on each sampling day.

In all cases the predicted EC_{20} was far below the USEPA chronic criterion for copper, and for all cases except one the predicted EC_{50} was below the criterion (criteria = 6.66, 6.58, and 6.49 for Experiments 1, 2, and 3, respectively; see Table 2.1). Upper confidence limits for predictions were usually below criteria for EC_{20}s, but only consistently below criteria for EC_{50} predictions for Experiment 3.

Leland and Carter (1985) studied various effects of Cu^{++} in streams using both natural and artificial substrates and obtained mixed results concerning periphyton abundance. On artificial substrates, treatment with 2.5 ug/L reduced standing stocks relative to control reaches, although treatment with 5 ug/L did not always differ significantly from controls. On natural substrates, however, treatment with 2.5, 5, or 10 ug/L did not result in significant reductions in biomass as compared to control levels, which the authors attributed to replacement of sensitive species by others more tolerant of Cu^{++}. In other studies, Rodgers *et al.* (1979) and Weber and McFarland (1981) found significant reductions in periphyton standing stocks due to continuous

treatment with 50 and 120 ug/L Cu^{++}, although lower concentrations were not utilized.

ii. Effects of Cu^{++} on growth rates and colonization

Transformed data (Figs. 2.7 A-C) for each experiment are presented as the natural log of biomass (ln AFDW/cm^2) versus sampling day. In such a plot, the slope of the regression line fitted to the linear portion of the plot is an estimate of the intrinsic (maximum) growth rate of the periphyton, and the calculated intercept of the regression with the dependent variable axis is interpreted as an estimate of the colonizing biomass (Dudley, 1977). For each experiment, the maximum growth rate periods in control streams were determined by visual examination of the plots. Growth rates observed for treatment stream periphyton were compared to control growth rates for periods during which roughly equivalent biomass was present in the streams. This allows growth rate comparisons to be made between periphyton mats which are similarly constrained by diffusion into and out of the mat. Comparisons made between periphyton mats of different densities are not valid because important factors that regulate growth, such as light and nutrient penetration into the mat and diffusion of wastes from the mat, are dissimilar and would overshadow effects due to Cu^{++} treatments.

Examination of results of statistical tests for equali

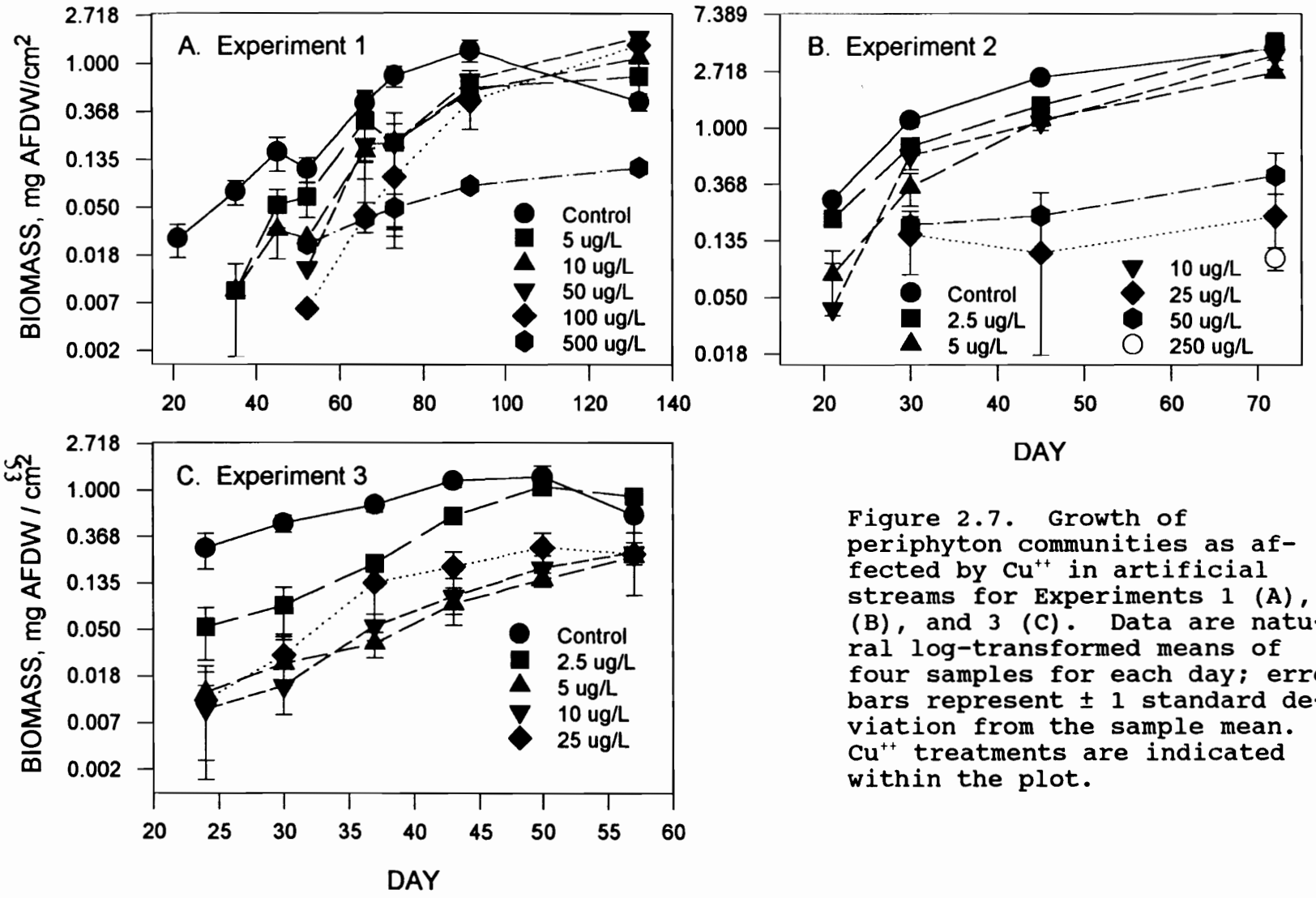

Figure 2.7. Growth of periphyton communities as affected by Cu^{++} in artificial streams for Experiments 1 (A), 2 (B), and 3 (C). Data are natural log-transformed means of four samples for each day; error bars represent ± 1 standard deviation from the sample mean. Cu^{++} treatments are indicated within the plot.

ty of slopes (Table 2.3) indicates that in Experiments 1 and 3 significant reductions in periphyton growth rates were not observed through 100 ug/L Cu^{++} treatment. In some cases, maximum observed growth rates in treatment streams exceeded control growth rates. In Experiment 2, statistically significant reductions in growth rates were observed in streams treated with ≥ 10 ug/L Cu^{++}. Collectively, these results may be a result of *a posteriori* hypothesis testing, as these comparisons were not contemplated during experimental design. As such, sampling regimens (designed for detection of significant differences in biomass) were not optimal for measurement of growth rates, and maximum growth rate in control streams may have occurred prior to periphyton sampling (especially in Experiments 2 and 3). Alternatively, shifts in community composition due to Cu^{++} treatment may have resulted in communities whose maximum growth rates are not decreased significantly as compared to controls. Mechanisms of metal tolerance (Reed and Gadd, 1989; Rai *et al.*, 1981) may not pose such extensive energetic requirements for substantial growth rate reductions to be observable at the concentrations tested. The dominance of *Achnanthes minutissima* due to Cu^{++} treatments at these light levels (Chapter 4) is the most probable explanation for these observations, as the EC_{50} for growth rate reduction of this diatom is 187 ug/L Cu^{++} (Watanabe *et al.*, 1988).

Table 2.3. Results of dummy variable linear regression analysis of maximum observed growth rates of periphyton mats for Experiments 1-3, using individual models LN (Biomass) = M (DAY) + B, where M is Slope and B is Intercept. P is the significance level of the T-test for the hypothesis $Parameter_{Treatment} = Parameter_{Control}$, except for Control streams which used the hypothesis $Parameter_{Control} = 0$.

A. Experiment 1

Treatment	Slope	P	Intercept	P
Control	0.07504	0.0002	-5.29260	0.0001
5 ug/L	0.10919	0.2550	-8.21451	0.0431
10 ug/L	0.06871	0.7552	-6.78350	0.0966
50 ug/L	0.09565	0.3392	-8.81342	0.0013
100 ug/L	0.11076	0.1013	-10.64085	0.0001

B. Experiment 2

Treatment	Slope	P	Intercept	P
Control	0.15750	0.0003	-4.59255	0.0001
2.5 ug/L	0.14248	0.7743	-4.60490	0.9927
5 ug/L	0.08249	0.0921	-3.56995	0.4276
10 ug/L	0.03922	0.0109	-1.66139	0.0299
25 ug/L	0.00623	0.0008	-2.14117	0.0671
50 ug/L	0.02558	0.0023	-2.85301	0.1599

C. Experiment 3

Treatment	Slope	P	Intercept	P
Control	0.07630	0.0001	-3.10041	0.0001
2.5 ug/L	0.08948	0.6385	-4.40398	0.3087
5 ug/L	0.07263	0.8194	-5.57747	0.0013
10 ug/L	0.06833	0.6201	-5.21476	0.0056
25 ug/L	0.06235	0.4105	-4.39123	0.0673

Significant differences between control and treatment regression intercepts were obtained for various concentrations in each of the experiments. Although objections may be raised to extrapolations of parameters prior to initial sampling (e.g., interpretation of regression intercepts as colonization), comparisons of intercept terms are useful for comparison of temporal development of periphyton communities. Reductions of regression intercepts as compared to control levels indicate temporal displacement of community development. Respective lowest observed effects concentrations (LOECs) of regression intercepts for the three experiments are 5 ug/L, 10 ug/L, and 5 ug/L. Chronic effects levels for these experiments, calculated as the geometric mean of the NOEC (no observed effects Cu^{++} concentration) and LOEC (APHA, 1992) using measured mean concentrations, are 1.02 ug/L, 8.00 ug/L, and 3.74 ug/L respectively. Mean chronic effects level for these experiments is 4.26 ug/L Cu^{++} ± 3.52 (sample standard deviation).

Literature reports for effects of Cu^{++} on algal growth rates were conducted exclusively with static tests, although these data confirm the present results. A growth chronic effects level of 100 ug/L was determined for the blue-green algae *Anaebaena variabilis* and *Anacystis nidulans* (Young and Lisk, 1972) and *Chroococcus paris* (Les and Walker, 1984). Among chlorophytes, growth of *Chlorella vulgaris* was reduced

by 50% after 33 days exposure to 180 ug/L (Rosko and Rachlin, 1977), while *Selenastrum capricornutum* exhibited a chronic effects level at 50 ug/L (Bartlett *et al.*, 1974). Garvey *et al.* (1991) found that the growth rate of *Chlamydomonas reinhardtii* was not reduced by treatment with 64 ug/L Cu^{++}. Results of chronic tests on organisms dependent on periphyton as a food resource (either directly or indirectly) indicate that growth rates of these organisms are generally more sensitive to Cu^{++} than algal taxa. Chronic effects levels such as 10.9 ug/L for the snail *Campeloma decisum* (Arthur and Leonard, 1970), 12.2 ug/L for the mayfly *Epeorus latifolium* (Hatakeyama, 1989), 20.9 for white suckers (McKim *et al.*, 1978), 13.6 for fathead minnow (Mount and Stephan, 1969), and 19 ug/L for rainbow trout (McKim *et al.*, 1978) have been determined. However, other species are more tolerant, such as smallmouth bass (517 ug/L growth NOEC; McKim *et al.*, 1978) or exhibit differential responses, such as bluntnose minnows (8.8 ug/L for reproductive impairment versus 119 ug/L growth NOEC; Horning and Neihesel, 1979). These collective values, however, are higher than those determined above which caused significant decreases in algal standing stocks in artificial streams. As such, effects on populations dependent on periphyton as a trophic resource could be impacted due to resource limitation in the absence of direct chemical effects.

In retrospect, it is not surprising that colonization is a more sensitive indicator of toxicity than growth rates. Lotic algae display colonization preferences based on light (Bothwell *et at.*, 1989) and nutrient levels (Hill and Knight, 1988), so reactions of colonizing cells to intolerable concentrations of Cu^{++} are not unexpected. Weber and McFarland (1981) found that Cu^{++} treatment of a small calcareous stream induced greater changes in community composition than in standing stocks of biomass. Additionally, Pratt *et. al.* (1987) found that protozoan colonization was more sensitive to Cu^{++} treatment than community biomass, with a chronic value of 6.7 ug/L.

Effects of Cu^{++} on temporal development of periphyton communities could have adverse effects on dependent consumers in natural systems. It has been suggested that disturbances are a primary controlling factor in aquatic ecosystems (Reice *et al.*, 1990). The timing and periodicity of such events, particularly spates, are especially critical in this respect. In reference to these experiments, if the periodicity of the disturbance is less than the time required for equilibration of biomasses due to Cu^{++} treatment, then treated (exposed) reaches would not equilibrate with control biomass levels. As such, Cu^{++} exposure combined with natural disturbances could affect dependent populations to a greater degree through resource limitations than ex-

pected from direct chronic toxicity measurements.

Conclusions

EC_{20} and EC_{50} values from biomass regressions were consistently lower than USEPA chronic criteria for copper. Comparison of maximum periphyton growth rates revealed that this parameter is a relatively insensitive indicator of toxicity, and significant reductions in maximum growth rate were detected in only one experiment. Temporal displacement of growth curves resulted in significant differences in periphyton standing crop. This displacement was interpreted as differential colonization of substrates in response to Cu^{++} treatments, and was reflected in differences between control and treatment regression intercepts.

Due to the relative insensitivity of algal growth rates to Cu^{++} as compared to effects on colonization and the implications of these effects on colonization, static tests may not be adequate predictors of effects of Cu^{++} introduced into lotic systems. As such, the utility of artificial stream studies to risk prediction for lotic systems is demonstrated. Additionally, a comparison of chronic effects concentrations observed here and in the literature suggests that periphyton biomass levels may be more sensitive to Cu^{++}

than many organisms dependent on periphyton as a food resource.

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Chapter 3. Interactions of light and copper on periphyton growth in artificial streams

Introduction

Periphyton communities (Steinman and McIntire, 1990) often provide a significant proportion of the trophic resources to aquatic consumers (Cummins, 1974; Vannote *et al.*, 1980). As such, effects on periphyton communities must be determined when evaluating potential environmental effects of a toxic compound. Despite the importance of these communities to the maintenance of higher trophic levels, they are not collectively considered in determining Water Quality Criteria (Stephan *et al.*, 1985; USEPA, 1985). In the case of copper, autotroph values are not considered in Criteria calculation, due to both conflicting and incomplete data (USEPA, 1985).

A factor which has not been widely explored in algal toxicity studies is how irradiance levels affect algal responses to toxicants. Many algal metabolic pathways are influenced by the quantity and quality of incident light (Lee, 1989). Periphyton production (Hynes, 1970) and temporal species distributions (Patrick, 1971) in natural systems are often affected by seasonality, although the singular effects of light and temperature are difficult to separate.

Standing crops of periphyton biomass (Rosemond, 1993) and successional trends (Steinman and McIntire, 1986) can be related to the irradiance received by the community. Diffusion of metal ions into periphyton mats has been found to be inversely proportional to mat density (Rose and Cushing, 1970), and differential responses of algal taxa to metals is well documented (Palmer, 1959; Rai *et al.*, 1981). As such, light levels may play a role in metal toxicity by influencing both the exposure rate of the metal to all cells in the mat and the taxonomic composition of periphyton species within the mat.

There are conflicting reports on the influence of irradiance levels in algal toxicity studies. Various studies have shown either positive (Kamp-Neilsen, 1969; Mayasich *et al.*, 1986; Azeez and Bannerjee, 1987), negative (Mayasich *et al.*, 1986), or no (Azeez and Bannerjee, 1987) relationship between toxicity and irradiance, depending on the alga and toxicant. As such, the influence of irradiance on algal responses is unresolved.

These experiments were designed to investigate effects of copper on periphyton community growth dynamics and standing stocks in artificial streams as influenced by irradiance.

Materials and Methods

Experiments were conducted in 10 artificial streams in the Ecosystem Simulation Laboratory at Virginia Tech. Streams were fiberglass ovals 0.93 meters on the long axis, 0.63 meters on the short axis, with channel width of 23 centimeters. Standpipes in each stream were adjusted to maintain 32 liters of water in each stream (depth approximately 6 cm). Streams were supplied with 1 L/minute municipal tap water dechlorinated with activated charcoal. Input rates were adjusted volumetrically daily. Water hardness, alkalinity, and pH were measured every third day; mean values for all measurements over the experiment were 49.4 mg/L $CaCO_3$, 37.2 mg/L $CaCO_3$, and 7.5, respectively. Total dissolved orthophosphate concentrations (ascorbic acid method; APHA, 1992) were approximately 0.85 mg/L PO_4^{3-}-P. Nitrate levels (determined by cadmium reduction method; APHA, 1992) were approximately 1.0 mg/L NO_3^--N. These high nutrient levels are a result of using a municipal water supply which could not be avoided. Residual chlorine concentrations were below detection using amperometric titration (< 0.01 mg/L; APHA, 1992). Ammonia concentrations were below detection using the phenate method (< 10 ug NH_3-N/L; APHA, 1992). Stream flow was maintained at 15 cm/sec by

motor driven paddlewheels.

Five Cu^{++} concentrations and two light levels were used in the study (one replicate per treatment). Target concentrations in treated streams were 2.5 ug/L, 5.0 ug/L, 10 ug/L, and 25 ug/L. Cu^{++} (as $CuCl_2$) was supplied to the streams by peristaltic pumps. Concentrations of stock solutions were made considering the pump flow rates (approximately 0.75 ml/min) and stream water input rate. Pump rates were measured gravimetrically on a weekly basis by collecting toxicant outflow for 20 minutes and new stock concentrations were adjusted accordingly. Water column total copper concentrations samples were measured every third day (Table 3.1) from samples taken upstream from paddlewheels. High light levels (400 $\mu M/m^2/sec$) were supplied by 1000 Watt halide lamps (ANSI #M47PA-1000/U; Philips Lighting Co., Somerset, NJ), while low light levels (40 $\mu M/m^2/sec$) were supplied by full-spectrum Vita-Lites®. Lighting regimen was 12:12 light:dark.

Unglazed square ceramic tiles (23 cm^2) were placed in the streams prior to dosing and served as sample units (as in McCormick and Stevenson, 1991). Streams were filled to capacity and an aliquot of $CuCl_2$ solution added to bring the stream $[Cu^{++}]$ to nominal concentrations. Stream inputs of water and Cu^{++} were then initiated, followed by addition of periphyton propagules. Propagules were supplied to the

Table 3.1. Means and standard deviations of copper concentrations, pH, water hardness, and alkalinity in artificial streams. Water chemistry was measured every third day (N = 20); temperature was measured daily. Copper is as total copper; hardness and alkalinity are mg/L as $CaCO_3$. Chronic criteria are calculated for each mean water hardness using the formula C_C = exp (0.8543[ln (hardness)] - 1.465) (USEPA, 1985).

A. High light streams

Treatment	[Cu^{++}], ug/L
Control	0.11 ± 0.050
2.5 ug/L	2.41 ± 0.51
5 ug/L	5.62 ± 0.60
10 ug/L	10.85 ± 0.64
25 ug/L	27.3 ± 2.20

B. Low light streams

Treatment	[Cu^{++}], ug/L
Control	0.11 ± 0.040
2.5 ug/L	2.58 ± 0.48
5 ug/L	5.43 ± 0.50
10 ug/L	10.77 ± 0.72
25 ug/L	26.05 ± 1.99

C. All streams

pH	Hardness	Alkalinity	T, °C
7.57 ± 0.19	49.6 ± 3.2	38.3 ± 3.9	22 ± 3.9

Chronic criterion
6.49 ug/L

streams by inoculation with a slurry of periphyton scraped from rocks in the New River in Montgomery County, VA and by passing system input water through a headbox containing periphyton-covered rocks before it entered the streams.

Periphyton was removed from tiles (N = 4 for each stream sampling) by scraping with stainless steel razor blades and suspended in 100 mls of deionized distilled water. Periphyton suspensions were homogenized using a hand-held mixer (bamix HANDIMIXER; Biospec Products, Inc., PO Box 722, Bartlesville OK 74005). Biomass was estimated using and ash-free dry weight (AFDW) measurements. AFDW estimates of biomass were determined by filtering a portion of the suspensions on pre-ashed, acid-washed, tared glass-fiber filters. Filters were then dried at 105°C for 24 hours and weighed. Organic matter on the filters was ignited at 500°C for one hour and reweighed. Wetting caused filters to adhere to weighing pans during the drying process, and weight loss due to dehydration of clays (APHA, 1992) was considered inconsequential in these samples, so rewetting and redrying steps were omitted. AFDW was calculated as dry weight minus ash weight. Periphyton growth was measured on six occasions (24, 30, 37, 43, 50, and 57 days) until sloughing of mats from control stream substrates was demonstrated. Chlorophyll *a* and pheophytin *a* contents of periphyton suspensions were determined spectrophotometrical-

ly according to APHA (1992).

Polynomial least-squares regression (Kleinbaum and Kupper, 1978) was used to detect trends in biomass responses to Cu^{++} dosing. Regression equations relating biomass levels observed in streams to measured Cu^{++} concentrations were fitted using polynomial (linear, quadratic, and cubic; alone and in combination) and logistic models for each experimental sampling day. The model which best described the response data for a particular experimental sampling day (i.e., yielding the greatest statistical significance and highest r^2) was used to predict Cu^{++} effect concentrations (EC_X) which would reduce control stream biomass by 20% and 50%. EC_{20} was chosen because it approximates the no-observed-effect-concentration in many cases (McCormick *et al., in press*). EC_{50} was used because of the prevalence of 50% effects reports (e.g., LC_{50}) in the literature. Confidence limits for predicted effect concentrations were calculated by determining the predicted Cu^{++} concentrations associated with the upper and lower 95% confidence limits associated with biomass predictions for the effect concentrations. Growth rate analyses were conducted using dummy variable regression (Ott, 1992) on natural log-transformed data. Data were analyzed using Statistical Analysis Software (SAS Institute, 1990).

Results and Discussion

Water column copper concentrations were generally 10% higher than nominal concentrations (Table 3.1). This was a result of decreases in stream inputs through the course of the day from impingement of organic matter within delivery faucets. Control stream copper concentrations never exceeded 0.3 ug/L, and treatment stream concentrations were generally within 20% of nominal ranges. Exceptions occurred in the 2.5 ug/L high light stream. After biomass had exceeded approximately 1.2 mg AFDW/cm^2 (after 52 days) maintenance of stream copper concentration became impossible due to rapid uptake by high standing stocks of periphyton. Biomass data from this period were not used in analyses.

The ratio of absorbances of chlorophyll *a* and pheophytin *a* was never less than 1.60, demonstrating that the periphyton was in good physiological condition (APHA, 1992).

i. Effects of Cu^{++} on periphyton accumulation

Biomass curves (Fig. 3.1) are plotted as biomass (mg AFDW/cm^2) versus sampling day. Similar growth responses are observed in individual stream plots. Biomass levels rose exponentially through time, eventually reaching a maximum level. At this point, the periphyton mat had begun to

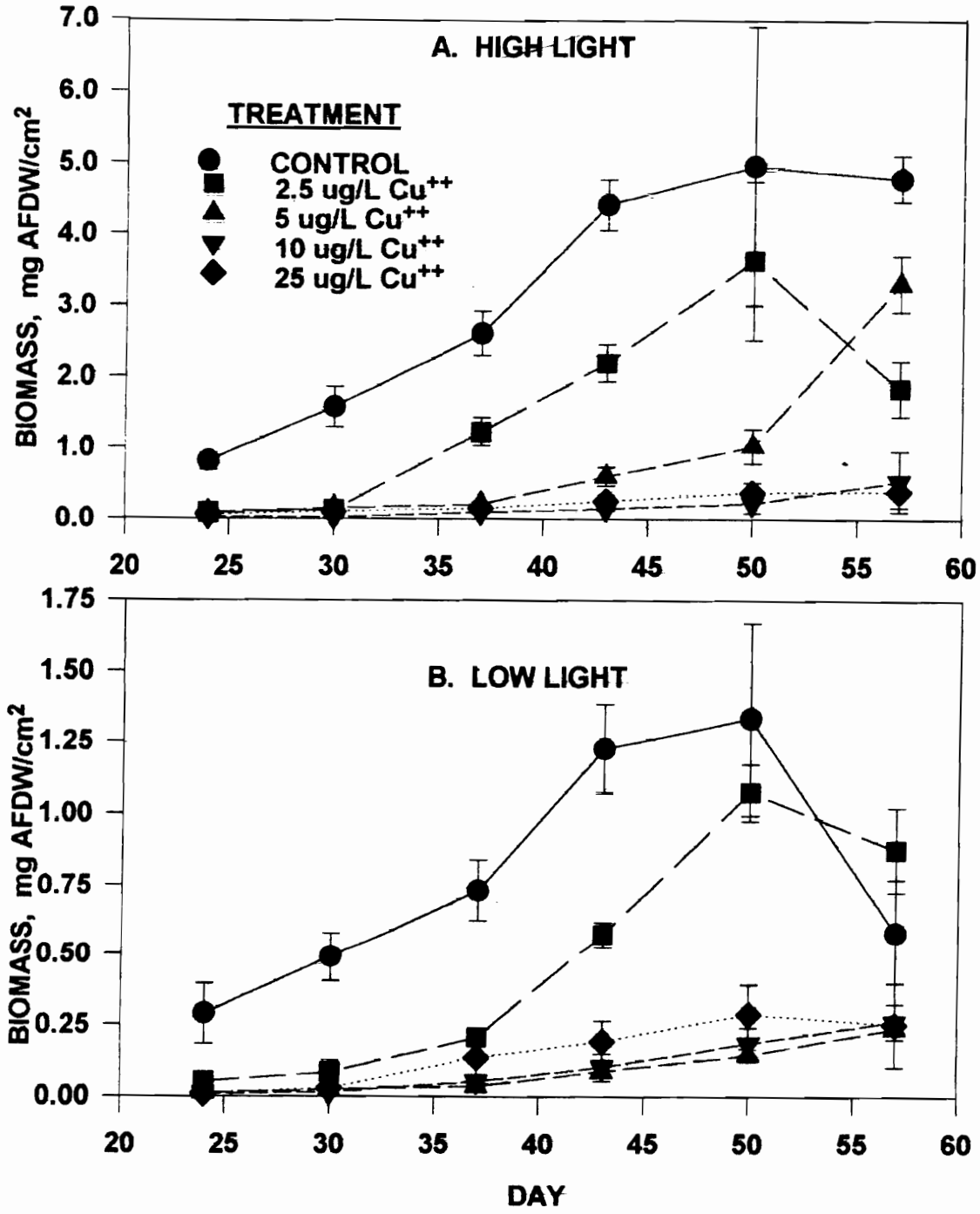

Figure 3.1. Growth of periphyton communities as affected by Cu^{++} in high (A) and low (B) light streams. Cu^{++} treatments are indicated within plots. Error bars represent ± 1 standard deviation from the sample mean (n = 4).

senesce, and portions of the mat sloughed off (especially in control and low Cu^{++} treatments). Data for sampling days after senescence was demonstrated in control streams were not included in analyses.

Biomass responses to Cu^{++} for each sampling day (Figs. 3.2-3.3) were generally best modelled by a combined linear/quadratic equation (Table 3.2A). All regressions were statistically significant ($P < 0.05$), and usually explained more than 90% of the variability observed in the data.

Predicted EC_{20} values were below the lowest Cu^{++} treatment in all cases (Table 3.2B). Lower confidence limits for these predictions were equal to or less than the control stream concentrations for all days. Upper confidence limits for EC_{20} predictions were below 1 ug/L except for regressions of Day 50 samples. Predicted EC_{50} values were below 3 ug/L for all days. Lower confidence limits for predicted EC_{50}s were always less than 1 ug/L, while upper confidence limits were usually below 5 ug/L. The highest upper confidence limits for these predictions were associated with the final two sampling days, and those of the final day were beyond the regression concentration domain.

In all cases the predicted EC_{20} and EC_{50} was far below the USEPA chronic criterion for copper (criterion = 6.49 ug/L; see Table 3.1). Upper confidence limits for predictions were below the criterion for eight of the ten EC_{20}

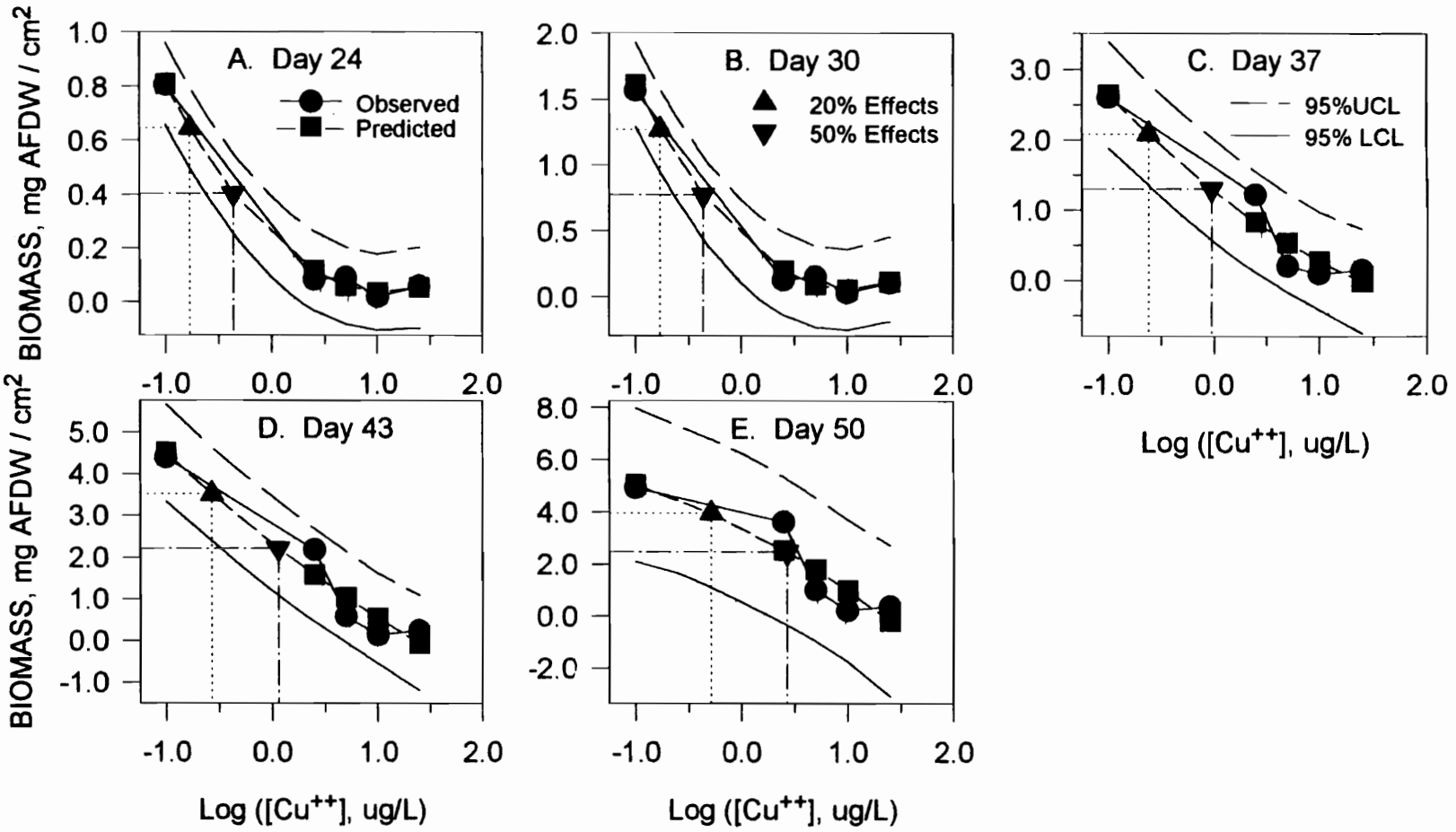

Figure 3.2. Biomass responses to Cu^{++} treatment by sampling day for high light streams. Observed and regression-predicted biomass levels are shown. 95% upper (UCL) and lower (UCL) confidence intervals for the regression are plotted. Predicted effect concentrations (EC_{20}, EC_{50}) are indicated by drop lines.

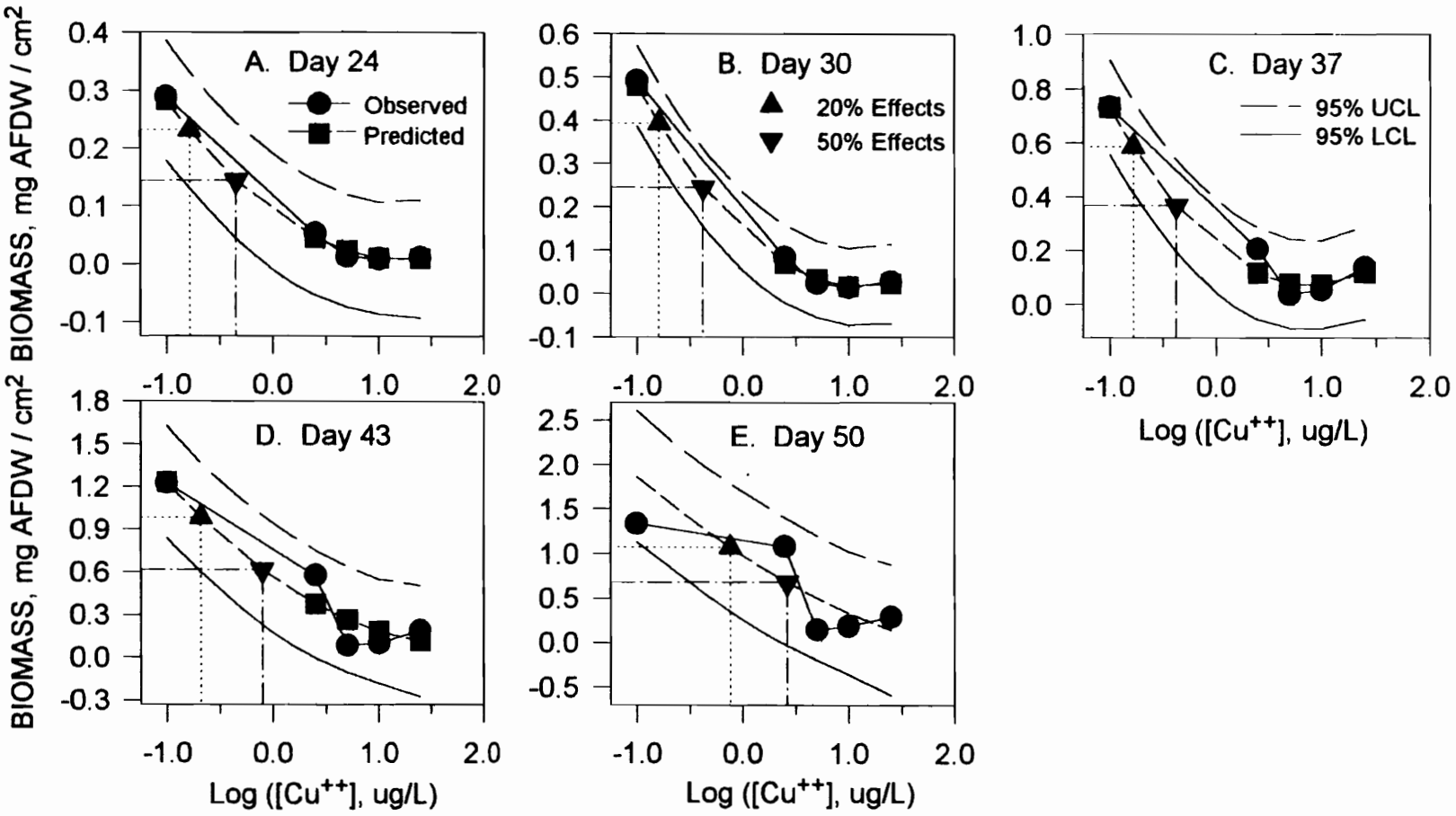

Figure 3.3. Biomass responses to Cu^{++} treatment by sampling day for low light streams. Observed and regression-predicted biomass levels are shown. 95% upper (UCL) and lower (UCL) confidence intervals for the regression are plotted. Predicted effect concentrations (EC_{20}, EC_{50}) are indicated by drop lines.

Table 3.2. Regression parameters (A), predicted effects concentrations, and prediction 95% confidence limits for effects concentrations for each sampling day for high and low light streams. For C, $Y = \beta_1 X + \beta_2 X^2 + \beta_3$, where X = Log ([$Cu^{++}$], ug/L); Y = observed biomass.

A. Regression parameters

LIGHT	DAY	MODEL	β_1	β_2	β_3	r^2	P-VALUE
H	24	C	-0.387	0.181	0.242	0.967	0.001
H	30	C	-0.779	0.387	0.448	0.957	0.001
H	37	C	-1.181	0.187	1.270	0.910	0.001
H	43	C	-1.978	0.192	2.336	0.924	0.001
H	50	C	-2.030	-0.388	3.411	0.699	0.001
L	24	C	-0.136	0.056	0.091	0.870	0.001
L	30	C	-0.231	0.103	0.145	0.953	0.001
L	37	C	-0.329	0.185	0.217	0.940	0.001
L	43	C	-0.524	0.147	0.561	0.874	0.001
L	50	C	-0.768	0.121	0.978	0.814	0.001

B. Predicted effects concentrations and confidence limits

LIGHT	DAY	EC_{20}	95% CL	EC_{50}	95% CL
H	24	0.17	(0.11, 0.30)	0.44	(0.24, 0.93)
L	24	0.17	(*, 0.55)	0.45	(0.14, 2.59)
H	30	0.18	(0.11, 0.32)	0.43	(0.24, 0.99)
L	30	0.16	(*, 0.28)	0.42	(0.23, 0.90)
H	37	0.24	(*, 0.85)	0.94	(0.26, 4.57)
L	37	0.17	(*, 0.33)	0.42	(0.20, 1.17)
H	43	0.27	(*, 0.93)	1.15	(0.33, 4.69)
L	43	0.21	(*, 0.84)	0.80	(0.20, 6.56)
H	50	0.52	(*, 9.00)	2.67	(*, *)
L	50	0.77	(0.11, *)	2.63	(0.32, *)

predictions and for seven of the ten EC_{50} predictions.

Leland and Carter (1985) studied various effects of copper in streams using both natural and artificial substrates and obtained mixed results concerning periphyton abundance. On artificial substrates, treatment with 2.5 ug/L reduced standing stocks relative to control reaches, although treatment with 5 ug/L did not always differ significantly from controls. On natural substrates, however, treatment with 2.5, 5, or 10 ug/L did not result in significant reductions in biomass as compared to control, which the authors attributed to replacement of sensitive species by others more tolerant of copper. In other studies, Rodgers *et al.* (1979) and Weber and McFarland (1981) found significant reductions in periphyton standing stocks due to continuous treatment with 50 and 120 ug/L Cu^{++}, although lower concentrations were not utilized.

ii. Effects of Cu^{++} on periphyton growth rate

Transformed data (Figure 3.4) are presented as the natural log of biomass (ln AFDW / cm^2) versus sampling date. Maximum growth rate periods in control streams were determined by visual examination of the plots. Growth rates observed for treatment stream periphyton were compared to control growth rates for periods during which roughly equivalent biomass was present in the streams (when possible).

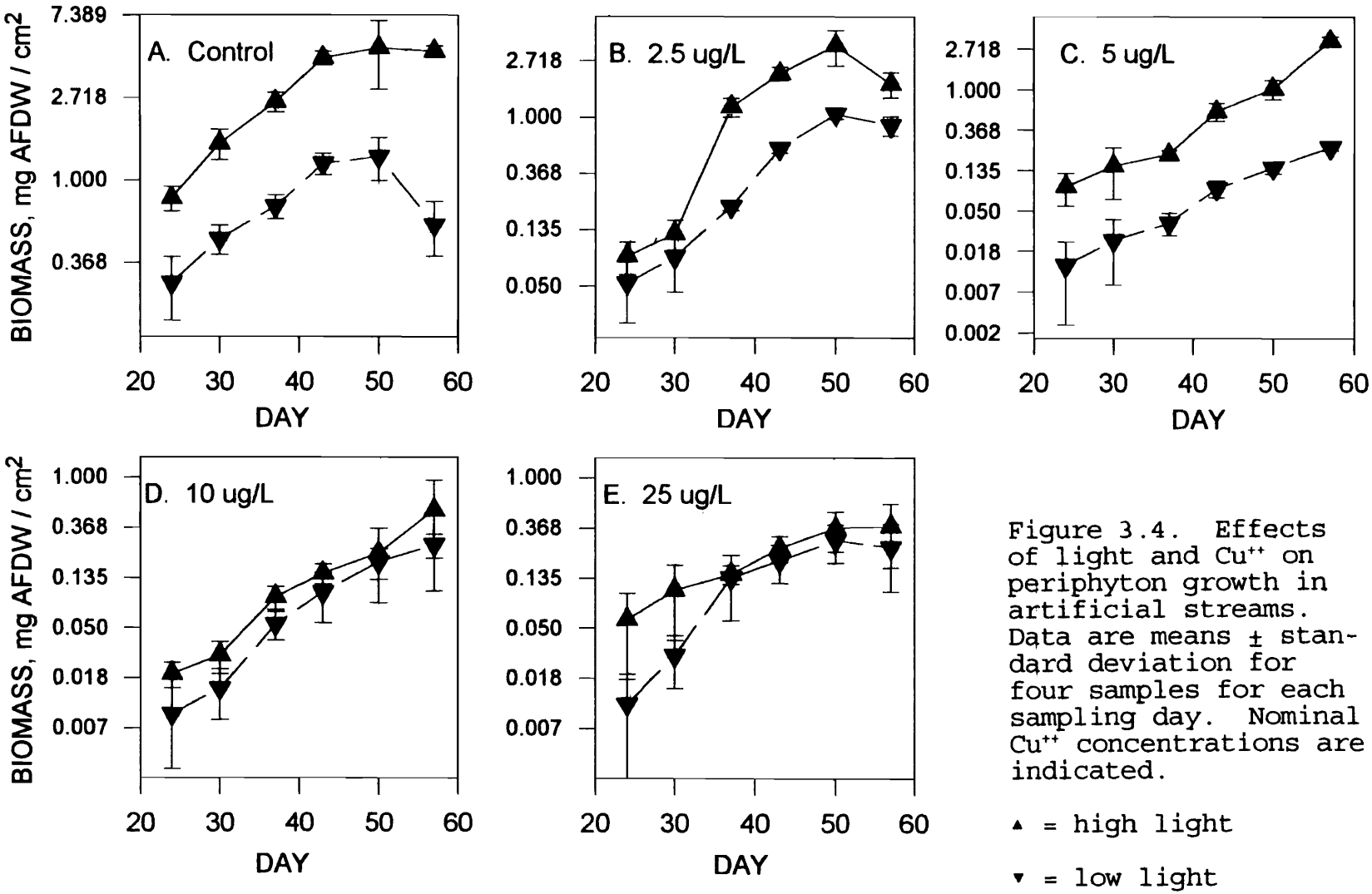

Figure 3.4. Effects of light and Cu^{++} on periphyton growth in artificial streams. Data are means ± standard deviation for four samples for each sampling day. Nominal Cu^{++} concentrations are indicated.

▲ = high light

▼ = low light

This allows growth rate comparisons to be made between periphyton mats which are similarly constrained by diffusion into and out of the mat. Comparisons made between periphyton mats of different densities are not valid because important factors which regulate growth, such as light and nutrient penetration into the mat and diffusion of wastes from the mat, are dissimilar and would overshadow effects due to Cu^{++} treatments.

Testing for equality of slopes (Table 3.3 A, B) indicates that significant reduction of periphyton growth rate due to Cu^{++} treatment was not observed. Regression slopes for treatment streams were not significantly different from control slopes for either high or low light streams. In two cases, maximum observed growth rates in treatment streams exceeded control growth rates. Collectively, these results may be a result of *a posteriori* hypothesis testing, as these comparisons were not contemplated during experimental design. As such, sampling regimens were not optimal for measurement of growth rates, and maximum growth rate in control streams may have occurred prior to periphyton sampling (especially in high light control). Alternatively, shifts in community composition due to Cu^{++} treatment may have resulted in communities whose maximum growth rates are not decreased significantly compared to controls. Mechanisms of metal tolerance (Reed and Gadd, 1989; Rai *et al.*, 1981) may

Table 3.3. Statistical analysis of effects of copper and light on periphyton growth rates from dummy variable analysis (ANOVA). A, B: Comparison of regression coefficients as affected by copper treatments in high and low light streams. T for H0: $m_T = m_C$ and $I_T = I_C$ are tests of the hypothesis that copper treatment slopes and intercepts are equivalent to control values. For control streams, the hypothesis Parameter = 0 was tested. C. Comparison of growth rate regression coefficients between paired streams on the basis of light treatment. T for H0: $m_H = m_L$ and $I_H = I_L$ are tests of equivalency between high and low light slopes and intercepts.

A. Comparison of growth rate regression coefficients between high light streams.

TREATMENT	SLOPE	T for H0: $m_T = m_C$	Pr > |T|	INTERCEPT	T for H0: $I_T = I_C$	Pr > |T|
CONTROL	0.08673	7.91	0.0001	-2.22277	-6.00	0.0001
2.5 ug/L	0.08124	-0.27	0.7894	-2.78466	-0.67	0.5064
5 ug/L	0.12280	1.85	0.0685	-5.92121	-4.16	0.0001
10 ug/l	0.07194	-0.97	0.3363	-5.08524	-4.59	0.0001
25 ug/L	0.06727	-0.95	0.3454	-4.38278	-2.57	0.0127

B. Comparison of growth rate regression coefficients between low light streams.

TREATMENT	SLOPE	T for H0: $m_T = m_C$	Pr > |T|	INTERCEPT	T for H0: $I_T = I_C$	Pr > |T|
Control	0.07630	8.47	0.0001	-3.10041	-10.18	0.0001
2.5 ug/L	0.08948	0.47	0.6385	-4.40398	-1.03	0.3087
5 ug/L	0.07263	-0.23	0.8194	-5.57747	-3.39	0.0013
10 ug/L	0.06833	-0.50	0.6201	-5.21476	-2.89	0.0056
25 ug/L	0.06235	-0.83	0.4105	-4.39123	-1.87	0.0673

C. Comparison of growth rate regression coefficients between light treatments.

TREATMENT	T for H0: $m_H = m_L$	Pr > |T|	T for H0: $I_H = I_L$	Pr > |T|
CONTROL	-1.12	0.2727	-2.78	0.0091
2.5 ug/L	0.35	0.7285	-1.51	0.1506
5 ug/L	0.37	0.7120	-1.71	0.1013
10 ug/l	-0.14	0.8931	-0.10	0.9224
25 ug/L	-0.19	0.8522	-0.01	0.9942

not pose such extensive energetic requirements for substantial growth rate reductions to be observable at the concentrations tested. The dominance of *Achnanthes minutissima* due to Cu^{++} treatments at these light levels (Chapter 4) is the most probable explanation for these observations, as the EC_{50} for growth rate reduction of this diatom is 187 ug/L Cu^{++} (Watanabe *et al.*, 1988).

Significant differences between control and treatment regression intercepts were obtained for streams receiving 5 ug/L Cu^{++} or more in both light treatments (except 25 ug/L Cu^{++} low light). Although objections may be raised to extrapolations of parameters prior to initial sampling (e.g., interpretation of regression intercepts as colonization), comparisons of intercept terms are useful for comparison of temporal development of periphyton communities. Reductions of regression intercepts as compared to control levels indicate temporal displacement of community development.

Chronic effects of a toxicant on a population are calculated as the geometric mean between the highest concentration of the toxicant which did not result in a statistically significant difference from the control response (NOEC) and the lowest tested concentration which did produce a significant difference from the control response (APHA, 1992). Chronic effects levels for regression intercepts

using measured mean concentrations, are 1.05 ug/L Cu^{++} for high light streams and 0.99 ug/L Cu^{++} for low light streams. Mean chronic effects level for both light treatments is 1.02 ug/L Cu^{++} ± 0.02 (sample standard deviation).

Literature reports for effects of Cu^{++} on algal growth rates were conducted exclusively with static tests, although these data confirm the present results. A growth chronic effects level of 100 ug/L was determined for the cyanophytes *Anaebaena variabilis* and *Anacystis nidulans* (Young and Lisk, 1972) and *Chroococcus paris* (Les and Walker, 1984). Among chlorophytes, growth of *Chlorella vulgaris* was reduced by 50% after 33 days exposure to 180 ug/L (Rosko and Rachlin, 1977), while *Selenastrum capricornutum* exhibited a chronic effects level at 50 ug/L (Bartlett *et al.*, 1974). Bringmann and Kühn (1980), however, found that the LOEC for total biomass of *S. capricornutum* in static tests was 1.1 mg/L Cu^{++}. Garvey *et al.* (1991) found that the growth rate of *Chlamydomonas reinhardtii* was not reduced by treatment with 64 ug/L Cu^{++}. Visviki and Rachlin (1994) found that *C. bullosa* was more sensitive to Cu^{++}, with an observed 96 hour growth EC_{50} of 50.7 ug/L Cu^{++}.

Results of chronic tests on organisms dependent on periphyton as a food resource (either directly or indirectly) indicate that growth rates of these organisms are generally more sensitive to Cu^{++} than algal taxa. Chronic ef-

fects levels such as 10.9 ug/L Cu^{++} for the snail *Campeloma decisum* (Arthur and Leonard, 1970), 12.2 ug/L for the mayfly *Epeorus latifolium* (Hatakeyama, 1989), 20.9 for white suckers (McKim *et al.*, 1978), 13.6 for fathead minnow (Mount and Stephan, 1969), and 19 ug/L for rainbow trout (McKim *et al.*, 1978) have been determined. However, other species are more tolerant, such as smallmouth bass (517 ug/L growth NOEC; McKim *et al.*, 1978) or exhibit differential responses, such as bluntnose minnows (8.8 ug/L for reproductive impairment versus 119 ug/L growth NOEC; Horning and Neihesel, 1979). These collective values, however, are higher than those determined above which caused significant decreases in algal standing stocks in artificial streams. As such, effects on populations dependent on periphyton as a trophic resource could be impacted due to resource limitation in the absence of direct chemical effects.

iii. Effects of light on periphyton growth rate

Comparison of regression parameters from high and low light streams by Cu^{++} treatments (Table 3.5 C) indicates that light level did not produce significantly different (ANOVA, $P > 0.27$) maximum growth rates between streams. Regression intercept comparisons indicate that a significant difference (ANOVA, $P < 0.01$) in regression intercept was proven only for control streams. This indicates that while

light is a strong determinant of colonization rate, the effect of light on colonization is less important when Cu^{++} levels increase.

iv. Effects of light on periphyton biomass

Biomass levels in high light streams were consistently higher than in low light streams (Fig. 3.4), but the difference between the two decreases with increasing Cu^{++} treatment. This effect is clear when the differences in biomass between high and low light treatments are compared between Cu^{++} treatments (Figure 3.5). This indicates that effects of reducing light on periphyton abundance are greater in the absence of additional stress. Increasing Cu^{++} levels produce proportionally less biomass in high light streams than in low light streams. This confirms Odum's (1985) hypothesis that efficiency of resource use decreases in stressed systems. However, Cu^{++} is not more toxic at high light levels, because predicted effects concentrations were similar (Table 3.2) for both light levels. This finding is in contrast to several literature reports. Kamp-Neilsen (1969) reported that toxicity of Cu^{++} to *Chlamydomonas pyrenoidosa* is related to light intensity. Azeez and Bannerjee (1987) found that acute (6 hour) Cu^{++} exposure reduced *Anacystis nidulans* chlorophyll *a* to a greater extent under illuminated conditions than in dark. This trend was not consistent with

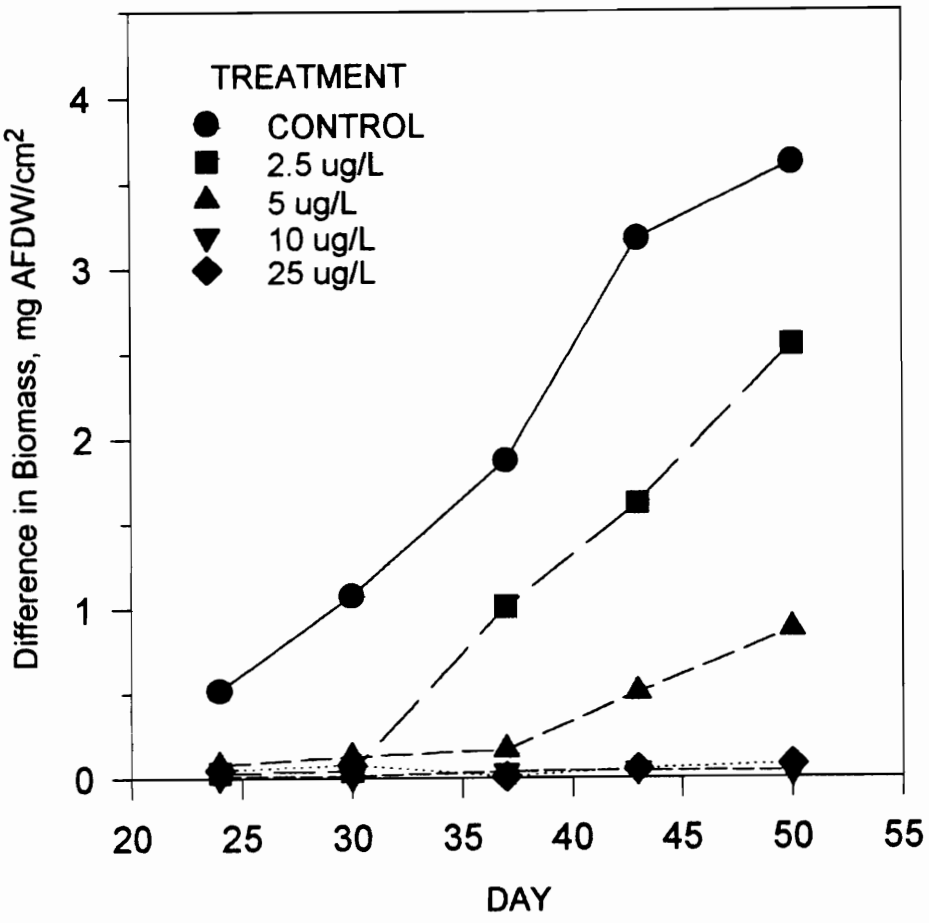

Figure 3.5. Difference in mean periphyton biomass between high and low light treatments for each Cu^{++} treatment. Differences are calculated as mean high light stream biomass minus mean low light biomass for each sampling day. Nominal Cu^{++} treatments are indicated in legend.

cadmium exposure to *Spirulina platensis*, however, as reductions in chlorophyll *a* were similar in light and dark treatments. The toxicity of the triazine herbicide atrazine to two species of algae is also affected by irradiance levels (Mayasich *et al.*, 1986). Atrazine is relatively more toxic to the diatom *Phaeodactylum tricornutum* at high light levels, while it is relatively more toxic to the eustigmatophyte *Nannochloris oculata* at low light levels.

Conclusions

Predicted EC_{20} and EC_{50} values from biomass regressions were consistently lower than USEPA chronic criteria for Cu^{++}, but were not affected by stream irradiance. Light levels affected biomass levels, but increasing Cu^{++} treatment reduced the importance of light. As such, light levels have relatively little impact on the toxicity of Cu^{++} to periphyton.

Maximum observed growth rates were not significantly affected by Cu^{++} or light treatments. Growth curve regression intercepts, interpreted as a measure of colonization, were significantly reduced by nominal Cu^{++} treatments of 5 ug/L in both light treatments. As such, colonization seems

to be a better indicator of sensitivity than growth rate, although it is not as sensitive as periphyton standing stock. Decreasing irradiance affected colonization only in the absence of Cu^{++}.

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Chapter 4. Interactions of copper and light on periphyton community composition in artificial streams

Introduction

Changes in periphyton community taxonomic composition can have adverse consequences for consumers in affected ecosystems. Grazing macroinvertebrates are often constrained in food selection by their mouthpart morphology (Cummins and Klug, 1979), and can discriminate between some algal species, demonstrating preferences for certain types (Lamberti and Resh, 1983). Other researchers have found that grazer preferences can be based on availability of the food source (Paine and Vadas, 1969). Thus, alteration of the taxonomic composition of periphyton communities could induce bottom-up alterations in dependent food webs.

The adverse effects of anthropogenic pollutants on biological diversity of lotic periphyton is well documented (e.g., Patrick, 1971; Rai *et al.*, 1981). Heavy metal pollution (particularly copper; Reed and Gadd, 1989) leads to a reduction of species diversity in natural systems, culminating in the dominance of a few tolerant algal forms. Leland and Carter (1984), for example, found that dominant

species were eliminated from streams dosed with low levels of copper, while higher levels of copper reduced species richness to half that of control levels. Examination of responses of individual taxa with community-level tests may preclude testing responses of individual species in multiple toxicity tests.

A factor which has not been widely explored in algal toxicity studies is how irradiance levels affect the responses of algae to toxicants. Periphyton production (Hynes, 1970) and temporal species distributions (Patrick, 1971) in natural systems are often affected by seasonality, although the singular effects of light and temperature are difficult to separate. The single greatest factor affecting the type and abundance of algae in aquatic systems is the quantity and quality of impinging irradiance (Lee, 1989). Variation of light while maintaining equivalent temperatures can thus elucidate important effects due to irradiance levels. Standing crops of periphyton biomass (Rosemond, 1993) and successional trends (Steinman and McIntire, 1986) can be related to the irradiance received by the community. Diffusion of metal ions into periphyton mats has been found to be inversely proportional to mat density (Rose and Cushing, 1970), and differential responses of algal taxa to metals is well documented (Palmer, 1959; Rai *et al.*, 1981). As such, light levels may play a role in metal toxicity by

influencing both the exposure rate of the metal to all cells in the mat and the taxonomic composition of periphyton species within the mat.

This study investigated periphyton community taxonomic responses to five concentrations of copper in artificial streams under two levels of irradiance. Particular points addressed are distributions of taxa and responses of dominant taxa within and between copper treatments as influenced by irradiance, and successional trends and interactions of taxa by copper and light treatment. Community composition was compared between treatments on the basis of taxon percentage of total sample biovolume on an areal basis instead of numbers of cells per unit area as this more realistically represents the availability of each taxon to grazing macroinvertebrates.

Materials and Methods

The artificial stream system used for these experiments has been previously described (Chapter 2). Briefly, periphyton communities were allowed to colonize and develop in ten artificial streams, each receiving one of five copper treatments (0, 2.5, 5, 10, or 25 ug/L Cu^{++}) under two irradiance levels (400 or 40 $\mu E/m^2/s$). On four sampling dates (30, 37, 50, and 57 days post-colonization),

subsamples from four substrate units from each stream were pooled into a common sample. Samples were mounted on slides using Hyrax® for diatom identification and quantification (Stevenson and Stoermer, 1981) or Taft's Syrup Medium (Stevenson, 1984) for non-diatom community composition. A minimum of 500 cells from each sample were identified to lowest possible taxonomic level. Non-diatom cells were identified and quantified from TSM mounts and diatoms enumerated (although not identified). An equivalent number of diatoms were then identified using Hyrax® mounts. The dimensions of one hundred cells of the five most abundant taxa were measured to the nearest 0.5 um, and mean cell biovolumes were calculated. Total sample algal biovolumes were calculated by summing biovolumes of observed taxa, and taxon sample percentage biovolume was calculated by dividing taxon sample biovolume by total sample algal biovolume. Mean taxon sample percentage biovolumes were calculated by averaging individual values from the four samples. Data analysis and statistical tests were conducted using Statistical Analysis Software (SAS, Inc.).

Results and Discussion

Five taxa were dominant in the artificial streams.

These taxa consisted of the cyanophycean *Oscillatoria* sp. and *Chroococcus* sp., the chlorophycean *Scenedesmus quadricauda* and the diatoms *Achnanthes minutissima* and *Navicula seminulum*. Although *S. quadricauda* is typically encountered in lentic habitats, its presence in these lotic mesocosms is not unusual (see Prescott, 1970). Other taxa were extremely rare, never accounting for more than 0.6% of the community (by number) in any sample. These species were mainly the diatoms *Nitzschia palea, Gomphonema parvulum, Cocconeis placentula, Synedra ulna,* and *Eunotia* sp., in order of frequency encountered. Reasons for paucity of taxa in these streams are not known. In retrospect, propagule supplies through headbox inputs were probably inadequate, as Patrick (1971) emphasized the importance of a large species pool of potential colonizers to maintenance of species richness of diatom communities. Nutrient levels in the streams may have been unacceptably high, as many algae are adversely affected by elevated levels of phosphate or nitrate due to exclusion by tolerant species. Additionally, it is possible that greater species richness existed at earlier stages of the experiment but was not observed due to timing of sampling regime and exclusion of species in later successional stages. However, biomass standing stocks in higher copper treatment levels were sparse and significantly lower than in control and lower copper treatment levels

prior to initial community composition sampling (see Chapter 3), so it was assumed that analysis of such samples would not provide meaningful data.

Biovolumes of taxa were calculated and plotted on an areal basis for high light and low light streams for each of the sampling dates (Figs. 4.1, 4.2). Conversion factors used to convert from cell number to biovolume were 14 um^3/cell for *Chroococcus*, 1 um^3/cell for *Oscillatoria*, 168 um^3/cell for *S. quadricauda*, 118 um^3/cell for *N. seminulum*, and 42 um^3/cell for *A. minutissima*. This conversion emphasizes the presence of taxa with larger cells and de-emphasizes the presence of taxa with smaller cells, and is appropriate because it normalizes the proportion of total biovolume comprised by a particular taxon to a fraction of available resource, the factor of importance from the perspective of a grazing macroinvertebrate. Comparison of communities based on taxon percent of sample biovolume (Figs. 4.3, 4.4) was necessary because of significant differences in areal periphyton biomass between treatments.

i. Effects of copper on taxonomic dominance

Species dominance within copper treatments was evaluated by combining data from all samples and comparing each taxon's mean percentage of total algal biovolume in each sample (Table 4.1). In control streams, *Chroococcus* sp. and *N. seminulum* were codominant, accounting for over 42% and

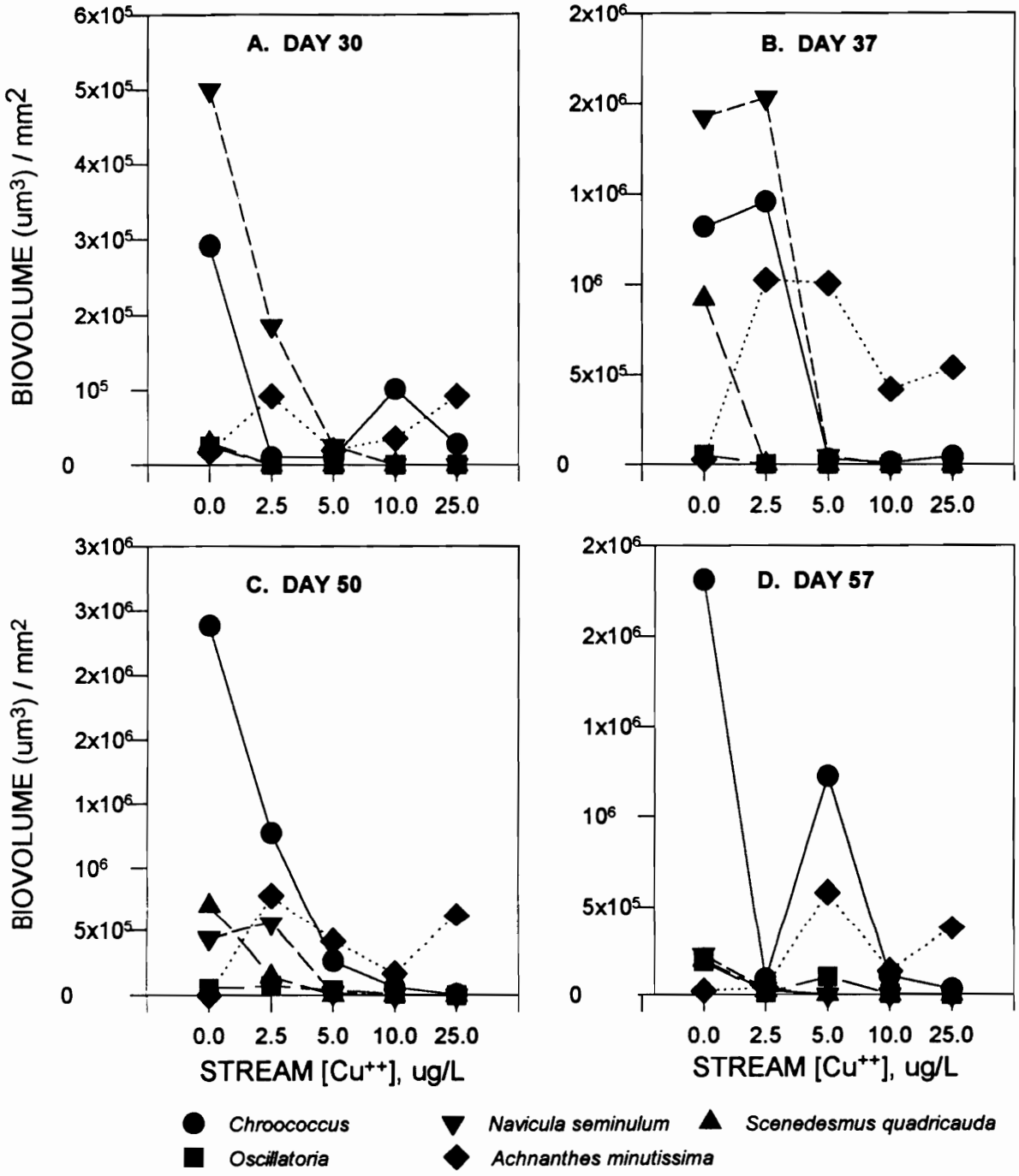

Figure 4.1. Stream community composition data per unit area from high light streams for each of the sample dates. Concentrations indicated are nominal doses of Cu^{++}.

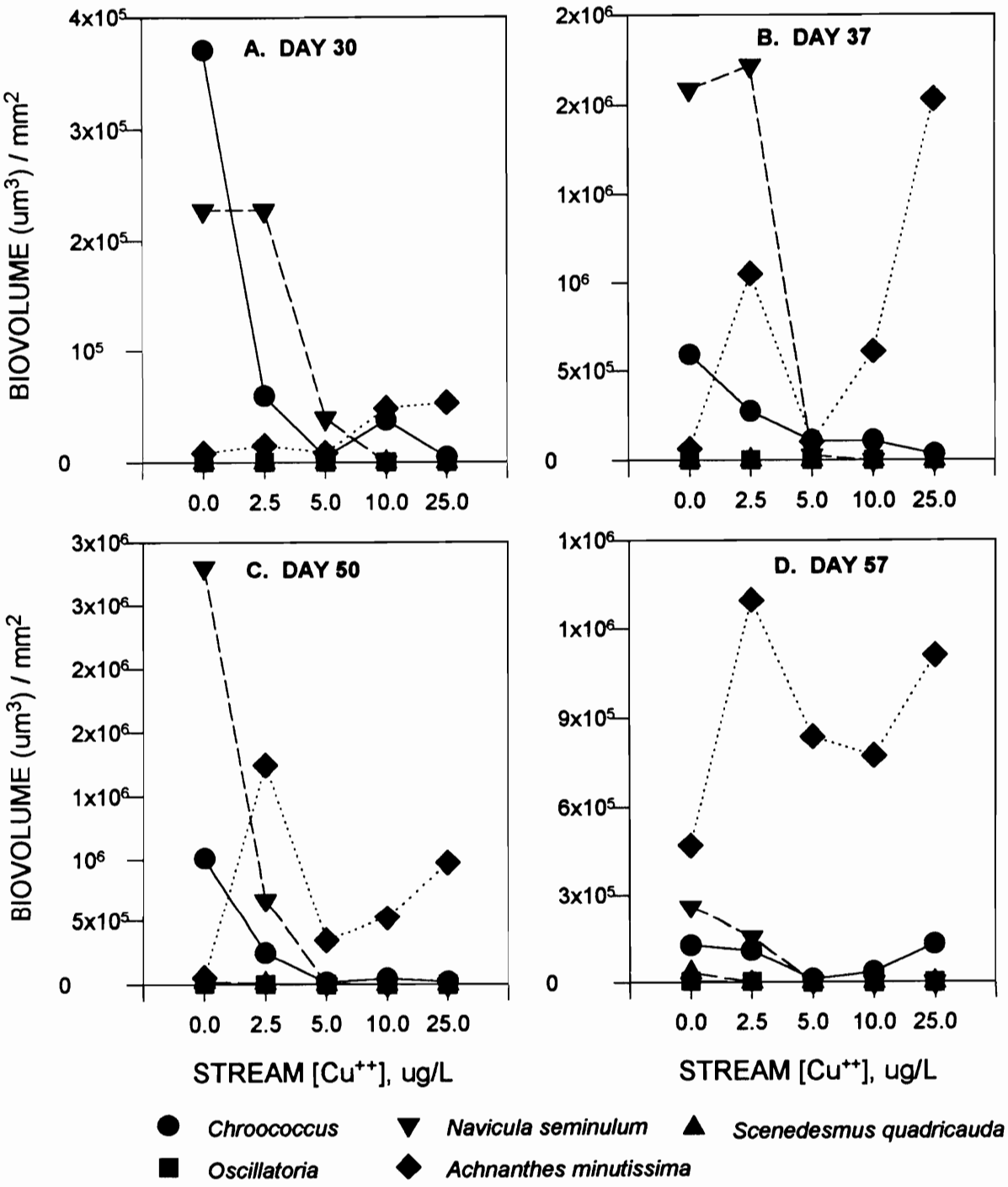

Figure 4.2. Stream community composition data per unit area from low light streams for each of the sample dates. Concentrations indicated are nominal doses of Cu^{++}.

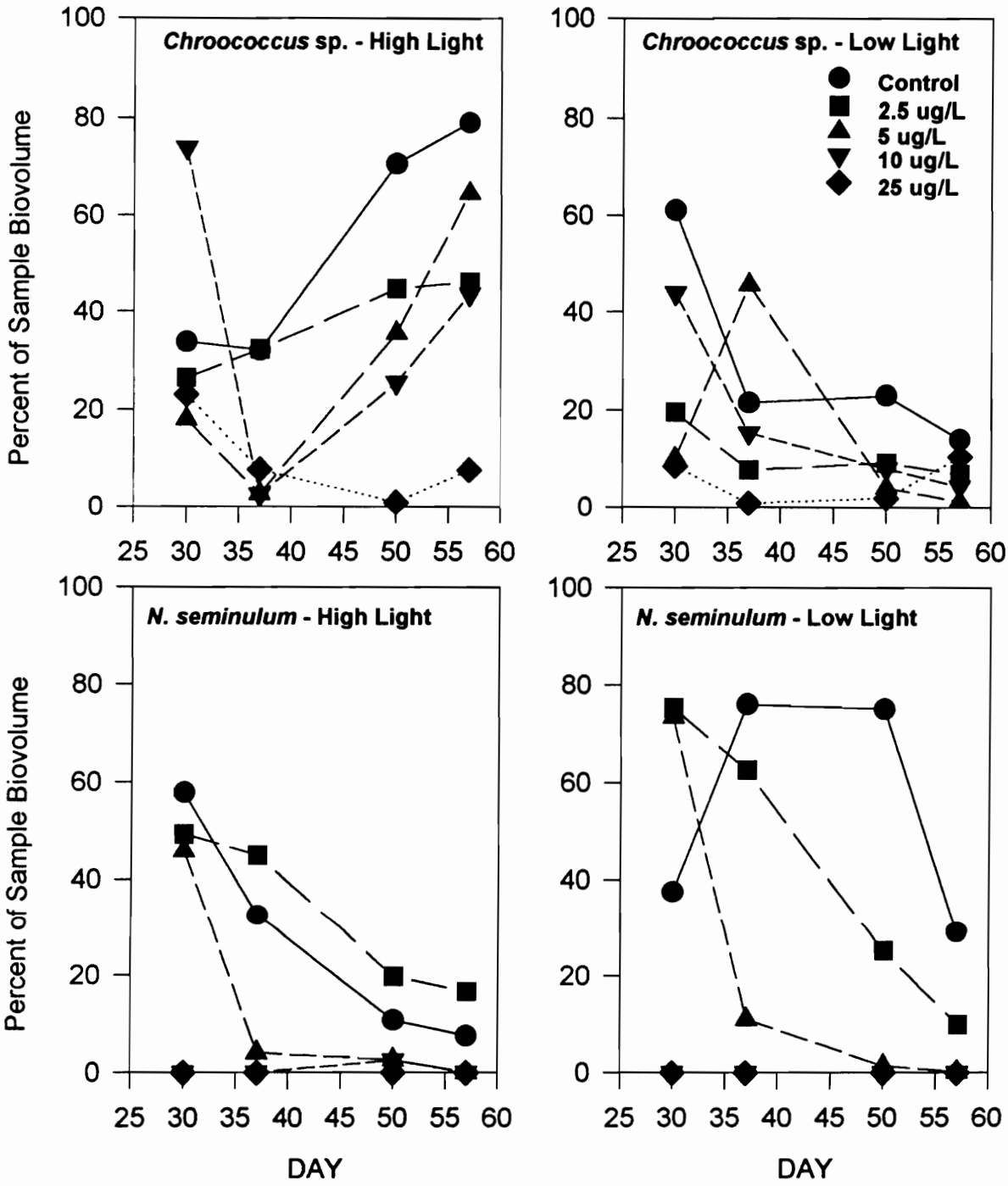

Figure 4.3. Treatment taxon percents of sample biovolume for *Chroococcus* sp. and *Navicula seminulum* by sampling day.

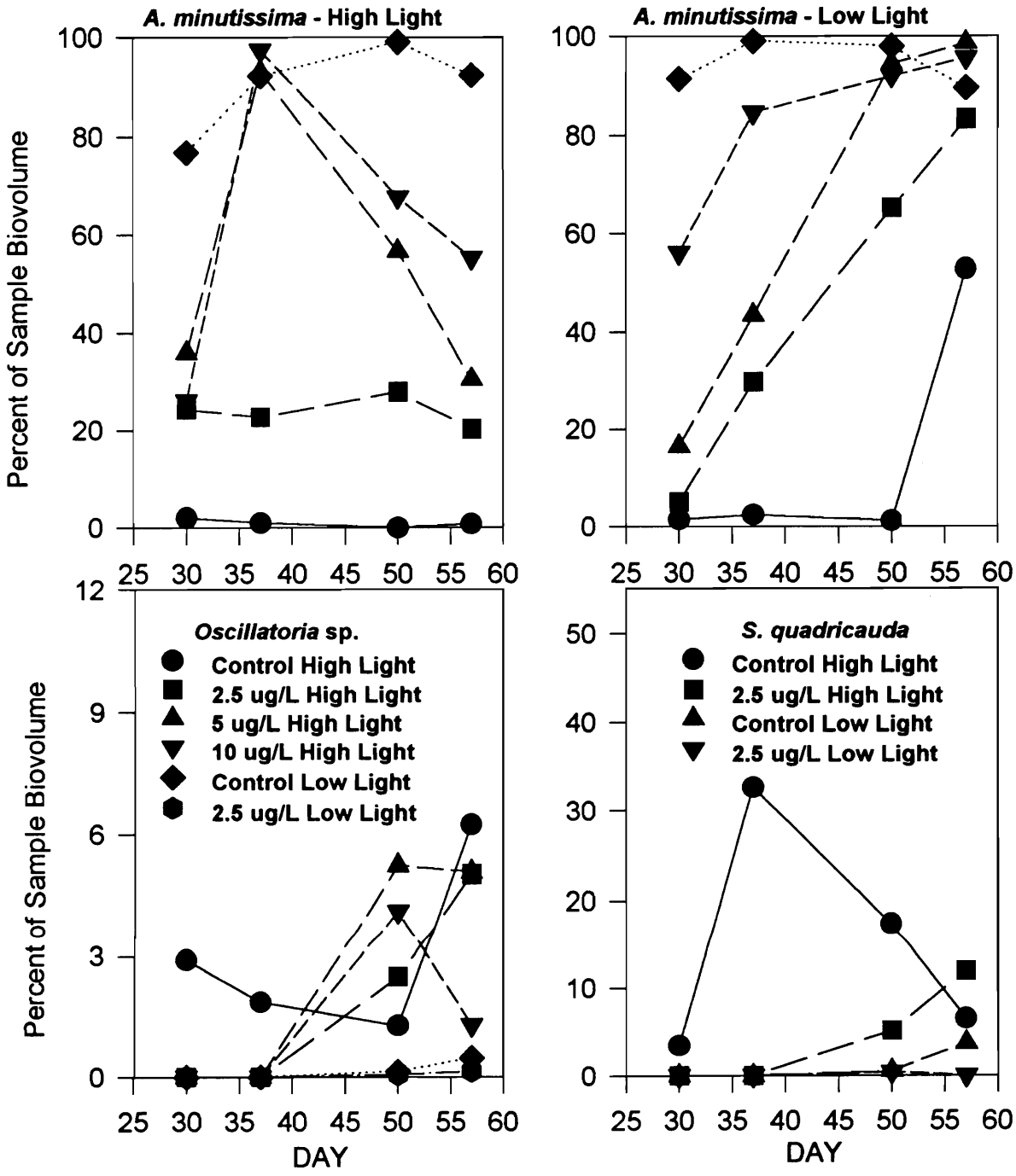

Figure 4.4. Treatment taxon percents of sample biovolume for *Achnanthes minutissima*, *Oscillatoria* sp., and *Scenedesmus quadricauda* by sampling day. Copper treatment plot symbols for *A. minutissima* are: control, ●; 2.5 ug/L, ■; 5 ug/L, ▼; 10 ug/L, ▲; and 25 ug/L, ♦. Symbols for *Oscillatoria* sp. and *S. quadricauda* plots are indicated.

Table 4.1. Comparison of mean percentage of algal species biovolumes observed within copper treatments using Fisher's Least Significant Difference. Means indicated by different levels are significantly different ($\alpha = 0.05$). LSD is the minimum difference between sample means required for a statistically significant difference for this α level. Mean is the mean percentage of total sample algal biovolume comprised by the indicated species.

A. Control streams: LSD = 0.1918

Species	Mean	T Grouping
Chroococcus sp.	41.87	A
Navicula seminulum	40.78	A
Scenedesmus quadricauda	8.03	B
Achnanthes minutissima	7.72	B
Oscillatoria sp.	1.61	B

B. 2.5 ug/L streams: LSD = 0.1763

Species	Mean	T Grouping
N. seminulum	37.95	A
A. minutissima	34.79	A
Chroococcus sp.	24.12	A
S. quadricauda	2.18	B
Oscillatoria sp.	0.97	B

C. 5 ug/L streams: LSD = 0.2205

Species	Mean	T Grouping
A. minutissima	58.69	A
Chroococcus sp.	22.72	B
N. seminulum	17.29	BC
Oscillatoria sp.	1.29	BC
S. quadricauda	0.00	C

D. 10 ug/L streams: LSD = 0.1612

Species	Mean	T Grouping
A. minutissima	71.89	A
Chroococcus sp.	27.11	B
N. seminulum	0.31	C
Oscillatoria sp.	0.68	C
S. quadricauda	0.000	C

E. 25 ug/L streams: LSD = 0.1574

Species	Mean	T Grouping
A. minutissima	80.93	A
Chroococcus sp.	7.63	B
S. quadricauda	0.00	B
Oscillatoria sp.	0.00	B
N. seminulum	0.00	B

41% respectively of the observed algal biomass in samples from these streams. Subdominant species were *S. quadricauda* (8%), *A. minutissima* (8%), and *Oscillatoria* sp. (2%). The lowest copper treatment level (2.5 ug/L Cu^{++}) induced a shift in species dominance. Samples were dominated by *N. seminulum* (38%), *A. minutissima* (35%), and *Chroococcus sp.* (24%). *S. quadricauda* (2%) and *Oscillatoria* sp. (1%) accounted for the remaining biomass. Communities in streams dosed with higher levels of copper were dominated by *A. minutissima*, which accounted for 59%, 72%, and 81% of the observed algal biovolume in samples from streams dosed with 5, 10, and 25 ug/L Cu^{++}, respectively, while *Chroococcus* sp. (23%, 27%, and 8%) was the primary subdominant species. Other species accounted for less than 20% of the observed algal biovolume at these copper levels.

The results of the present study are in agreement with previously published data. The most thorough study of copper effects on lotic periphyton community composition was conducted by Leland and Carter (1984). These researchers found that algal species composition in stream reaches dosed with 2.5 ug/L Cu^{++} differed from control reaches, due to replacement of the control codominant *Lyngbya* sp. by *A. minutissima*. This trend was noticed in all reaches treated with copper. However, of the 22 most abundant taxa in control reaches, only one (*Lyngbya* sp.) was negatively impacted

by 2.5 ug/L CU^{++}, while four taxa were reduced in absolute abundance by 5 ug/L Cu^{++} and 16 were reduced by 10 ug/L Cu^{++} (the highest treatment level). In contrast to the present study, Weber and McFarland (1981) found that *N. seminulum* became more abundant in stream reaches dosed with 120 ug/L Cu^{++} (only treatment level). However, water hardness in that study averaged greater than 250 mg/L as $CaCO_3$. Gustavson and Wängberg (1995) found significant reductions (> 50%) in phytoplankton species richness in enclosures dosed with 15 ug/L Cu^{++}, although there was no reduction in species richness in enclosures dosed with 5 ug/L Cu^{++}.

Without data concerning relative nutritional value or macroinvertebrate selectivity for the taxa observed in this study, predictions of adverse effects on grazing communities based on observed changes in periphyton community composition are not possible. However, Leland *et al.* (1989) found that aquatic insect community composition was less sensitive to copper exposure than periphyton. Of the 37 most abundant macroinvertebrate taxa, none declined in abundance at continuous exposure to 2.5 ug/L Cu^{++} although significant changes in periphyton community composition were noted. Nineteen macroinvertebrate species declined at 5 ug/L Cu^{++}, although it is not possible to distinguish between effects due to direct toxicity of copper on the macroinvertebrates and effects due to altered trophic resources.

ii. Responses of taxa to copper treatments

The responses of individual taxa to copper treatments were evaluated by comparing the mean percentage of total sample algal biovolume each species composed between copper treatments (Table 4.2). The dominance of *Chroococcus* sp., which was codominant in control streams, was not significantly reduced by copper concentrations through 10 ug/L Cu^{++}. *N. seminulum*, the other codominant taxon in control streams, was significantly reduced by 5 ug/L Cu^{++}. Other species were also affected by copper treatment. Dominance of *A. minutissima* significantly increased and the proportion of *S. quadricauda* significantly decreased in all streams treated with copper as compared to control streams. *A. minutissima* composed a low proportion of biomass in control streams but became dominant or codominant as a result of all copper treatments. The abundance of *S. quadricauda*, while not dominant in control streams, significantly decreased as a result of all copper treatments, and did not occur in samples from streams receiving more than 2.5 ug/L Cu^{++}. Abundance of *Oscillatoria* sp., which did not dominate in any treatment, was not statistically reduced as a result of any of the copper treatments, although it did not occur in streams receiving 25 ug/L Cu^{++}. These results can be used to rank the relative sensitivities of the taxa to copper. In order of decreasing sensitivity, *S. quadricauda* >

Table 4.2. Comparison of effects of copper on each species' mean percentage of total sample algal biovolume using Fisher's Least Significant Difference (paired T-tests). Means indicated by different levels are significantly different ($\alpha = 0.05$). All comparisons are between 8 samples. LSD is the minimum difference between sample means required for a statistically significant difference for this α level. Mean is the mean percentage of total sample algal biovolume comprised by the indicated species.

A. *Chroococcus* sp.: LSD = 0.2076

$[Cu^{++}]$, ug/L	Mean	T Grouping
0	41.87	A
10	27.11	A B
2.5	24.12	A B
5	22.72	A B
25	7.63	B

B. *Navicula seminulum*: LSD = 0.2039

$[Cu^{++}]$, ug/L	Mean	T Grouping
0	40.78	A
2.5	37.95	A
5	17.29	B
10	0.31	B
25	0.00	B

C. *Achnanthes minutissima*: LSD = 0.2379

$[Cu^{++}]$, ug/L	Mean	T Grouping
25	92.37	A
10	71.89	A B
5	58.69	B
2.5	34.79	C
0	7.71	D

D. *Oscillatoria* sp.: LSD = 0.0181

$[Cu^{++}]$, ug/L	Mean	T Grouping
0	1.61	A
5	1.29	A
2.5	0.97	A
10	0.68	A
25	0.00	A

E. *Scenedesmus quadricauda*: LSD = 0.0557

$[Cu^{++}]$, ug/L	Mean	T Grouping
0	8.03	A
2.5	2.18	B
5	0.00	B
25	0.00	B
10	0.00	B

Oscillatoria sp. > *N. seminulum* > *Oscillatoria* sp. > *A. minutissima*.

The resistance of *A. minutissima* to copper is well documented, and is often a dominant species in polluted rivers, especially those impacted by heavy metals (Watanabe *et al.*, 1988). In the present study, as in Leland and Carter (1984), it was the primary replacement species in communities from streams treated with copper.

iii. Interaction of light and copper effects on periphyton communities

Dominances of taxa within copper treatments on the basis of mean percentages of sample biovolumes within copper treatments were compared between high and low light streams to determine how light affected copper effects on taxonomic distribution (Table 4.3). Mean taxon distributions within streams were compared using pairwise T-tests ($\alpha = 0.05$). Pairwise T-tests were used to test for significant differences in taxon distributions between high and low light streams for each copper treatment (Table 4.4). Comparisons were made on the difference in percent biovolume of each taxon in high and low light streams for each sampling day. In control streams, decreasing light reduced the dominance of *Chroococcus* sp. relative to *N. seminulum*, although differences between taxon dominances in paired samples were not

Table 4.3. Comparison of species dominance (biovolume mean percentage of sample total) within copper treatments between high and low light streams. Within light levels, taxa means of samples (N = 4) were grouped using Fisher's LSD (paired T-tests). (continued next page)

A. Control streams

HIGH LIGHT			SPECIES		LOW LIGHT		
T Grouping	Std Dev	Mean			Mean	Std Dev	T Grouping
A	24.40	53.86	1	3	54.44	24.67	A
B	23.26	27.11	3	1	29.87	21.16	AB
CB	13.21	15.00	5	4	14.47	25.51	CB
CB	2.21	3.07	2	5	1.06	1.79	C
C	0.85	0.96	4	2	0.16	0.23	C

B. $[Cu^{++}]$ = 2.5 ug/L

HIGH LIGHT			SPECIES		LOW LIGHT		
T Grouping	Std Dev	Mean			Mean	Std Dev	T Grouping
A	9.56	37.39	1	4	45.81	35.11	A
A	16.80	32.71	3	3	43.19	30.83	A
A	3.19	23.77	4	1	10.84	5.93	B
B	5.65	4.26	5	5	0.10	0.20	B
B	2.41	1.88	2	2	0.05	0.07	B

C. $[Cu^{++}]$ = 5 ug/L

HIGH LIGHT			SPECIES		LOW LIGHT		
T Grouping	Std Dev	Mean			Mean	Std Dev	T Grouping
A	28.40	54.08	4	4	63.30	40.05	A
BA	26.49	30.20	1	3	21.45	34.98	B
BC	21.93	13.13	3	1	15.25	20.59	B
BC	2.99	2.58	2	2	0.00	0	B
C	0	0.00	5	5	0.00	0	B

Table 4.3 (continued) For species, 1 = *Chroococcus* sp., 2 = *Oscillatoria* sp., 3 = *Navicula seminulum*, 4 = *Achnanthes minutissima*, and 5 = *Scenedesmus quadricauda*. Mean is the mean percentage of sample algal biovolume comprised by the indicated taxon; Std Dev is the sample standard deviation of the mean. Means indicated by different letters are significantly different ($\alpha = 0.05$).

D. $[Cu^{++}] = 10$ ug/L

HIGH LIGHT					LOW LIGHT		
T Grouping	Std Dev	Mean	SPECIES		Mean	Std Dev	T Grouping
A	29.59	61.65	4	4	82.13	17.94	A
A	30.12	36.36	1	1	17.87	17.94	B
B	1.96	1.36	2	3	00.00	0	C
B	1.25	0.63	3	2	00.00	0	C
B	0	0.00	5	5	00.00	0	C

E. $[Cu^{++}] = 25$ ug/L

HIGH LIGHT					LOW LIGHT		
T Grouping	Std Dev	Mean	SPECIES		Mean	Std Dev	T Grouping
A	9.40	90.17	4	4	71.69	4.74	A
B	9.40	9.83	1	1	5.43	4.74	B
C	0	0.00	3	3	0.00	0	B
C	0	0.00	2	2	0.00	0	B
C	0	0.00	5	5	0.00	0	B

Table 4.4. Comparison of light effects on means of sample differences of algal taxon percents of total algal sample biovolumes by copper treatments. For each comparison (N = 4), taxon percents of total sample biovolume in high and low light streams were compared using paired T-tests under the hypothesis that biovolume percents were equal. ND represents comparisons not conducted due to lack of taxa in indicated copper treatment.

A. Control streams

SPECIES	T: High = Low	Pr>|T|
Chroococcus sp.	1.169831	0.3266
Oscillatoria sp.	2.879498	0.0636
N. seminulum	-1.50744	0.2288
A. minutissima	-1.05388	0.3693
S. quadricauda	1.981785	0.1418

B. $[Cu^{++}]$ = 2.5 ug/L

SPECIES	T: High = Low	Pr>|T|
Chroococcus sp.	3.653137	0.0354
Oscillatoria sp.	1.564062	0.2158
N. seminulum	-1.45415	0.2419
A. minutissima	-1.23252	0.3055
S. quadricauda	1.474939	0.2367

C. $[Cu^{++}]$ = 5 ug/L

SPECIES	T: High = Low	Pr>|T|
Chroococcus sp.	0.668578	0.5516
Oscillatoria sp.	1.731601	0.1818
N. seminulum	-1.25538	0.2982
A. minutissima	-0.34406	0.7535
S. quadricauda	ND	ND

D. $[Cu^{++}]$ = 10 ug/L

SPECIES	T: High = Low	Pr>|T|
Chroococcus sp.	1.630483	0.2015
Oscillatoria sp.	1.391345	0.2583
N. seminulum	1.000000	0.3910
A. minutissima	-1.76525	0.1757
S. quadricauda	ND	ND

E. $[Cu^{++}]$ = 25 ug/L

SPECIES	T: High = Low	Pr>|T|
Chroococcus sp.	1.100767	0.3514
Oscillatoria sp.	ND	ND
N. seminulum	ND	ND
A. minutissima	-1.10077	0.3514
S. quadricauda	ND	ND

statistically significant ($P < 0.33$ and 0.23, respectively). In high light streams dosed with 2.5 ug/L Cu^{++}, *Chroococcus* sp. was a codominant taxon, accounting for a mean percentage of 37% of the total algal biovolume in samples. However, in low light streams dosed at this copper level this taxon became subdominant to *A. minutissima* and *N. seminulum*, representing only 11% of the mean total algal biovolume. This was the only taxon whose distribution was significantly different ($P < 0.036$) within copper treatments as a result of decreasing light levels. In the low light streams, *Chroococcus* sp. was replaced by *A. minutissima* relative to high light streams. This trend also occurred in 5 ug/L streams, with high light *Chroococcus* sp. reduced to subdominance in low light stream. Its decrease was supplanted by an increase in dominance of *A. minutissima*. Although present in high light streams, *Oscillatoria* sp. was not detected in samples from low light streams, although this absence was not statistically significant due to low distributions in the high light treatment. Reduction in light excluded both *Oscillatoria* sp. and *N. seminulum* in the 10 ug/L treatments. Light treatment did not induce significant changes in community composition in streams dosed with 25 ug/L Cu^{++}. From these results, it appears that only *Chroococcus* sp. was definitively more sensitive to copper when irradiance was reduced, although this could be true for

Oscillatoria sp. and *N. seminulum*.

It is possible that individual algal cells and taxa are more susceptible to toxicants when less energy is available for use in ameliorating its effects. At irradiance levels below compensation, growth is not possible. Above compensation, excess energy is available for growth and reproduction. Added toxicants stress organisms, increasing respiration and the amount of energy needed for maintenance, thus decreasing energy available for growth (Selye, 1956). This has the net effect of raising the compensation point for individual taxa. Conflicting evidence exists to support this hypothesis. Kamp-Neilsen (1969) reported that toxicity of copper to *Chlamydomonas pyrenoidosa* is related to light intensity. Azeez and Bannerjee (1987) found that acute (6 hour) copper exposure reduced *Anacystis nidulans* chlorophyll *a* to a greater extent under illuminated conditions than in dark. This trend was not consistent with cadmium exposure to *Spirulina platensis*, however, as reductions in chlorophyll *a* were similar in light and dark treatments. The toxicity of the triazine herbicide atrazine to two species of algae is also affected by irradiance levels (Mayasich *et al.*, 1986). Atrazine is relatively more toxic to the diatom *Phaeodactylum tricornutum* at high light levels, while it is relatively more toxic to the eustigmatophyte *Nannochloris oculata* at low light levels.

iv. Successional trends within streams

Several of the taxa exhibited trends through time (Figs. 4.3, 4.4). In general, *Chroococcus* sp. increased in dominance in high light streams with time, and decreased in dominance in low light streams. *N. seminulum* decreased in dominance in high light streams with time, and *A. minutissima* increased in dominance in low light streams.

Simple linear regressions of taxon percent biomass against time for each of the species and treatments were conducted (Table 4.5). Of the fifty total possible regressions (five taxa X five copper treatments X two light treatments), seven individual taxon regressions were statistically significant ($P < 0.05$), and explained a large fraction (> 90%) of the observed variation in the taxon's percentage of sample biovolume. Thirteen of the possible regressions were not conductible due to lack of the specific taxon in any of the samples from the particular treatment, seven were not significant due to presence of the taxon in the final two samplings only, and two were not significant due to presence of the taxon in only one sample from the particular treatment. Of the statistically significant regressions, six were from three streams in which pairs of taxa changed relative to each other. In high light control and 2.5 ug/L Cu^{++} streams, *Chroococcus* sp. increased and *N. seminulum* decreased in proportion over the course of the experiment.

Table 4.5. Significant linear regressions of taxon biovolume percents of sample totals versus time within copper and light treatments. Regressions used the model Biovolume % = m (Day) + b, where m is Slope and b is Intercept. SE is the standard error of the indicated estimate. Slope is expressed as change in sample percent biovolume per day, while Intercept is expressed as sample percent biovolume. Prob>F is the probability that the slope of the regression is equal to zero.

[Cu^{++}]	Light	Species	Intercept ± SE	Slope ± SE	Prob>F	R-square
Control	High	*Chroococcus* sp.	-29.5 ± 17.6	1.91 ± 0.39	0.0396	0.9225
Control	High	*N. seminulum*	106.5 ± 16.8	-1.83 ± 0.37	0.0395	0.9225
2.5 ug/L	High	*Chroococcus* sp.	3.9 ± 4.4	0.77 ± 0.10	0.0157	0.9688
2.5 ug/L	High	*N. seminulum*	91.1 ± 9.1	-1.34 ± 0.20	0.0221	0.9562
2.5 ug/L	Low	*N. seminulum*	152.5 ± 5.9	-2.51 ± 0.13	0.0028	0.9945
2.5 ug/L	Low	*A. minutissima*	-78.8 ± 5.4	2.87 ± 0.12	0.0017	0.9965
5 ug/L	Low	*A. minutissima*	-76.4 ± 20.4	3.21 ± 0.45	0.0195	0.9614

Exchange rates (decrease in *N. seminulum* and increase in *Chroococcus* sp.) observed in the control stream were equivalent. The exchange rate in the 2.5 ug/L Cu^{++} stream for *Chroococcus* sp. was only half that for *N. seminulum*, with the balance accounted for by an increased (although statistically not significant) presence of *S. quadricauda* and *Oscillatoria*. In the low light 2.5 ug/L Cu^{++} stream, *N. seminulum* significantly decreased in dominance as *A. minutissima* increased in dominance at similar rates through the experiment.

Conclusions

Comparison of community composition data as biovolume percentages shows that both light and copper treatments affected the taxonomic composition of periphyton communities in these mesocosms. The lowest copper treatment (2.5 ug/L) induced development of communities significantly different from control communities on the basis of dominant taxa. Relative taxonomic sensitivities in these streams was concluded to be *S. quadricauda* > *Oscillatoria* sp. > *N. seminulum* > *Oscillatoria* sp. > *A. minutissima*, in order of decreasing sensitivity. Reduction in irradiance level also induced significant changes in community composition. *Chroococcus* sp. was negatively impacted by irradiance reduc-

tion, benefitting *A. minutissima*.

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Chapter 5. Effects of copper on periphyton phosphorus, copper, and protein content

Introduction

Toxicological tests for regulatory purposes are based (in part) on observed mortality of individuals as a function of toxicant concentration in laboratory settings. Extrapolation of these results to environmental situations may not be valid for predictions of ecosystem responses to toxicant exposure (Cairns, 1983), as deleterious effects on various important ecological processes may be affected at toxicant levels below those predicted by laboratory tests. Reductions in trophic resources could have negative effects on organisms in the absence of direct toxicological effects.

Grazing macroinvertebrates, like all other consumers, have dietary requirements for numerous macro- and micronutrients. Primary macronutrients required include fixed carbon, nitrogen, and phosphorus, although specific dietary requirements of these compounds are not well documented. Stressed organisms may have different dietary requirements or nutritional needs than unstressed organisms. For instance, metal toxicity may lead to increased need for various amino acids such as cysteine due to increased

metallothionein production (see Abdel-Mageed and Oehme, 1990).

Heavy metals may also affect primary producers in a similar manner. Detoxification of metals through polyphosphate production (Sicko-goad and Stoermer, 1979; Kuwabara, 1985; Pettersson *et al.*, 1986; Twiss and Nalewajko, 1992; Verma *et al.*, 1993) or metallothionein binding (reviewed by Rai *et al.*, 1981) leads to increased need for phosphorus or nitrogen by these organisms. Additionally, such phosphate-based mechanisms of detoxification generally lead to higher internal polyphosphate and metal concentrations. These studies, however, were conducted *in vitro* using single species of algae. Such mechanisms have not been demonstrated to increase phosphorus concentrations of periphyton communities.

Effects of nutritional uptake of metals such as copper on aquatic consumers have been studied (e.g., Chang and Sibley, 1993). These results generally show that heavy-metal exposure via food uptake is less toxic to higher trophic levels (e.g., predators) than aqueous exposures when the two can be studied. Additionally, although such metals do increase in concentration in primary consumers, biomagnification up the trophic chain generally does not occur (Kiffney and Clements, 1993).

The present study was designed to investigate effects

of Cu^{++} exposure on the nutritional content of periphyton communities, and determine if any detected alterations would constitute additional risks to aquatic consumers beyond environmental exposure. It was hypothesized that phosphorus content of the periphyton would be related to copper bioconcentration due to polyphosphate-mediated Cu^{++} detoxification.

Materials and Methods

Experimental protocols for growth and sampling of periphyton communities have been previously described (Chapter 2). Briefly, periphyton communities were allowed to colonize and develop in ten artificial streams, each receiving one of five Cu^{++} treatments (0, 2.5, 5, 10, or 25 ug/L Cu^{++}) under two irradiance levels (40 or 400 $\mu E/m^2/s$). Periphyton phosphorus content was determined using the ascorbic acid method (APHA, 1992) from aliquots of persulfate-digested periphyton suspension. Periphyton protein content was determined spectrophotometrically using the method of Bradford (1976) from periphyton aliquots digested in 10% NaOH (Rausch, 1981). Data for periphyton community protein content were obtained during previous experiments under low light conditions (Chapter 2, Experiment 2). Periphyton copper bioconcentration was

determined by graphite furnace atomic absorption (Perkin Elmer 1100) from AFDW residues digested by refluxing in 50% HNO_3 (APHA, 1992). Preliminary experiments showed no significant difference between this method and using whole periphyton samples. Standards for all measurements were digested and analyzed as samples. Data was analyzed using Statistical Analysis Software (SAS Institute, 1990).

Samples were analyzed for nutrient and copper content from four sampling dates. Periphyton biomass on the first sampling date (Day 23) was not sufficient for analysis with techniques used here due to the fact that samples were divided for determination of several different parameters (AFDW and community composition, in addition to analyses discussed here). Phosphorus subsamples for Day 43 were accidently contaminated and rendered unusable, so data for copper bioconcentrations for this day were not included.

Results and Discussion

i. Periphyton phosphorus concentrations

Periphyton phosphorus concentrations (Figure 5.1) were not related to copper concentrations or sampling day in either light treatment (ANOVA $P > 0.09$). In high light streams, periphyton levels were somewhat inversely related to copper concentrations, although this relationship was

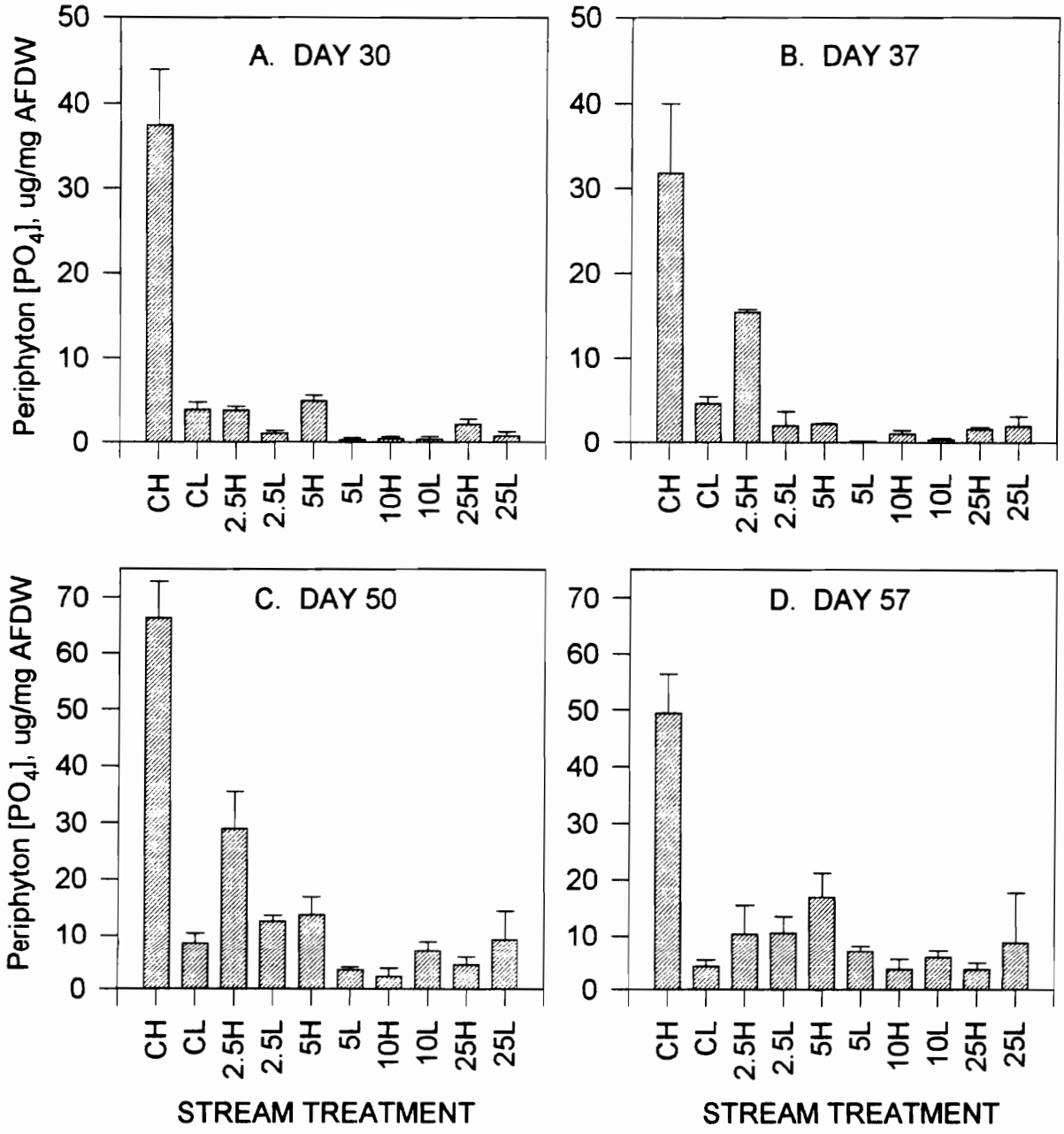

Figure 5.1. Periphyton phosphorus contents after 30, 37, 50, and 57 days of growth in relation to stream copper amendments in high (A) and low (B) light streams. Day 30 values are means of six samples, while other dates are means of 4 samples. Error bars are ± 1 standard deviation. Copper treatments (C, 2.5, 5, 10, and 25) and light treatments (H, L) are indicated.

not statistically significant. Periphyton phosphorus concentrations in control streams and 2.5 ug/L treatments were each significantly higher (Duncan's Multiple Range; $\alpha = 0.05$) than in other treatments. High light control stream periphyton had significantly higher phosphorus than low light control stream periphyton for all sample days (T-test, $P < 0.001$). In low light streams, periphyton from streams receiving 2.5, 5, or 10 ug/L Cu^{++} had significantly higher phosphorus (Duncan's Multiple Range; $\alpha = 0.05$) in samples from days 50 and 57 than the first two sample days. Other relationships were not statistically significant. It is possible that increased periphyton phosphorus content was not observed due to lack of polyphosphate detoxification mechanisms in the major taxa observed in these streams (*Chroococcus* sp., *Oscillatoria* sp., *Scenedesmus quadricauda*, *Navicula minima*, and *Achnanthes minutissima*), because they taxa have not been previously demonstrated to form polyphosphate bodies in response to metal exposure (Cembella *et al.*, 1984). Additionally, changes in periphyton community composition over the course of the experiment may have contributed to variability in P content. Kesler (1982) found that phosphorus content of periphyton in a small pond was more strongly related to water column total phosphorus when high numbers of filamentous algae were present.

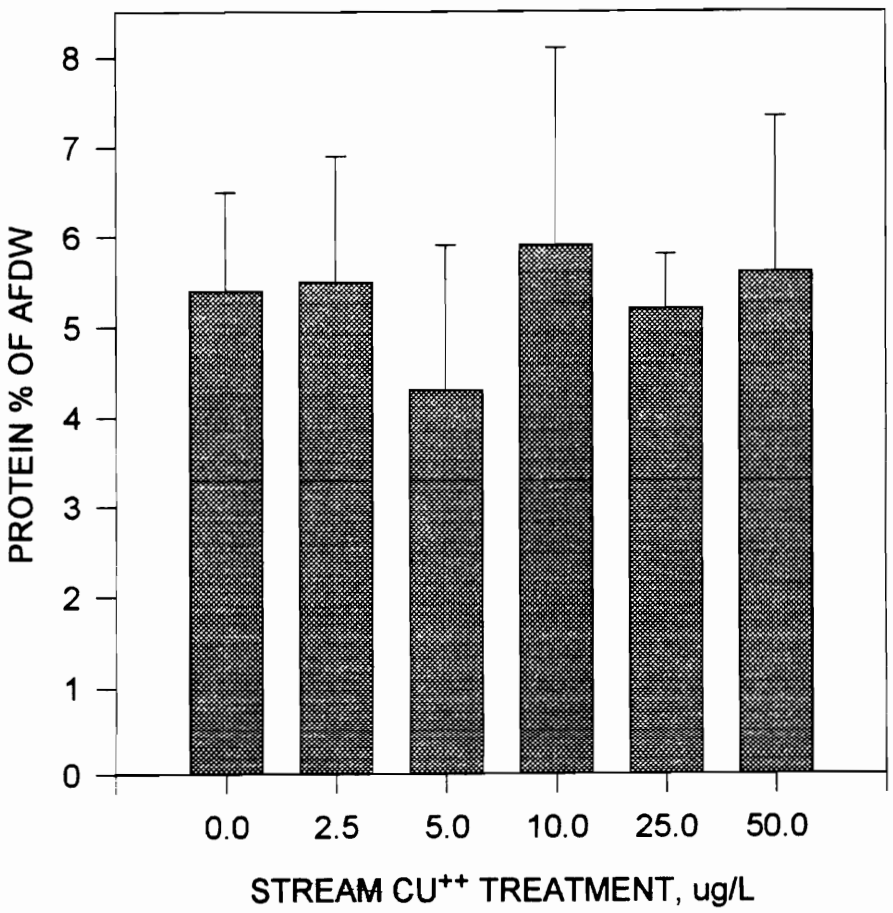

Figure 5.2. Periphyton community protein content as a function of [Cu^{++}] in low light streams. Data are from artificial stream experiments conducted in 1992. Bars are means of estimates from four samples ± 1 standard deviation.

ii. Periphyton protein content

Data for periphyton community protein contents as a percentage of AFDW (Figure 5.2) demonstrated that this parameter did not vary significantly (ANOVA $P > 0.83$) due to copper treatments. Clark *et al.* (1982) investigated effects of 50 ug/L Cu^{++} on periphyton communities in artificial streams. In copper-treated streams, protein (2% NaCl soluble) on an areal basis did not differ significantly from control streams. Percent protein of the community (AFDW) was usually higher than in controls, although all values were between 3-8%. It is possible that these percentages would change if calculated as a percentage of dry weight, as was observed by Clark *et al.* (1982). This would occur if diatom densities were high enough that frustule masses caused dry weight and AFDW estimates to vary between samples. Reduced overall availability due to decreased periphyton biomass (as with total phosphorus availability) are the main effects on protein.

iii. Periphyton copper bioconcentration

Copper bioconcentrations (Fig. 5.3) were linearly related to water column copper concentrations. Measurements conducted on Day 30 and Day 37 were much higher than on subsequent sampling dates. This is probably due to extremely low biomass measurements at that time, and small errors in biomass determination led to large errors in

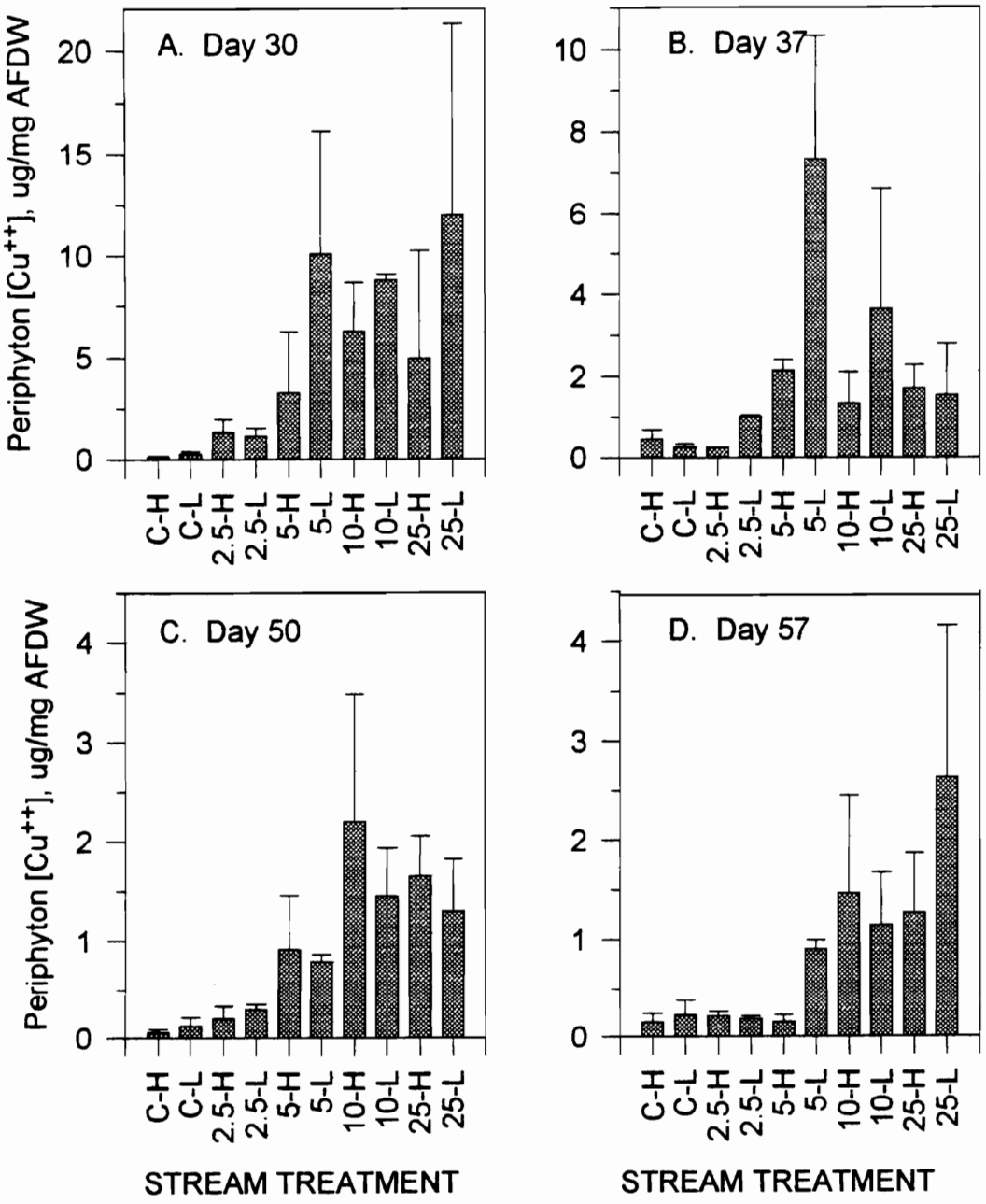

Figure 5.3. Periphyton community copper bioconcentration as a function of light and copper treatments. Data are means of either six samples (Day 30) or four samples (other dates) ± 1 standard deviation. Copper treatments (C, 2.5, 5, 10, and 25) and light treatments (H, L) are indicated.

copper bioconcentration calculations. Copper bioconcentration factors (bioconcentrations divided by water column concentrations) were approximately 10^6 ($10^{5.56} \pm 10^{5.39}$; mean ± 1 std. dev.) in control streams as opposed to approximately 10^5 ($10^{5.10} \pm 10^{4.78}$; mean ± 1 std. dev.) observed in copper dosed streams. The difference (higher bioconcentrations in control stream periphyton) is due to the extremely low water column copper concentrations in control streams.

Hatakeyama (1989) studied the chronic effects of copper toxicity on survival, growth, and emergence of the mayfly *Epeorus latifolium*. Effects due to food-borne copper were examined in the absence of water column exposures by placing larvae and previously-exposed periphyton into undosed stream microcosms. Other larvae received both water column and food-borne copper exposure. Dual exposure tests found that chronic reductions in larval growth rate occurred between 10 and 15 ug/L Cu^{++} (algal concentration 110-240 ug Cu^{++}/g algae). Similar reductions in growth due to food-borne copper were found when larvae were fed algae containing 1140 ug Cu^{++}/g algae. In the present experiment, such algal copper bioconcentrations were found after 50 days exposure to between 5 and 10 ug/L Cu^{++}.

Conclusions

Detoxification of copper via polyphosphate formation (as evidenced by increased periphyton phosphorus content) was not observed in these experiments, and phosphorus content was not related to copper bioconcentration. As such, it is determined that the taxa of algae observed in these experiments do not use this detoxification method. Contrary to expectations, an inverse relationship between periphyton phosphorus content and copper concentration was observed for early sampling days. Periphyton protein content was not related to copper treatment.

Periphyton copper concentrations which have deleterious effects on mayfly growth were observed for stream copper concentrations below 10 ug/L Cu^{++}. Effects of copper on nutrient (phosphorus, protein) contents of periphyton communities seem to be mainly on reduced availability due to decreased biomass rather than on actual concentrations within the community. As such, these are relatively insensitive parameters for predicting adverse effects on aquatic consumers.

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Chapter 6. Effects of copper on leaf processing and nutritional suitability to a shredding macroinvertebrate

Introduction

The majority of stream trophic structure can be supported by allochthonous inputs, especially leaf material (Cummins, 1974). The relative importance of allochthonous inputs for supplying energy to higher trophic levels is dependent on the nature of the body of water, surrounding watershed, and season (e.g., Vannote *et al.*, 1980).

Leaf material which enters streams is processed by a consortium of microorganisms (Suberkropp and Klug, 1976). These consumers are responsible for the majority of the enzymatic digestion of the leaf. This partial digestion of leaves by aquatic microbiota has been termed "conditioning" (Boling *et al.*, 1975). During leaf conditioning, the quality of the leaves as food sources for aquatic macroinvertebrates is increased. Concentrations of nitrogen, phosphorus, and utilizable carbon compounds generally increase, and the leaf matrix becomes softer (Webster and Benfield, 1986). Macroinvertebrates are able to detect the degree of conditioning of leaves, and prefer those with greater conditioning (Cargill *et al.*,1985).

To a large extent, current methods of environmental

assessment do not address impacts on detritus-based processes (Stephan *et al.*, 1985). Relatively little research has been conducted to determine if detritus processing, especially the processing of allochthonous organic material, is negatively impacted in stressed streams. Alterations of total available nutrients or energy from these sources could restrict secondary production in affected aquatic systems. Impairment of leaf conditioning from an introduced toxicant could cascade into declines in populations of higher trophic levels (shredders, collectors, gatherers, and predators) beyond those expected due to the immediate effects of the toxic substance. Diminished food resources could exacerbate the initial physiological effects of the stressor on these higher organisms. Therefore, effects on these ecological processes must be considered as well as effects on higher trophic levels.

The present study examined several parameters related to leaf conditioning to determine effects due to addition of Cu^{++} to conditioning media. These parameters included leaf conditioning rates, phosphorus content, nitrogen content, and leaf Cu^{++} content. Additionally, the suitability of treated leaves as a nutritional resource for a shredding macroinvertebrate was investigated by comparing lipid contents of stonefly larvae fed leaves from these treatments.

Materials and Methods

i. Leaf conditioning treatments

Leaves from four species of tree were used: red maple (*Acer rubrum*), pin oak (*Quercus palustris*), weeping willow (*Salix babylonica*), and smooth alder (*Alnus serrulata*). Leaves were collected at abscission and air-dried on lab benchtops for 7 days. Leaves (10 g) were conditioned in 2 L conditioning medium amended with Cu^{++} (0, 5, 50, and 500 ug/L) in 4L Erlenmeyer flasks in an environmental chamber maintained at 15°C. Conditioning medium was adapted from Suberkropp *et al.* (1983) (contents listed in Table 6.1) and adjusted to pH 7.0. This formula reduced medium water hardness from 300 mg/L to 50 mg/L to minimize the interaction of copper with $CaCO_3$ (USEPA, 1985). Equivalent concentrations of nitrate, phosphate, and sulfate were maintained. Medium was made in 64 L batches, and culture media were refreshed every 48 hr. Heterotrophic microorganisms were introduced to the cultures by adding 10 ml of homogenized stream leafpack material at each refreshment. Cultures were aerated by aquarium pumps during the incubations. Extent of leaf conditioning was estimated by measuring leaf penetrances (Feeny, 1970; D'Angelo *et al.*, 1991) of 10 leaves (five measurements per leaf) of each species from each treatment every 7 days. Initial penetrances were determined

Table 6.1. Comparison of ingredients (g/L) used in conditioning media by Suberkropp *et al.* (1983) and in the present study. Equivalent concentrations of major ions (excluding Ca^{++} and Mg^{++}) were maintained.

INGREDIENT	**Suberkropp *et al.***	**PRESENT STUDY**
KNO_3	1.01	1.01
NaCl	0.11	0.11
KH_2PO_4	0.41	0.41
K_2HPO_4	0.52	0.52
$MgSO_4 \circ 7H_2O$	0.49	0.082
$CaCl_2 \circ 2H_2O$	0.15	0.025
Na_2SO_4	---	0.235

from leaves soaked for 24 hours in deionized distilled H_2O. Leaves used for penetrance measurements were discarded due to contamination from the penetrometer (brass rod) and handling. Leaf conditioning rates were determined using a negative exponential model (rate = - d(ln penetrance)/dt) analogous to leaf breakdown rate (Jenny *et al.*, 1949; Olson, 1963; Webster and Benfield, 1986).

ii. Leaf chemical analyses

a. Phosphorus Leaf phosphorus content was measured from triplicate leaf samples powdered with a mortar and pestle. Samples (≈20 mg) and standards were suspended in 2.0 ml deionized distilled (DDI) H_2O and digested by addition of 0.5 g potassium persulfate, 0.500 ml 10 N H_2SO_4, and autoclaved for 30 min. Leaf particles were still evident after the first digestion so the procedure was repeated. Samples were neutralized with 5N NaOH and volumes raised to 20 ml. Phosphate concentrations were measured using the ascorbic acid method (APHA, 1992) using 1000 µl of digested samples, 5 ml DDI H_2O, and 1000 µl of the combined ascorbic acid reagent.

b. Nitrogen Total Kjeldahl nitrogen was measured in samples submitted to the Forage Testing Laboratory at Virginia Tech. Only single estimates of leaf N could be obtained due to dearth of leaf sample remaining following feeding experiments and sample size required for testing

protocol.

c. Copper Leaf Cu^{++} content was estimated from triplicate samples (≈ 20 mg) digested in 500 µl concentrated metals-grade HNO_3 and diluted 1:10. Standards were treated as samples. Cu^{++} concentrations in digests and standards were measured by graphite furnace atomic absorption spectrophotometry using a Perkin Elmer 1100.

iii. Stonefly feeding experiments

Stonefly larvae (*Pteronarcys* sp.; Plecoptera: Pteronarcyidae) were collected from White Rocks Branch in the Jefferson National Forest in Giles County, VA. Middle instars (approximately 1.5 cm length) were collected from under stones and in leaf packs using a D-frame kick net and transported to the Ecosystem Simulation Laboratory at Virginia Tech. Twenty-six larvae were immediately sacrificed to serve as lipid content controls. Stoneflies were randomly distributed between feeding treatments, using 10 larvae per treatment. Each larva was placed in a 1-pint HDPE food container containing 500 ml continuously aerated dechlorinated tap H_2O which was refreshed twice per week. Feeding experiments were conducted in an environmental chamber at 12.5°C. Stoneflies were fed treated leaves daily. The amount of leaf offered to each larvae varied according to the amount of leaf remaining unconsumed from the previous day.

iv. Stonefly lipid content

Stonefly production (weight gain) proved too variable to allow estimation of leaf nutritional suitability, so lipid contents of larvae were measured. Following feeding experiments, larvae were starved 24 hr to clear their digestive tracts and oven-dried for 24 hr at 95°C. It was assumed that potential losses due to lipid volatization would be a constant fraction, so that any such losses would not affect differences between treatments. Total lipids were extracted using a 2:1 mixture of chloroform and methanol (Folch *et al.*, 1957). Extracts were not washed to obtain estimates of polar lipid derivatives. Each larva was extracted three times with 3 ml of extractant for 24 hr followed by a final extraction with 1 ml. Final extracts were clear, indicating that all lipids were extracted.

Results and Discussion

i. Leaf conditioning treatments

Initial leaf penetrances varied between species, ranging from 275.7 ± 72.3 g/5 mm^2 for maple, 379.3 ± 49.4 g/5 mm^2 for alder, 460.9 ± 58.5 g/5 mm^2 for oak, and 466.9 ± 30.5 g/5 mm^2 for willow. Initial penetrances were

significantly different between all species (ANOVA; $p < 0.0001$) except oak and willow (ANOVA; $p = 0.6006$).

Leaf conditioning (Figures 6.1, 6.2) rates were compared using dummy variable linear regression analysis of natural log-transformed penetrance measurements versus degree-days (Table 6.2). Comparison of control regression slopes indicates that leaf species were conditioned at significantly different rates (Table 6.2A). Willow leaves were conditioned at the fastest rate ($m = -0.0064$/degree-day), followed by alder (-0.0052/degree-day), maple (-0.0027/degree-day), and oak (-0.0019/degree-day). Conditioning half-lifes, statistical time for penetrance measurements to decrease by 50% ($= \ln 2 / |\text{slope}|$), indicate that conditioning microorganisms reduce willow penetrance by 50% in 109 degree days, while 132, 255, and 370 degree-days are required for alder, maple, and oak, respectively. The observed conditioning rates are not related to initial penetrances. Willow had the highest rate of conditioning and the highest initial mean penetrance, while oak was conditioned at the slowest rate although it had the second highest initial mean penetrance. Maple, which had the lowest initial penetrance was conditioned at the second lowest rate.

These rates for leaf conditioning are higher than published rates of leaf processing in natural systems.

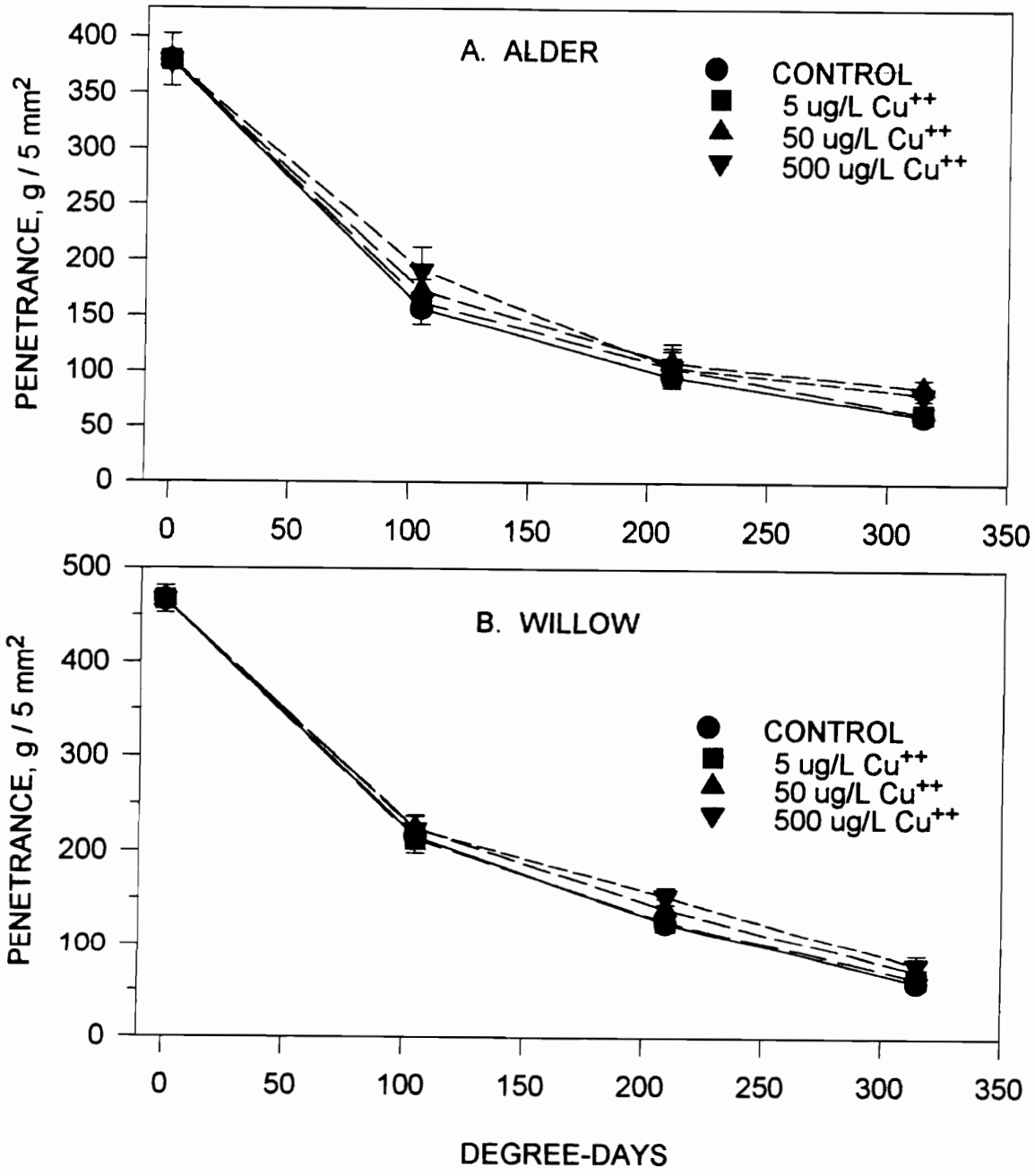

Figure 6.1. Conditioning of alder and willow leaves (as measured by leaf penetrance) in response to copper treatments in artificial microcosms. Copper treatments are indicated in legend. Values are means ± 95% confidence limits for each treatment on each sampling date.

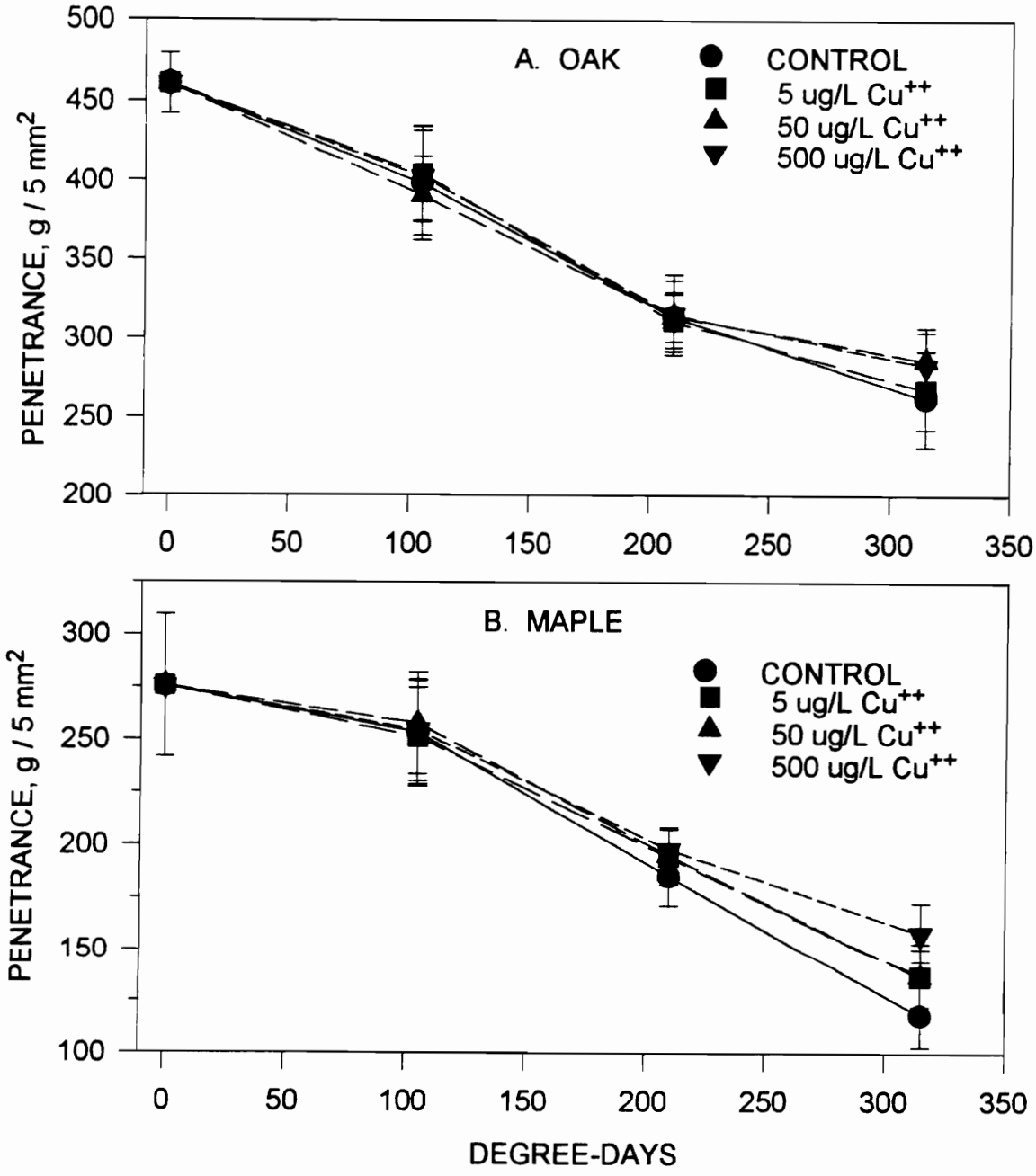

Figure 6.2. Conditioning of oak and maple leaves (as measured by leaf penetrance) in response to copper treatments in artificial microcosms. Copper treatments are indicated in legend. Values are means ± 95% confidence limits for each treatment on each sampling date.

Table 6.2. Comparison of conditioning rates between (A) and within (B-E) species as affected by conditioning media copper amendment. Regressions used an inverse exponential model of decreasing penetrance versus degree-day and were compared via dummy variable analysis. Between species comparisons were made between control groups for each leaf type; T for H0: $m_1 = m_2$ and $P_{I,1} = P_{I,2}$ indicate tests of hypotheses for equivalent slopes and initial mean penetrances between leaf types. Within species, Pr > |T| indicates results of statistical tests for equivalency between control and treatment regression slopes (for CONTROL, H_o: SLOPE = 0 was tested).

A. Comparison of regression parameters between leaf types

COMPARISON	T for H0: $m_1 = m_2$	Pr > |T|	T for H0: $P_{I,1} = P_{I,2}$	Pr > |T|
WILLOW, ALDER	4.41	0.0001	6.7524	0.0001
WILLOW, MAPLE	12.63	0.0001	10.8957	0.0001
WILLOW, OAK	16.78	0.0001	0.5264	0.6006
ALDER, MAPLE	8.71	0.0001	5.2889	0.0001
ALDER, OAK	12.73	0.0001	-5.3522	0.0001
OAK, MAPLE	3.00	0.0031	10.6724	0.0001

B. ALDER (R^2 = 0.800695; F = 288.11; Pr > F 0.0001)

TREATMENT	SLOPE	Pr > |T|
CONTROL	-0.005234686	0.0001
5 ug/L Cu^{++}	-0.005354574	0.7006
50 ug/L Cu^{++}	-0.004420303	0.0092
500 ug/L Cu^{++}	-0.004742379	0.1126

C. MAPLE (R^2 = 0.497455; F = 49.78; Pr > F = 0.0001)

TREATMENT	SLOPE	Pr > |T|
CONTROL	-0.002717309	0.0001
5 ug/L Cu^{++}	-0.002198211	0.1323
50 ug/L Cu^{++}	-0.002246120	0.1716
500 ug/L Cu^{++}	-0.001736254	0.0046

D. OAK (R^2 = 0.594670; F = 92.64; Pr > F = 0.0001)

TREATMENT	SLOPE	Pr > |T|
CONTROL	-0.001872642	0.0001
5 ug/L Cu^{++}	-0.001827357	0.8171
50 ug/L Cu^{++}	-0.001600280	0.1647
500 ug/L Cu^{++}	-0.001729201	0.4750

E. WILLOW (R^2 = 0.936546; F = 849.73; Pr > F = 0.0001)

TREATMENT	SLOPE	Pr > |T|
CONTROL	-0.006367627	0.0001
5 ug/L Cu^{++}	-0.006167334	0.3636
50 ug/L Cu^{++}	-0.005887761	0.0303
500 ug/L Cu^{++}	-0.005535934	0.0002

Processing rates measure loss of mass from leaf packs, including losses due to microbial respiration, shredder consumption, and erosion of leaves by transported particles, and individual experimental designs can have significant effects on the processing rate (Benfield *et al.*, 1979). Webster and Benfield (1986) tabulated mean leaf processing rates for many families of plants from literature reports. Approximate mean rates of 0.005/d for Betulaceae (alder) and Salicaceae (willow), 0.004 for Aceraceae (maple), and 0.0025/d for Fagaceae (oak) were determined. Conditioning rates observed in the present study were 0.096/d for willow, 0.079/d for alder, 0.041 for maple, and 0.028 for oak. Published rates for individual species used in this study include 0.0047 and 0.0013 for *S. babylonica* (Hanlon, 1982), 0.0041 for *A. serrulata* (Witkamp and Frank, 1969), 0.0062 for *A. rubrum* (Petersen and Cummings, 1974), and 0.0008 for *Q. palustris* (Day, 1982). Differences between the present experiment and results of others are due to the difference in parameters studied, although the trends between species are consistent with literature reports.

ii. Effects of copper on leaf conditioning

Amendment of conditioning media with 5 ug/L Cu^{++} did not significantly reduce conditioning rates (Table 6.2B-E), as regression slopes were not significantly different from

control slopes for any of the species ($P > 0.05$). Treatment with 50 ug/L Cu^{++} significantly reduced conditioning rates for alder ($P < 0.01$) and willow ($P < 0.04$), although not for maple or oak. Treatment with 500 ug/L Cu^{++} significantly reduced conditioning rates for maple ($P < 0.005$) and willow ($P < 0.002$), although not for alder ($P > 0.10$) or oak ($P > 0.47$). On the basis of these results, there is a weak trend relating copper susceptibility to conditioning rate. Willow conditioning was most affected by copper treatments, as it was significantly reduced by both 50 and 500 ug/L Cu^{++} treatment, while oak leaf conditioning was not significantly reduced by copper treatment. Maple leaf conditioning, which occurred at an intermediate rate, was reduced by 500 ug/L Cu^{++} treatment. Effects on alder leaf conditioning were mixed, showing significant reduction at 50 but not 500 ug/L Cu^{++} treatment. Examination of data indicates that although 500 ug/L Cu^{++} treatment reduced alder leaf conditioning as compared to control, higher variance in penetrance measurements for this species precluded statistical detection of significant rate reduction.

Calculations of chronic effects on leaf conditioning rates as the geometric mean of NOEC and LOEC (APHA, 1992) indicate that copper chronic effects concentration for alder and willow leaf conditioning is 15.8 ug/L, and 158 ug/L for maple. A chronic effects concentration for oak leaf

conditioning cannot be calculated due to lack of significant effects for any concentration tested.

Leland and Carter (1985) studied the effects of copper treatment on leaf processing in streams, and found that microbial respiration on willow and quaking aspen leaves conditioned at 10 and 15 ug/L Cu^{++} was reduced as compared to controls, although observed decreases were not statistically significant. Bringmann and Kühn (1980) observed a reduction in bacterial reproduction at 30 ug/L Cu^{++}, although the decrease was slight (3%). Coal ash effluent also impedes leaf processing as determined by ATP content and leaf disk weight loss (Forbes and Magnuson, 1980), attributed in part to higher concentrations of chromium, barium, and aluminum in exposed leaves. In soft water (hardness < 11 mg/L), cadmium concentrations of 5 ug/L significantly decreased leaf processing rates and microbial colonization of mixed-species leaf packs (Giesy, 1978).

Growth of leaf colonizing bacteria on agar plates amended with copper (Figure 6.3) was studied. This experiment allows determination of the effects of copper bound to organic matter on the growth of microorganisms. Amendment of agar with 1.00 mg/L Cu^{++} did not significantly reduce the growth of microbial colonies, although growth was statistically decreased ($P < 0.001$; ANOVA) by 10.0 mg/L Cu^{++}. Hornor (1984) found that microbial respiration in

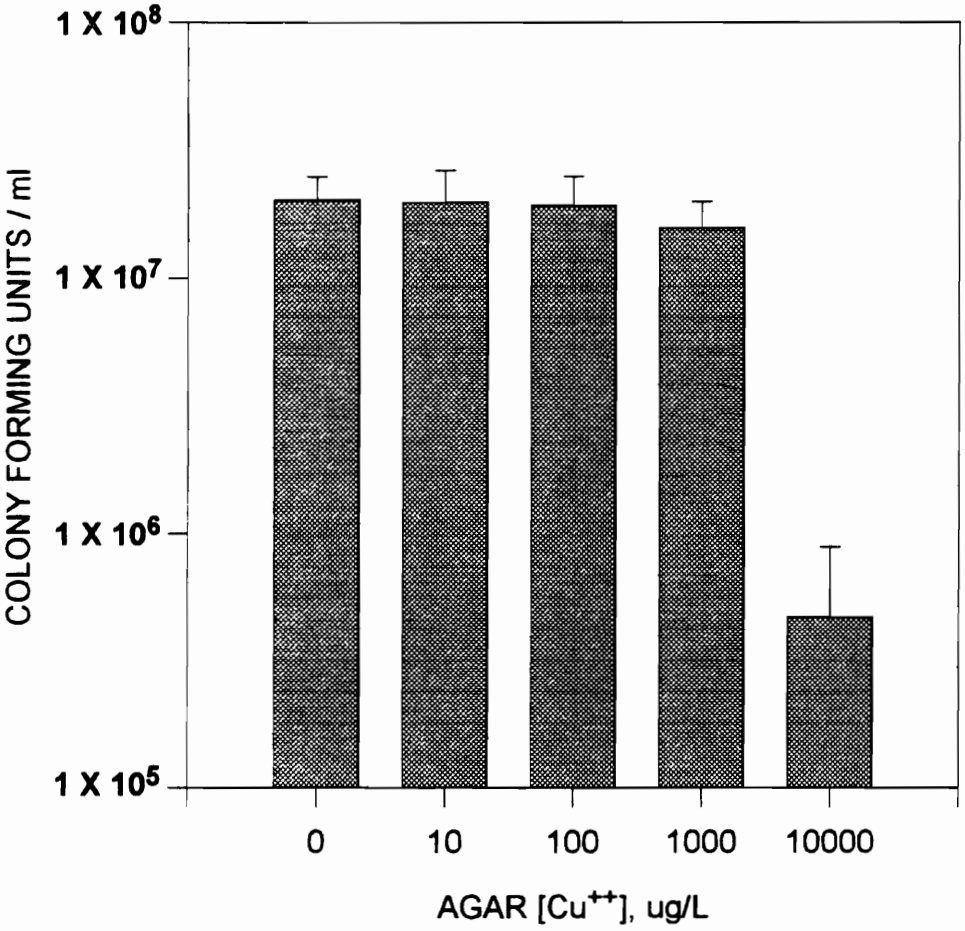

Figure 6.3. Growth of aquatic leafpack-inhabiting microorganisms on plate count agar amended with Cu^{++}. Growth was quantified by counting the number of colony-forming units (CFU) which reproduced to yield visible colonies after 96 hours of incubation. Data are means of six replicates ± 1 standard deviation.

artificial streams treated with 26 mg/L Zn^{++} consumed only 31% less O_2 than reference microcosms, indicating that bacteria and fungi can be extremely resistant to added metals.

iii. Leaf phosphorus content

Initial leaf phosphorus measurements were not significantly different between alder, maple, and oak (1.13-1.38 ug PO_4/mg leaf; ANOVA $P > 0.45$), while willow ($[PO_4^-]$ = 1.83 ug/mg) was significantly higher (ANOVA $P < 0.05$) than alder and oak (Figure 6.4). Conditioning rates were correlated with initial phosphorus content ($\rho = -0.52893$; $P < 0.036$).

Phosphorus contents of leaves were increased by conditioning in all cases. Final concentrations in oak leaves were significantly lower (ANOVA $P < 0.001$) than in other species, a reflection of their slower conditioning rates. Within species, control leaf phosphorus contents were significantly higher than treated leaves only for maple (ANOVA $P < 0.03$) due to high variability in determinations. However, there was a trend of higher phosphorus content in control leaves than treated ones.

iv. Leaf nitrogen content

Willow leaves had the highest initial nitrogen content (1.1%; Figure 6.5), significantly greater (ANOVA, $P < 0.05$)

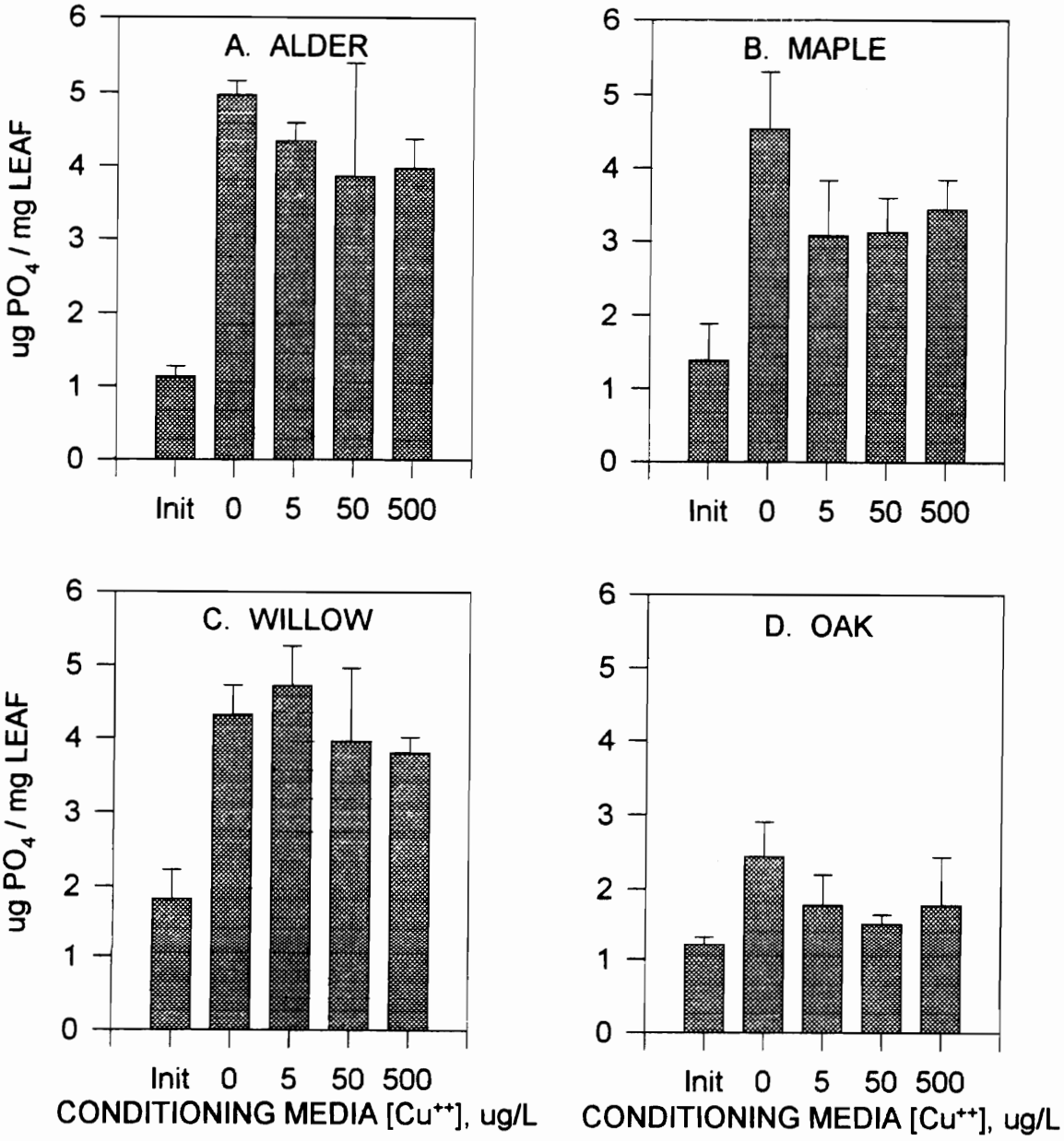

Figure 6.4. Mean leaf phosphorus contents in conditioning experiments after 21 days. Initial leaf values are indicated by "Init", while copper treatments are as indicated in legend. Error bars are ± sample standard deviation.

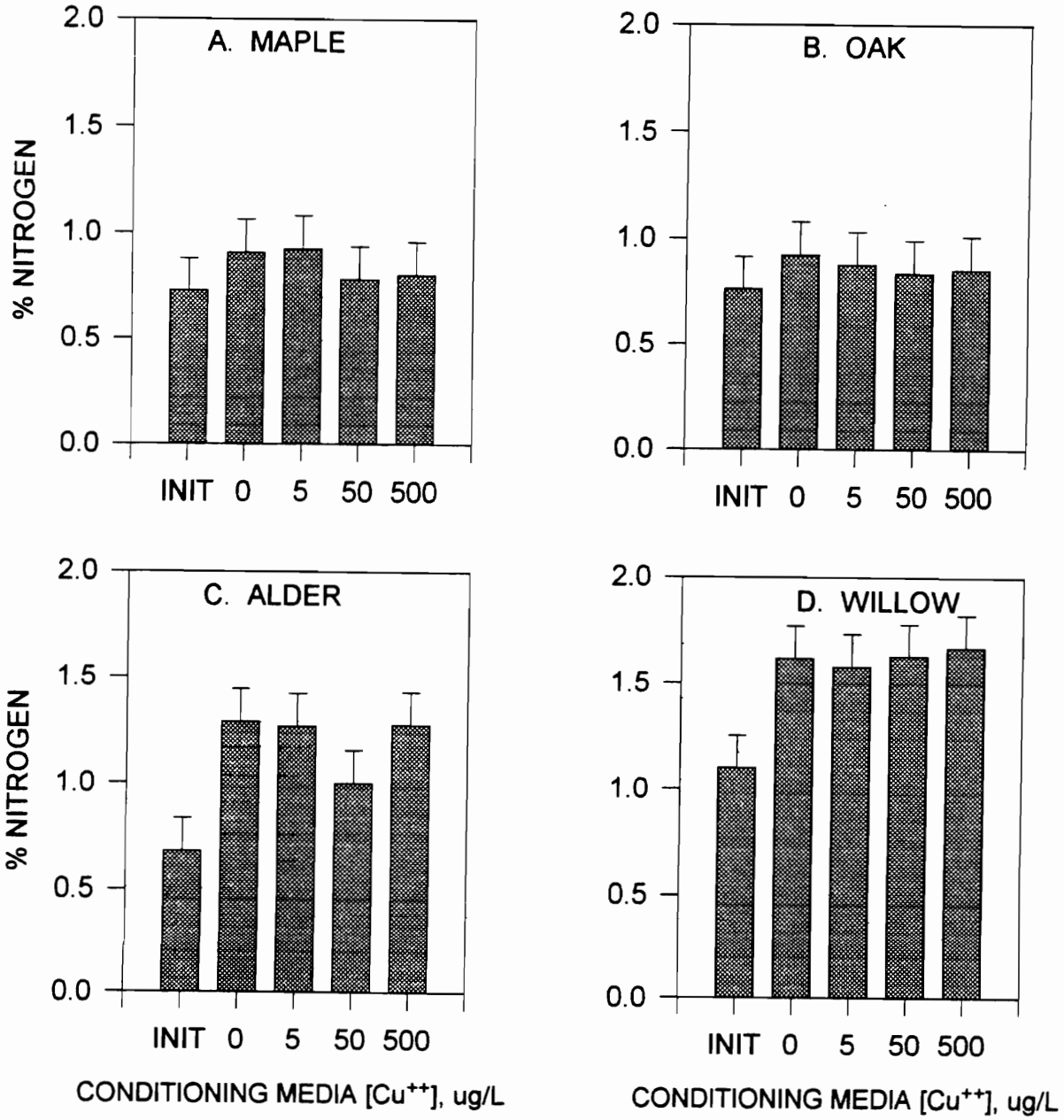

Figure 6.5. Nitrogen content of leaves in conditioning experiments after 21 days. Data result from analysis of single replicates; error bars are ± population standard deviation. Initial values are indicated by "Init", while copper treatments are as indicated in legend.

than oak (0.76%), maple (0.76%), and alder (0.68%). Oak, maple, and alder nitrogen contents were not significantly different (ANOVA, $P > 0.65$).

Many researchers (e.g., Kaushik and Hynes, 1971) have shown that leaf breakdown rates are greater for leaves with higher nitrogen content. The present results are in agreement with past reports. Conditioning rates were strongly correlated with initial nitrogen content ($\rho = -0.59929$; $P < 0.015$).

Nitrogen contents of alder and willow leaves increased significantly (ANOVA, $P < 0.05$) due to conditioning, although final values for maple and oak were not significantly greater than initial levels. Leaf nitrogen contents usually increase as conditioning proceeds (Kaushik and Hynes, 1971; Bärlocher *et al.*, 1978), and these data confirm results obtained from conditioning rate determinations. Willow and alder leaves were conditioned at significantly higher rates than maple and oak leaves, and these differences are reflected in both changes in leaf nitrogen content and final nitrogen contents. Both alder and willow leaves had significantly higher nitrogen contents ($P < 0.005$) than either oak or maple.

Copper treatments did not have significant effects on leaf nitrogen contents (ANOVA $P > 0.05$), despite the differences in conditioning rates which resulted from copper

treatments. Leland and Carter (1985) found that leaf C:N ratios were decreased as a result of treatment with 2.5 ug/L Cu^{++} as compared to controls, but differences were not evident between copper treatments ranging up to 15 ug/L. Total nitrogen contents were not reported.

v. Leaf copper content

Prior to treatment, maple leaves had significantly higher copper content ($P < 0.05$; Duncan's Multiple Range; Table 6.3A) than other leaves. Initial copper measurements did not differ significantly between alder, oak, and willow. Conditioning significantly increased leaf copper concentrations above initial values in all treatments ($P < 0.05$; T-test; Figure 6.6). Leaf copper bioaccumulations were strongly related to copper treatments (ANOVA $P < 0.0001$; $R^2 > 0.99$; Table 6.3B). Additionally, leaf types accumulated copper at significantly different rates (ANOVA $P < 0.01$; Figure 6.C) except alder and maple (ANOVA $P > 0.09$). Copper bioaccumulation was not related to conditioning rate (ANOVA $P > 0.50$), although oak leaves did have the lowest rate for each parameter. Leland and Carter (1985) did not compare effects on leaf processing to leaf copper concentrations, as leaf copper bioaccumulations were not measured. Giesy (1978) compared leafpack cadmium bioaccumulations and processing as affected by 28 weeks of processing in control

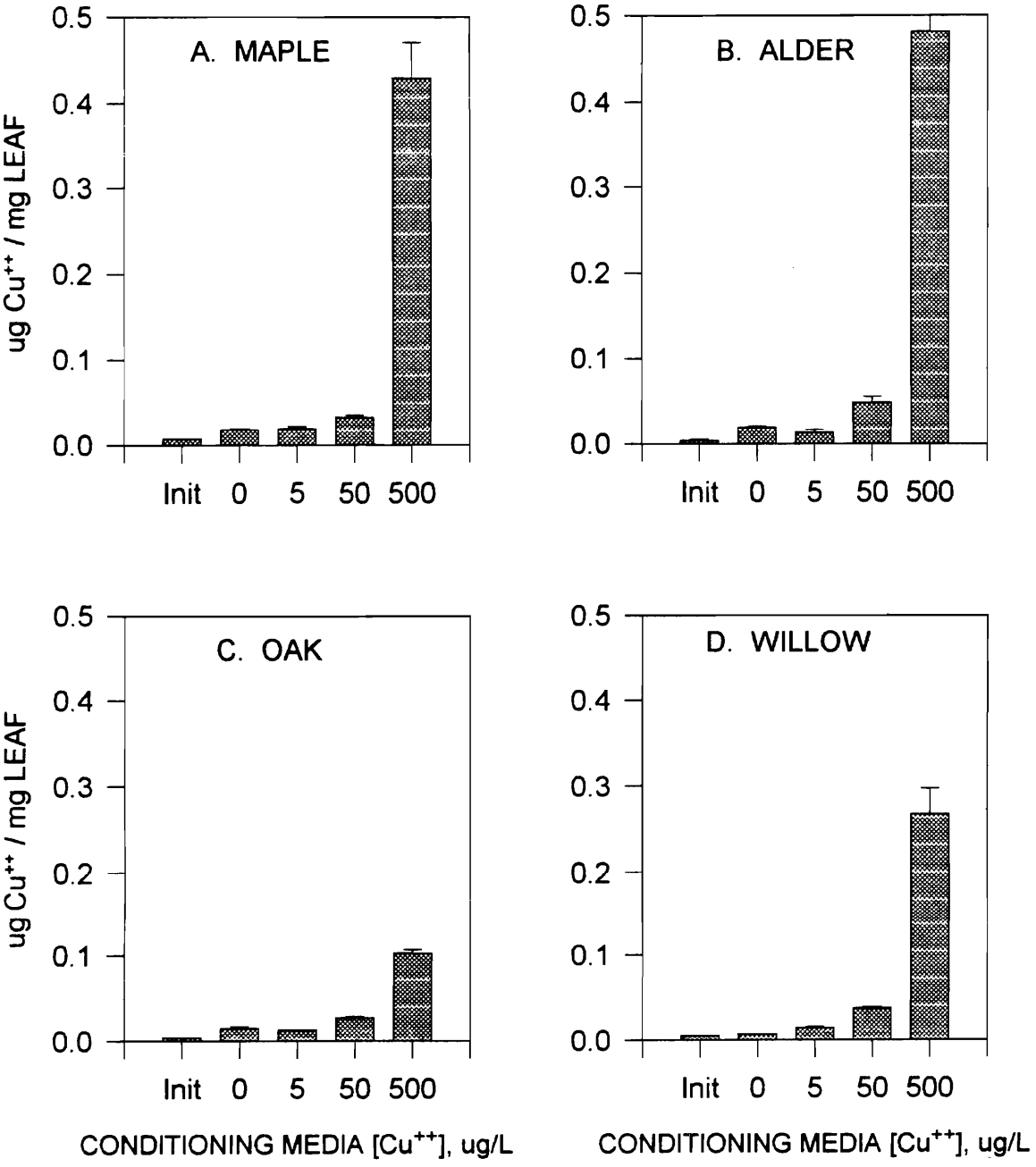

Figure 6.6. Copper contents of leaves in conditioning experiments after 21 days. Initial values are indicated by "Init"; copper treatments are indicated in legend.

Table 6.3 Statistical treatment of leaf copper measurements. Analyses are based on triplicate samples from each treatment.

LEAF	Duncan Grouping	Mean	N
Maple	A	0.0077200	3
Willow	B	0.0045143	3
Alder	B	0.0043667	3
Oak	B	0.0040340	3

A. Duncan's Multiple Range Test for initial leaf copper concentration. Means with the same letter are not significantly different ($\alpha = 0.05$).

LEAF	SLOPE	INTERCEPT	R^2	P
ALDER	0.0009028152	0.0105010540	0.995840	0.0001
MAPLE	0.0008400793	0.0079056653	0.987116	0.0001
OAK	0.0001785049	0.0143241107	0.994238	0.0001
WILLOW	0.0005153677	0.0095383096	0.997573	0.0001

B. Linear regressions of leaf copper bioconcentrations using the model Leaf $[Cu^{++}]$ = m (Treatment $[Cu^{++}]$) + b, where leaf concentrations are ug Cu^{++}/mg leaf and treatment concentrations are ug/L. N = 12 for each regression..

Comparison	T for H0: $m_1 = m_2$	Pr > |T|
Alder, Maple	-1.77	0.0926
Alder, Oak	-38.23	0.0001
Alder, Willow	2.94	0.0082
Maple, Oak	-19.25	0.0001
Maple, Willow	-10.34	0.0001
Oak, Willow	36.96	0.0001

C. Results of dummy variable analysis for equality of slopes (m_i) between leaf copper bioconcentration regressions.

and cadmium-treated stream microcosms. Control leaves contained 2.8 ug Cd^{++}/g leaf, while exposures to 5 and 10 ug/L Cd^{++} produced leaf concentrations of 8.5 and 18.4 ug/g, respectively. Higher bioconcentrations were observed in the present study (treatment means of 11.7 and 14.7 ug Cu^{++}/g leaf for control and 5 ug/L Cu^{++} treatments). However, less than 24% of the original leaf material remained in Giesy's leafpacks when Cd^{++} determinations were conducted, and the majority of the bound metal may have been lost with more labile leaf material.

vi. Stonefly lipid content

Results of stonefly feeding experiments (Figure 6.7) indicated that copper treatments did not reduce the nutritional suitability of leaves as measured by consumer lipid content. Leaf copper contents in the ranges obtained in study (< 5 ug Cu^{++}/mg leaf in 500 ug/L treatments) are below the food-borne copper concentration (> 1.1 ug/mg) which Hatakeyama (1989) determined to be detrimental to another aquatic insect.

When treatments are combined, differences in nutritional suitability between leaf species (ANOVA; N = 163, F = 4.43, Pr > F = 0.0021) are evident. Lipid contents of stoneflies fed alder (8.29%) or oak (7.99%) did not differ significantly from control stoneflies (9.45%),

while stoneflies fed maple (6.75%) or willow (6.15%) were significantly lower. Although larvae were not quantity-limited in food, mean lipid content of stoneflies as a percentage of dry weight declined slightly from initial values in all treatments except for alder controls. As such, these leaf species alone (especially willow, maple, and oak) may not be suitable resources for these insects. For many individuals, lipid content was extremely low (occasionally < 2%). Additionally, mortality up to 20% was observed in some treatments, and only two treatments suffered no mortality of individuals. Mortality was not related to leaf type or treatment.

Conclusions

The four leaf species were conditioned at significantly different rates, and contained different concentrations of nitrogen, phosphorus, and copper both initially and as a result of conditioning. Conditioning rates were related to both initial leaf nitrogen and phosphorus contents. Conditioning of three of the leaf species studied was impaired by treatment with copper. Within leaf species, copper treatment did not significantly affect leaf phosphorus or nitrogen contents. Leaf copper contents were significantly related to copper treatments. When nutritional suitability

of leaves was investigated with stonefly feeding experiments, greater effects were observed between leaf species than between copper treatments within species. Leaf copper treatments did not affect stonefly lipid contents. As such, these parameters (leaf processing, nutrient content, and nutritional suitability) are less sensitive indicators of copper effects than other parameters.

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Chapter 7. Summary of effects of copper in artificial aquatic mesocosms.

In this study, the chronic effects of copper on important aquatic ecosystem processes and attributes were studied. In Chapter 2, the effects of copper on periphyton abundance, growth rate, and colonization were examined, and this approach was broadened to include the effects of light in Chapter 3. Effects of copper and light on periphyton community composition were analyzed in Chapter 4 and on periphyton nutrient content in Chapter 5. In Chapter 6, the effects of copper on leaf conditioning and nutrient content and the suitability of treated leaves to a shredding macroinvertebrate were examined. Results from these individual studies are combined here (Table 7.1) and compared to various literature reports of chronic toxicity levels (Table 7.2) to determine the relative sensitivity of these parameters. Comparison of chronic toxicity reports with acute toxicity reports (Table 7.3) indicates that acute toxicity levels are generally several orders of magnitude higher than chronic toxicity levels.

For each parameter, lowest observed effects concentrations (LOECs) are used to calculate chronic effects

Table 7.1. Lowest observed effects concentrations (LOEC) and chronic effects concentrations (CEC) for parameters examined in this study. NOEC = no observed effects significantly different from controls. CEC values calculated as the geometric mean between LOEC and NOEC. For copper content, values obtained were compared to data of Hatakeyama (1989).

Parameter	LOEC, ug/L	CEC, ug/L
A. Periphyton		
Biomass	< 1[A]	< 1
Growth rate	11.14	8.00
Colonization	5.43	3.74
Community composition	2.41	0.66
Phosphorus content	5.41	3.68
Protein content	53.46 NOEC	>37.68
Copper content	10.85	7.81
B. Leaf processing		
Conditioning rate	50	15.81
Phosphorus content	500 NOEC	> 158
Nitrogen content	500 NOEC	> 158
Copper content	500 NOEC	> 158
Stonefly lipids	500 NOEC	> 158

[A]Predicted EC_{20}

Table 7.2. Chronic toxicity values for copper to various freshwater aquatic organisms. Chronic values are calculated as the geometric mean of the NOEC and LOEC. Copper is reported as the cation.

Parameter or species	[Cu^{++}], ug/L	Endpoint	Reference
Leaf C:N	2.5	LOEC	Leland and Carter, 1985
Leaf microbial respiration	15	NOEC	Leland and Carter, 1985
Periphyton community composition	2.5	LOEC	Leland and Carter, 1985
Periphyton biomass (natural substrates)	10	NOEC	Leland and Carter, 1985
Anabaena variabilis (cyanophyte)	100	Growth	Young and Lisk, 1972
Anacystis nidulans (cyanophyte)	100	Growth	Young and Lisk, 1972
Chroococcus paris (cyanophyte)	100	Growth	Les and Walker, 1984
Chlorella pyrenoidosa (chlorophyte)	100	Growth	Steeman-Nielson and Kamp-Nielson, 1970
Chlorella vulgaris (chlorophyte)	180	33D EC_{50}	Rosko and Rachlin, 1977
Chlamydomonas reinhardtii (chlorophyte)	64	Growth NOEC	Garvey et al., 1991
Chlamydomonas bullosa (chlorophyte)	50.7	96H EC_{50}	Visviki and Rachlin, 1994
Selenastrum capricornutum (chlorophyte)	50	Growth	Bartlett *et al.*, 1974
Phytoplankton species richness	15	LOEC	Gustavson and Wängberg,1995
Protozoan community colonization	6.6	LOEC	Cairns *et al.*, 1980
Daphnia magna (cladoceran)	13.63	Chronic	Chapman *et al.**
Epeorus latifolium (mayfly)	12.2	Chronic	Hatakeyama, 1989
Epeorus latifolium (mayfly)	820 ug/g	Chronic food	Hatakeyama, 1989
Campeloma decisum (snail)	10.88	Chronic	Arthur and Leonard, 1970
Macroinvertebrate community	5	LOEC	Leland *et al.*, 1989
Oncorhynchus mykiss (rainbow trout)	19.01	Chronic	McKim *et al.*, 1978
Salvelinus fontinalis (brook trout	31.1	Chronic	McKim *et al.*, 1978
Salvelinus fontinalis (brook trout)	12.9	Chronic	McKim and Benoit, 1971
Salvelinus namaycush (lake trout)	30.5	Chronic	McKim *et al.*, 1978
Esox lucius (northern pike)	60.4	Chronic	McKim *et al.*, 1978
Catostomus commersoni (white sucker)	20.9	Chronic	McKim *et al.*, 1978
Micropterus dolomieui (smallmouth bass)	517.4	Growth NOEC	McKim *et al.*, 1978
Pimephales promelas (fathead minnow)	13.6	Chronic	Mount and Stephan, 1969
Pimephales notatus (bluntnose minnow)	8.8	Reproduction	Horning and Neihesel, 1979
Pimephales notatus (bluntnose minnow)	119	Growth NOEC	Horning and Neihesel, 1979
Lepomis macrochirus (bluegill)	28.3	Chronic	Benoit, 1975

Table 7.3. Selected acute toxicity values for copper to various freshwater organisms. Copper is reported as the cation.

Species	$[Cu^{++}]$, ug/L	Endpoint	Reference
Cyclops abyssorum (copepod)	2500	48H LC_{50}	Baudouin and Scoppa, 1974
Daphnia hyalina (cladoceran)	5	48H LC_{50}	Baudouin and Scoppa, 1974
Eudiaptomus padanus (zooplankton)	500	48H LC_{50}	Baudouin and Scoppa, 1974
Gammarus fasciatus (amphipod)	210	48H LC_{50}	Judy, 1979
Acroneuria lycorias (stonefly)	8300	24H LC_{50}	Warnick and Bell, 1969
Oncorhynchus mykiss (rainbow trout)	500	24H LC_{50}	Smith and Heath, 1979
Oncorhynchus mykiss (rainbow trout)	500	96H LC_{50}	Mayer and Ellersieck, 1986
Notemigonus chrysoleucus (golden shiner)	250	24H LC_{50}	Smith and Heath, 1979
Lepomis macrochirus (bluegill)	2400	24H LC_{50}	Smith and Heath, 1979
Lepomis macrochirus (bluegill)	900	96H LC_{50}	Thompson *et al.*, 1980
Lepomis macrochirus (bluegill)	221	96H LC_{50}	Mayer and Ellersieck, 1986
Lepomis *cyanellus* (green sunfish)	878	96H LC_{50}	Mayer and Ellersieck, 1986
Carassius auratus (goldfish)	2900	24H LC_{50}	Smith and Heath, 1979
Ictalurus punctatus (channel catfish)	2500	24H LC_{50}	Smith and Heath, 1979
Trichogaster trichopterus (blue gourami)	91.2	96H LC_{50}	Roales and Perlmutter, 1974

concentrations. When more than one LOEC was determined for a parameter the lowest value is used. For periphyton biomass effects, the lowest predicted EC_{20} value was used.

Due to factors discussed previously (Chapter 2), the application of results from static testing may not be applicable to lotic ecosystems. Better risk estimates result from the use of lotic testing protocols. However, field experiments are most often unsuitable or impractical, due to the expense of setting up such studies, need for multiple tests and replicates, ecological effects associated with environmental testing, and potential interference from biotic and abiotic factors. The need for use of artificial systems to alleviate these problems is evident.

The experiments in this dissertation generated much new information concerning the effects of Cu^{++} on important ecological processes within artificial systems. Extreme sensitivity of periphyton biomass levels to Cu^{++} was demonstrated at Cu^{++} concentrations far below USEPA chronic criteria. Periphyton colonization was found to be more sensitive to Cu^{++} than growth rates. Additionally, irradiance levels did not influence Cu^{++} toxicity within the light levels tested. Periphyton community composition was also shown to be extremely sensitive to Cu^{++}, and deleterious effects were demonstrated at Cu^{++} concentrations far below USEPA chronic criteria. The relative sensitivities of

observed taxa to Cu^{++} was quantified and ranked. Nutritional content of periphyton was found to be insensitive to Cu^{++} treatment. Detrimental dietary effects levels of Cu^{++} due to bioaccumulation was found to occur at concentrations higher than chronic criteria. Polyphosphate mechanisms for Cu^{++} detoxification were not observed in the artificial stream periphyton taxa. Autochthonous production was found to be more sensitive to Cu^{++} than conditioning of allochthonous matter. The utility of microcosms for studying leaf conditioning was demonstrated. Effects of Cu^{++} on conditioning of three leaf species were shown to occur at concentrations much higher than chronic criteria.

When compared to results of chronic toxicity studies from the literature, periphyton effects levels observed in these experiments are more sensitive indicators of chronic copper toxicity than values for other algal measures and for chronic and reproductive impairment effects on potential consumers of periphyton. As such, Cu^{++} exposure could affect dependant populations to a greater degree through resource limitations than expected from direct chronic toxicity measurements. This provides strong evidence for the importance of including effects on periphyton in water quality criteria calculation and suggests that the Chronic Criterion for copper should be revised.

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copper, chromate, zinc, and cyanide to freshwater fish: Effect of different temperatures. Bull. Environm. Contam. Toxicol. 22:113-119.

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CURRICULUM VITAE

Jay L. Comeaux

April, 1996

Personal Data

Address:	4812 Baumgardner Road Maryville, TN 37801 (423) 984-5703
Date of Birth:	May 17, 1962
Place of Birth:	New Iberia, Louisiana
Marital Status:	Married to Lisa Cheryl Bailey of Wise, VA

Education

Ph.D., Zoology (in progress), Virgina Tech, Blacksburg, VA
Dissertation topic: Effects of Copper on Benthic Communities in Artificial Microcosms
Major Professor: Dr. John Cairns, Jr.

M.S., Microbial Ecology, 1989, Virginia Tech, Blacksburg, VA
Thesis title: Transfer of Plasmids By Genetically-Engineered *Erwinia carotovora*
Major Professor: Dr. John Cairns, Jr.

B.S., Zoology, 1987, University of Southwestern Louisiana, Lafayette, Louisiana. Minors in Mathematics and Chemistry.

Professional Employment

Present - Unemployed

10/94 - 10/95 Senior Laboratory Technologist, University of Tennessee Dept. of Animal Science/Veterinary Medicine. Responsibilities included development of novel toxicity tests utilizing protozoan and bacterial species, investigations of the ecology of *Tetrahymena pyriformis*, QSAR studies utilizing *T. pyriformis* and *E. coli*, preparation and review of funding proposals. Immediate supervisor: Dr. T. W. Schultz

6/94 - 12/94	Instructor, Hiwassee College Division of Biological Sciences. Lecturer and Laboratory Instructor of Human Anatomy and Physiology.
1/94 - 5/94	Instructor, General Biology, Virginia Tech. Duties include all aspects of instruction and administration of a General Biology lecture for 335 students.
1993-4	Research Technician, University Center for Environmental and Hazardous Materials Studies, Virginia Tech. Primary researcher on grant "Ecosystem Effects of Abandoned Mine Lands in the Clinch and Powell Rivers of Virginia".
1992	Graduate Teaching Assistant, Biology Department, Virginia Tech. Laboratory Instructor for Principles of Biology
1989-92	Presidential Fellowship VPI&SU, Blacksburg, Virginia
1989	Graduate Research Assistant, Biology Department, Virginia Tech. Transfer of Recombinant Genes and Plasmids in Microcosms.
1987-88	Graduate Teaching Assistant, Biology Department, Virginia Tech. Laboratory Instructor for Principles of Biology and General Biology.
1985-87	Student Intern, Louisiana Department of Wildlife and Fisheries, New Iberia, Louisiana.
1982	Remedial Mathematics Instructor, Louisiana State University.

Professional and Honorary Societies

Virginia Academy of Science
AAAS
Omicron Delta Kappa
Society for Environmental Toxicology and Chemistry

Selected Service Activities

Vice-President, Graduate Student Assembly, 1990-92

Representative, University Commission on Graduate Studies, 1991-93

Advisor, Virginia Academy of Science Committee on Recombinant DNA Testing

Member, Graduate Honor System Constitution Revision Committee

Member, Graduate Student Assembly Constitution Revision Committee

Publications

Schultz, T.W., and J.L. Comeaux. *In press.* Structure-activity relationships for isothiocyanates. Bull. Environ. Contam. Toxicol.

Comeaux, J.L., C.D. Pooranampillai, G.H. Lacy, and V.K. Stromberg. 1990. Transfer of genes to other populations and analysis of associated potential risks. pp. 132-145 *in* Marois, J.J., and G. Bruening (*Eds.*), Risk Assessment in Agricultural Biotechnology: Proceedings of the International Conference. Cooperative Extension Division, University of California, Division of Agriculture and Natural Resources, Regents of the University of California, Berkeley, CA, 224 pp.

Comeaux, J.L., and J. Cairns, Jr. In preparation. The design and function of an inexpensive device for the measurement of water velocity.

Comeaux, J.L., G.H. Lacy, and J. Cairns, Jr. In preparation. Transfer of plasmids by genetically-engineered *Erwinia carotovora.*

Abstracts and Presentations

Comeaux, J.L., and J. Cairns, Jr. 1994. Effect of copper on production, nutritional content, and community composition of periphyton. Poster presentation at North American Benthological Association Annual Meeting, 24-29 May 1994, Orlando, FL.

Comeaux, J.L., and J. Cairns, Jr. 1993. Effect of copper on community composition and nutritional content of periphyton in laboratory mesocosms. Platform presentation at Society for Environmental Toxicology and Chemistry Fourteenth Annual Meeting, November 14-18, Houston, TX.

Comeaux, J.L., and J. Cairns, Jr. 1993. Effect of copper on lotic periphyton production and nutritional content. Third International Conference: Aquatic Ecosystem Health and the Ecological Significance of Bioassay Techniques. May 24-28, Blacksburg, VA

Comeaux, J.L., and J. Cairns, Jr. 1992. Responses of periphytic algae to copper stress in artificial streams. Poster presentation at Society for Environmental Toxicology and Chemistry Thirteenth Annual Meeting, November 6-11, Cincinnati, OH.

Comeaux, J.L., M.E. Arnegard, and J. Cairns, Jr. 1992. Design and function of an artificial stream system for the study of periphyton responses to stress. Poster presentation at Society for Environmental Toxicology and Chemistry Thirteenth Annual Meeting, November 6-11, Cincinnati, OH.

Comeaux, J.L., M.E. Arnegard, and J. Cairns, Jr. 1992. Design and function of an artificial stream system for the study of periphyton responses to stress. Poster presentation at Fourth Algal Ecology Consortium, Lake Pymatuning Laboratory (University of Pittsburgh Field Station), Linesville, PA, April 24-26, 1992.

Comeaux, J.L. and J. Cairns, Jr. 1991. Leaf processing in a stream impacted by heavy metal effluents. Platform presentation at Society for Environmental Toxicology and Chemistry Twelfth Annual Meeting, November 3-7, Seattle, WA.

Comeaux, J.L. 1991. Nutrient uptake by a zinc-stressed diatom community. Presented at North American Diatom Symposium XI, Clemson University, October 23-25.

Comeaux, J.L., G.H. Lacy, and J. Cairns, Jr. 1989. Plasmid and gene transfer by genetically-engineered *Erwinia carotovora* subsp. *carotovora* in soil, *in vitro*, and *in planta*. Poster presented at the American Society for Microbiology National Meeting, New Orleans LA, May 14-18.

Honors, Awards, and Grants

1993 Carl Zipper, J.L. Comeaux, and J. Cairns, Jr. Proposal "Ecosystem effects of abandoned mine lands in the Clinch and Powell Rivers of Virginia" funded for $26,000 by The Nature Conservancy and Powell River Project.

1993 SETAC Travel Award, $300.00 to help defray costs associated with presentation at The Society for Environmental Toxicology and Chemistry's Fourteenth Annual Meeting, November 14-18, Houston, TX. Matched by VPI&SU Biology Department.

1992 Elected to Omicron Delta Kappa, National Honor and Service Fraternity

1990 Bruce Parker and J.L. Comeaux. Proposal for developing an Aquatic Ecology Colloquium submitted to Miles Horton Center, VPI&SU funded for $4000.00.

1990 J.L. Comeaux and J. Cairns, Jr. Proposal "Impairment of Detritus Processing in Streams Stressed by Heavy Metals" funded for $995.00 by Virginia Academy of Science Small Projects Research Fund. Matching award of $500.00 by VPI&SU Biology Department.

1989 Presidential Fellowship, Graduate School, VPI&SU
1989 Graduate Student Assembly Travel Fund Award, $100.00 awarded for travel to ASM National Meeting.

1989 J.L. Comeaux and J. Cairns, Jr. Proposal "Transfer of Recombinant Genes in Soil" funded for $490.00 by Sigma Xi Grants-in-Aid of Research Award. Matching award of $490.00 by VPI&SU Biology Department.

1989 J.L. Comeaux and J. Cairns, Jr. Proposal "Transfer of Recombinant Genes *in planta*" funded for $200.00 by VPI&SU GSA Graduate Research Development Project. Matching award of $200.00 by VPI&SU Biology Department.