CHANGES IN RESPIRATION RATES AND BIOMASS ATTRIBUTES OF EPILITHON DUE TO EXTENDED EXPOSURE TO ZINC

by

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(ABSTRACT)

The purpose of this research was to determine the influence of extended dosing of zinc on the carbon cycling and biomass characteristics of freshwater epilithon. Experiments were conducted in artificial streams continuously dosed with 0.00, 0.05, or 1.00 mg Zn liter^{-1} for 20 to 30 days during summer and fall, 1984 and 1985. Repeated measurement of epilithon structure and function included estimates of ^{14}C-glucose respiration, ^{14}C-glutamate respiration, O_2 and CO_2 flux rates, ash-free dry weight (AFDW), protein, carbohydrate, and algal pigment concentrations, and total and zinc-tolerant colony forming units. An increase in epilithic glucose respiration per unit biomass consistently occurred 5 to 10 days after dosing with 1.0 mg Zn liter^{-1} was started. At the same time significantly lower epilithon biomass occurred in the high dosed streams relative to controls in 3 out of 4 studies. Although algal pigment concentrations were lowest in the high dose streams at the midpoint of the studies, the

chlorophyll a-to-pheophytin a ratio remained high, indicating that the minimal algal population was not senescing *in situ*. After 30 days, the epilithon dosed with 1.0 mg Zn $liter^{-1}$ had higher AFDW, protein, and carbohydrate concentrations than the other treatments. By 20 days, the high zinc treatment showed evidence of more total and zinc-tolerant colony forming units and lower rates of O_2 and CO_2 flux than epilithon from control streams. The high rates of glucose respiration were characteristic of epilithic communities stressed by 1.0 mg Zn $liter^{-1}$, and this response was not apparently due to *in situ* senescence of zinc-sensitive cells; the results suggested that epilithic biomass was washed out of the systems, not being degraded *in situ*. The development of unique epilithon communities that are acclimated to prolonged zinc exposure is evident in the eventual recolonization of the artificial surfaces, glucose respiration rates that are comparable to controls, and presence of zinc-tolerant heterotrophs.

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and periodically reminded me of the important

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GENERAL INTRODUCTION

Heavy metal pollution in freshwaters due to industrial waste, mine drainage, and combustion of coal is a major factor influencing the viability of aquatic ecosystems. Unlike most organic wastes, metals are not degraded and accumulate in biotic and abiotic components of the environment in significant quantities (Forstner and Wittmann, 1981). Although many metals are required in trace amounts by all organisms, toxic concentrations in waters and sediments can affect cells primarily by denaturing proteins (Gadd and Griffiths, 1978).

Most studies of the effects of heavy metals on bacteria and algae have involved pure cultures and lethal metal concentrations (Gadd and Griffiths, 1978; Babich and Stotzky, 1980). The goal of many of these studies has been to determine the various mechanisms of microbial resistance to metal ions or to obtain information regarding lethal metal concentrations for a particular organism. This type of research increases the understanding of microbial physiology and industrial processes, yet it is important to consider the potentially complex response of natural microbial communities undergoing stress from pollutants.

Heavy metals significantly affect aquatic microbial communities by determining which species are present and the activities of the microflora. Singleton and Guthrie (1977) observed increases in total colony forming units and a decrease in the diversity of mixed bacterial populations in the presence of 2.0 mg Cu liter^{-1} or 0.04 mg Hg liter^{-1}. Hornor and Hilt (1985) showed that zinc tolerance in heterotrophs is correlated with zinc concentrations in stream sediments, thus suggesting the role that metals play in determining the nature of a microbial population. The effect of heavy metals on microbial activity in aquatic systems has been determined primarily in planktonic and sediment systems. These studies have demonstrated decreases in glucose assimilation and respiration (Albright and Wilson, 1974), biological oxygen demand (Berkuen, 1982), and photosynthesis and nitrification (Mills and Colwell, 1977) associated with elevated heavy metal concentrations. Most of these have been short-term studies (≤3 days).

Zinc is a common heavy metal in aquatic systems due to natural and anthropogenic sources such as discharge at mine sites and smelters, municipal, residential, and industrial effluents, soil erosion, road surface runoff, and corrosion of zinc alloys and galvanized surfaces (Spear, 1981). In addition, zinc is a required trace element for many organisms. It is essential for the function of enzymes such

as carbonic anhydrase and DNA and RNA polymerases (Lehninger, 1982). Although it is required in biological systems, zinc is toxic at higher concentrations. The U.S. EPA (1980) has set 0.180 mg liter^{-1} total recoverable zinc as a limit in water, yet in some areas concentrations may exceed these guidelines (Weatherley *et al.*, 1980).

Despite its abundance and potential toxicity, the effect of zinc on epilithon has received limited attention. A study by Williams and Mount (1965) in outdoor streams dosed with zinc indicated that in zinc enriched systems the periphyton community changed from one composed mainly of autotrophs to a heterotrophic community. At high concentrations of the metal (9.0 mg liter^{-1}) fungi and slime-producing bacteria were dominant. Whitton (1980) cited several studies that indicated a predominance of mucilaginous algae in zinc enriched streams. This was recently corroborated in studies by Foster (1982a, b) dealing with algal species that colonize historically metal contaminated streams. These studies dealt with changes in epilithic species caused by zinc or metals but have largely ignored the long-term effect of zinc on nontaxonomic structure and function of the epilithon. There is little information regarding how zinc affects important epilithon processes such as the cycling of essential and sometimes limiting nutrients (e.g. carbon) *in situ*. The impact of this metal on indicators of biomass quality and

quantity in epilithic microbial communities has not been determined. Therefore, the purpose of this study was to measure the effect of continuous, long-term dosing of 0.00 (control), 0.05, and 1.0 mg Zn liter^{-1} on carbon cycling and ash-free dry weight, protein, and carbohydrate concentrations in epilithon.

Using artificial streams to implement continuous dosing of zinc, the objectives of this study were to determine on a periodic sampling basis:

1) the rate of ^{14}C-glucose respiration in epilithon during summer and fall seasons, and
2) the quality and quantity of epilithic biomass using ash-free dry weight, protein, and carbohydrate during the summer and fall seasons.

The term 'epilithon' was selected to describe the complex community of algae, bacteria, fungi, protozoans, and invertebrates that colonize submerged surfaces. Unlike more common terms (e.g. periphyton), 'epilithon' is nonspecific regarding the biota present. Artificial streams were chosen for this study since they offer greater experimental control than actual <u>in situ</u> studies and have many of the characteristics of the systems that they have been designed to model (Shriner and Gregory, 1984). Initially, horizontally placed

porcelain tiles were used as surfaces for colonization, but vertically situated glass rods were ultimately used since they allowed epilithic colonization without excessive sedimentation. Farris (1986) described the artificial stream design (Fig. 1). Each stream has an approximate volume of 27 liters. When a 1.2 liter per min influent water flow rate is used (as in these studies) the water in each stream is renewed approximately every 23 min. The estimated submerged surface area, including glass rods, equals 0.64 m^2. Preliminary studies suggested that sampling 0, 2, 5, 10, 20, and 30 days after initiation of zinc dosing was suitable for recognizing early changes in community activity. Later sample days were widely spaced to assess habituation to zinc.

Use of radiolabelled organic substrates is an important method for determining the rate of microbial activity in aquatic systems (Wright and Hobbie, 1966; Wright, 1978; Gocke, 1977). The procedure has been applied in studies measuring heterotrophic activity under conditions of environmental stress. Sayler et al., (1979) noted that glucose uptake by freshwater microbial communities was unaffected by polychlorinated biphenyls and phenanthrene. Albright and Wilson (1974) found that heterotrophic activity (glucose uptake and mineralization) decreased during microbial exposure to sublethal concentrations of metals (Cu and Zn). These studies represent short term, acute dosing assays. Little

is known regarding the effect of chronic stress on heterotrophic activity in relatively realistic assay conditions.

The method applied in the current research measured glucose respiration ($^{14}CO_2$ release) using the tracer approach (Williams and Askew, 1968). Glucose is highly labile and used by a variety of organisms and, since it has been used in many similar studies, it was chosen for this study for comparative purposes. Unlike the kinetic approach which uses several substrate concentrations, the tracer method cannot determine Vmax or the natural substrate concentration (Wright, 1978). The tracer method does provide the turnover time of the chosen substrate and it is considered accurate in deriving this single parameter (Gocke, 1977). Since this study involved so many samples on a given day (≥36), the use of the kinetic approach with multiple substrate concentrations was deemed logistically impractical.

Gross measurements of epilithic biomass provide information regarding structural changes in the quality of epilithic communities and also may be used for consideration of rate functions on a per unit biomass basis. Ideally, one would use plate counts or epifluorescent counts as indicators of heterotrophic biomass. Again, the number of treatments and replicates used in this study made this approach impractical.

The determination of ash-free dry weight was chosen as an accepted estimate of total organic material in the epilithon (American Public Health Association, 1985).

More information may be derived from assessments of epilithon biochemical composition. The relative proportion of protein and carbohydrate in the epilithon may effect the health of higher trophic level organisms that subsist on the epilithon (McMahon et al., 1974). Analysis of total protein has been used as an indicator of microbial biomass in several studies (Rausch, 1981; Brock and Brock, 1967; Bott and Brock, 1970; Clark et al., 1982). The study by Rausch (1981) offers an extensive review of protein extraction and quantification methods. He recommends the micro-biuret method of protein measurement (Itzhaki and Gill, 1964), and this method was selected for the present studies since it is similar to that commonly used for bacterial protein analysis (Herbert et al., 1971).

Carbohydrate has previously been used as a measurement of bacterial and/or algal biomass (Hitchcock, 1982; Clark et al., 1982; Haug et al., 1973). Clark et al., (1982) found that the carbohydrate concentration of the epilithon increased when the population was dominated by heterotrophs and cyanobacteria, the community composition shifting as a result of copper or chlorine treatment or the approach of maximal

seasonal temperatures. The anthrone method has been used to measure hexose carbohydrates in environmental samples (Clark et al., 1982; Cuhel et al., 1983) and was used in the present study.

Chapter I of this manuscript deals with two, 30-day studies carried out during 1984 to address the previously stated objectives. Among the most significant results common to both studies was evidence of higher glucose respiration rates per unit area or biomass five days after beginning metal amendments in epilithon dosed with 1.0 mg Zn $liter^{-1}$. Although other effects of zinc dosing were evident during the two studies, I wished to determine the cause of this increase in glucose respiration during the next phase of studies in these systems.

The response of marine bacterial populations to elevated copper concentrations has been examined by Vaccaro et al., (1977). Copper-tolerant heterotrophs exhibited increased activity (glycine utilization) apparently due to decreased biomass and extracellular release of organic compounds by copper-sensitive phytoplankton. The 1985 artificial stream experiment (Chapter II) represented the first opportunity to test a similar response of epilithic microorganisms to zinc stress.

The objectives of the second phase of this study were to determine the effect of chronic zinc dosing on:

1) the glucose-to-glutamate respiration ratio in the epilithon as an indicator of algal health,
2) the rates of flux of O_2 and CO_2 in the epilithon to measure the overall respiratory capacity not associated with specific organic compounds,
3) the chlorophyll a-to-pheophytin a pigment ratio in the epilithon as a direct measure of algal senescence in situ, and
4) the presence of zinc-tolerant heterotrophs in the epilithon.

AFDW and protein concentrations were again determined as the basic biomass structural measurements. This information was important for comparative purposes between the 1984 and 1985 studies.

The artificial streams and glass rod substrates were used as previously described with the following changes. Studies were shorter (only 20 days) since the critical time to test the immediate response of the epilithon to zinc was early in the dosing period. Also, the number of sampling days was reduced to 0, 5, 10, and 20 and the 0.05 mg Zn $liter^{-1}$

treatment was not performed. These changes were necessary to accomodate more analyses.

Measurement of the glucose-to-glutamate utilization ratio was used in studies by Griffiths et al., (1982) and Gillespie et al., (1976) as an indicator of the physiological health of an algal community. The concept is based on the assumption that carbohydrates (e.g. glucose) are secreted by healthy algal populations whereas free amino acids (e.g. glutamate) are released in greater quantities by senescing organisms. The studies cited suggested that in marine systems, algal die-off due to seasonal changes led to a decrease in the glucose-to-glutamate utilization ratio as the associated heterotrophs fine-tuned their metabolism to take advantage of the available organics. This method had not been applied in toxicological work, but it was anticipated that 1.0 mg Zn $liter^{-1}$ would induce an effect analogous to that of the seasonal influence noted in the work of others (i.e. a decrease in the glucose-to-glutamate respiration ratio). Analysis of respiration of trace quantities (<1.0 µg $liter^{-1}$) of ^{14}C-glucose and ^{14}C-glutamate were carried out as previously described for experiments with glucose alone.

The rates of O_2 and CO_2 flux provided information regarding the overall epilithic respiratory capacity in the different treatments. Gas chromatography has previously been used to

measure respiratory gas flux in sewage sludge (Hornor and Mitchell, 1981) and sediments (Stringfellow, 1984). This method was chosen due to the ease of handling as many as 18 samples per day and because measurements determining differences in gas concentrations may be made over incubation periods as short as 5 h. Determination of AFDW directly in the analysis vessel provided final values of μg O_2 consumed (or CO_2 evolved) per h per mg AFDW.

Chlorophyll a, along with other pigments, has been used as a structural indicator of algal populations (Strickland and Parsons, 1968; Holm-Hansen and Riemann, 1978). Pheophytin a is the primary degradation product of chlorophyll a and is present in relatively higher concentrations in stressed relative to healthy algal populations. A decrease in the chlorophyll a-to-pheophytin a ratio early in the dosing scheme would give evidence of decline of algae in general. This data would complement information derived from the glucose-to-glutamate respiration ratios.

Hornor and Hilt (1985) determined the presence of zinc-tolerant bacteria in sediments using 1/4 strength Nutrient Agar amended with zinc. Increasing concentrations of zinc allowed a relative estimate of zinc tolerance in bacteria obtained from sites differing in the degree of metal exposure. A similar method was used in the present study to

derive the number of total and zinc-tolerant colony forming units at the beginning and end of a 20-day study. The expectation was that zinc-tolerant bacteria would become more prevalent in epilithon exposed to continuous dosing with zinc.

This work represented the first study of the effect of moderate zinc concentrations on biomass quality and quantity and organic carbon cycling of freshwater epilithon. Its value lies in assessing the effect of a common and important heavy metal on processes and characteristics of the epilithic biomass that help determine the health of the entire river. Since this study was replicated and designed to run continuously, it offered an opportunity to note significant trends in these important communities and test hypotheses derived from studies in marine systems.

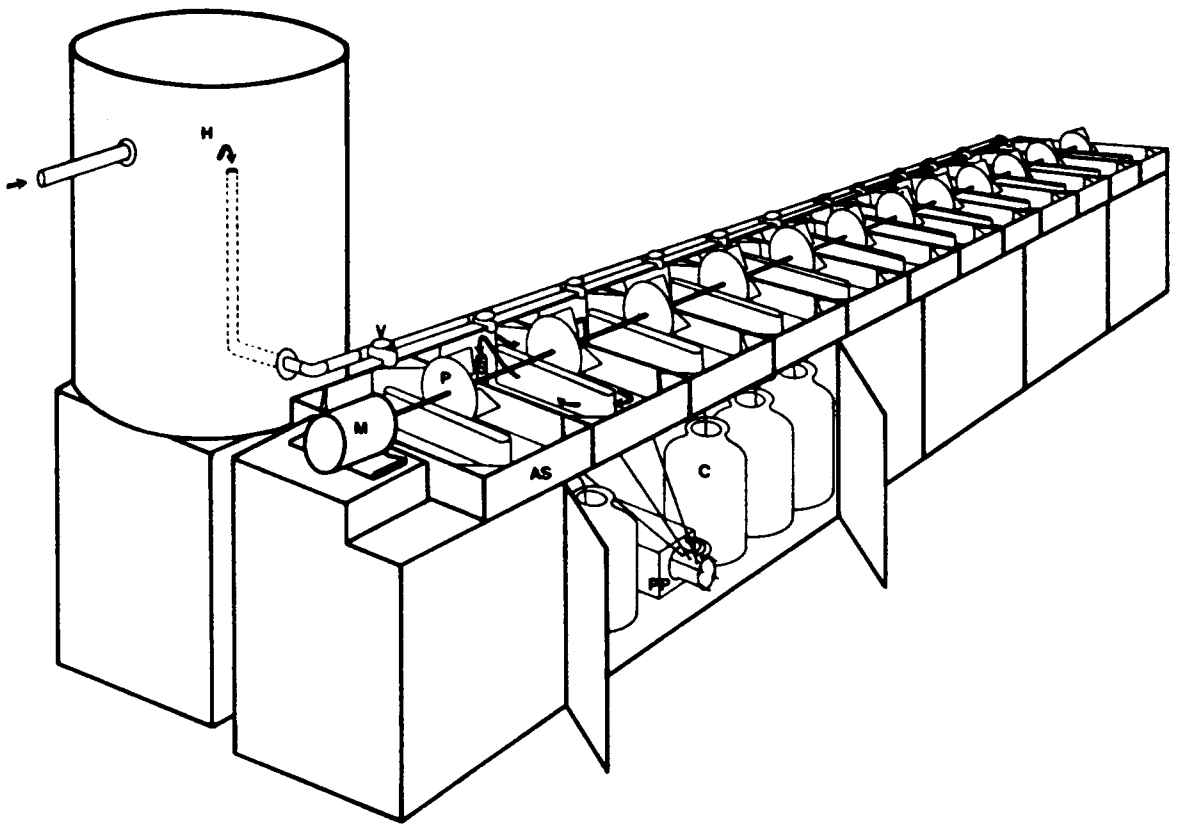

Figure 1. Diagram of artificial stream design. Individual streams (AS) receive river water through valves (V) delivered from a common headbox (H). Circulating water eventually leaves the stream through a standpipe (S). Paddlewheels (P) driven by a motor (M) maintain a continuous current in each stream. Peristaltic pumps (PP) delivers concentrated dissolved metal solutions from carboys (C) to selected streams.

CHAPTER I

EPILITHON EXPOSURE TO ZINC:
1. ORGANIC CARBON AND BIOMASS DYNAMICS

ABSTRACT

Artificial streams were used to study the effect of chronic zinc dosing on organic carbon cycling and biomass of epilithic microbial communities during June-July and September-October, 1984. The streams, containing glass rods colonized with epilithon, were dosed with 0.00, 0.05, and 1.00 mg Zn $liter^{-1}$. Glucose respiration, ash-free dry weight (AFDW), protein, and carbohydrate concentrations were measured 0, 2, 5, 10, 20, and 30 days after initiating zinc dosing. Maximum glucose respiration rates per cm^2 and per mg protein occurred on the 5th day in streams dosed with 1.0 mg Zn $liter^{-1}$. By the 30th day, greater biomass, noted by elevated AFDW, protein, and carbohydrate measurements, developed in epilithon dosed with 1.0 mg Zn $liter^{-1}$ compared to other treatments. By the 30th day, epilithon dosed with 1.0 mg Zn $liter^{-1}$ had relatively more carbohydrate than protein, whereas the opposite was true in undosed epilithon. High rates of glucose respiration in streams dosed with 1.0 mg Zn

liter^{-1} suggested a transient stimulation of activity in zinc-resistant heterotrophs, possibly at the expense of zinc-sensitive epilithic organisms. By the end of the study, high biomass values and shifts in the relative concentration of protein and carbohydrate in the epilithon dosed with 1.0 mg Zn liter^{-1} indicated important structural changes in the community due to the addition of metal.

INTRODUCTION

The epilithon or periphyton in streams consists of a diverse assemblage of microorganisms including algae, bacteria, fungi, and protozoans. The biomass of these communities is usually much greater than that of the plankton in streams (Hynes, 1970), and the quality of the epilithon can determine the physiological health of higher organisms dependent on this community as a source of food (McMahon et al., 1974). These structural and functional properties as well as nutrient cycling capabilities define the critical role of the epilithon within the stream ecosystem.

Williams and Mount (1965) studied the effect of zinc on epilithic communities and determined that 9.0 mg Zn $liter^{-1}$ causes a shift from predominately autotrophic to heterotrophic communities. This represents the only observation of the effects of zinc on epilithon and did not determine the subtle influence of extended zinc dosing on nontaxonomic structure and function in these systems. Zinc represents a model toxicant because it is abundantly distributed in aquatic systems due to both natural and anthropogenic sources (Spear, 1981). In addition to its role as a metal pollutant, zinc is required as a trace element by many organisms (Lehninger, 1982). Although levels of dissolved zinc are estimated to be relatively low ($<10^{-6}$ M or

0.07 mg liter^{-1}) even in industrialized rivers (Shiller and Boyle, 1985), in some cases the concentration of this metal may exceed the U.S.EPA limit of 0.180 mg liter^{-1} total recoverable zinc at hardnesses of 50 mg $CaCO_3$ liter^{-1} (U.S.EPA, 1980; Weatherley et al., 1980).

Albright and Wilson (1974) noted an increase in glucose turnover time in natural microbial assemblages treated with 0.05 mg Zn liter^{-1} during short-term (3-day) studies suggesting that low zinc concentrations may cause lethal and sublethal changes in aquatic microorganisms. Additionally, the anionic, polysaccharide matrix produced by the epilithon chelates heavy metals (Foster, 1982; Lock et al., 1984) and thus removes metals from surface waters or effluents (Filip et al., 1979; Sterritt and Lester, 1979). In this way, toxic elements may enter the base of the food chain (Patrick and Loutit, 1976).

The objectives of the present study were to determine the effect of 0.05 and 1.0 mg Zn liter^{-1} (0.77 and 15.30 µM) on organic carbon cycling and ash-free dry weight (AFDW), protein, and carbohydrate concentrations of the epilithic microbial community in outdoor artificial streams during summer and fall seasons.

MATERIALS AND METHODS

Study site and stream design. Glass rods (5.4 cm^2 surface area) separated by ≥ 2.2 cm were incubated in an attached, vertical position in river-fed artificial streams along the New River at Glen Lyn, Virginia, U.S.A. Design of the artificial streams was presented by Farris (1986). Nine artificial streams were used, representing three replicates of each of the three zinc treatments (0.00, 0.05, and 1.00 mg Zn $liter^{-1}$ additions). The rods were colonized with epilithon in the streams two weeks before starting metal dosing. One day before dosing with zinc, snails from the New River were placed in the streams (approximately 80-110 snails per m^2) as part of an additional experiment (Farris, 1986). The 30-day dosing periods were started on June 23, 1984 (June-July study) and again on September 7, 1984 (September-October study) with functional (glucose respiration) and structural (AFDW, protein, and carbohydrate) characteristics of the epilithon measured 0, 2, 5, 10, 20, and 30 days after starting zinc dosing. The streams were cleaned, painted, and recolonized between studies.

Glucose respiration. Rods with intact epilithon were removed randomly from three zones in each stream and placed into 250-ml jars containing 60 ml water from the corresponding

stream. Respiratory rates for glucose were determined using a modification of the method of Williams and Askew (1968). A trace amount of [U-^{14}C] glucose (specific activity: 345 µCi per mmole; 0.44 µg glucose liter^{-1} final concentration) was added to the jars containing the glass rods. The contents were mixed and the jars were sealed and incubated in the dark at stream temperature with gentle shaking. Killed controls were used to correct for abiotic production of $^{14}CO_2$. A 5-15 min incubation was used so that <10% of the labeled substrate would be respired. Biological activity was stopped by the injection of 2-ml of 6N H_2SO_4. A dry filter paper in each jar was saturated with 0.15-ml of phenylethylamine allowing absorption of $^{14}CO_2$. $^{14}CO_2$ absorption occurred during 1.5 h of gentle shaking after which filter papers were placed into 10-ml cocktail (4 g PPO and 0.1 g POPOP liter^{-1} toluene) and counted by liquid scintillation. The sample channels ratio method was used to correct for quench.

Ash-free dry weight, protein, and carbohydrate determinations. Epilithon from each stream was scraped from 2-5 glass rods and homogenized in a Waring blendor. The resulting slurry was stored at -20° C for later measurement of AFDW, protein, and carbohydrate. AFDW was determined by subtracting the combusted weight (1 h at 500° C) from the dry weight (dried to constant weight at 105° C) for samples of

the replicated zinc treatments (American Public Health Association, 1980). Protein was extracted from the slurries at 80° C in 0.5N NaOH for 10 min and the procedure repeated three times per sample (Rausch, 1981). Protein was analyzed using the micro-biuret method with bovine serum albumin as a standard (Itzhaki and Gill, 1964). Recovery of bovine serum albumin was 90% after extraction. Carbohydrate was determined by the phenol-sulfuric acid method (Herbert et al., 1971).

Water chemistry. Temperature, pH, alkalinity, hardness, conductivity, and ammonia were measured in each stream during the studies by standard methods (American Public Health Association, 1980). Total zinc was measured by flame atomic absorption. At the beginning, middle, and end of each study, nitrate, sulfate, and phosphate were measured using ion chromatography (Dionex) (Tabatabai and Dick, 1983).

Statistical methods. A repeated measures or split plot design (without replication or blocking) was used to analyze the experiment (Milliken and Johnson, 1984). Zinc was the between subject (whole plot) factor and time was the within subject (subplot) factor. All epilithon structural and functional characteristics and water chemistry measurements

were compared by treatment on a given study day using analysis of variance (ANOVA) (Sokal and Rohlf, 1981). Duncan's multiple range tests were performed after noting significance using ANOVA to determine significance among treatments on a given study day. The α=0.05 level was used for all statistical tests.

RESULTS

Temperatures in the streams increased from 24° C during the first two days to 29-30° C by the end of the June-July study (Table 1). During September-October, temperatures at the beginning (27° C) were higher than at the end of the study (17-18° C). Additionally, during the late summer study, the streams were subjected to decreasing daylight. During each study, pH was significantly lower in streams receiving the high zinc dose. Total zinc levels in control streams ranged from below detectable limits (<0.025 mg liter^{-1}) to 0.05 mg liter^{-1}. Although the means for total zinc in control streams and streams dosed with 0.05 mg Zn liter^{-1} were not significantly different during either study, they were consistently different than values obtained from streams dosed with 1.0 mg Zn liter^{-1}. Phosphate concentrations were always below detectable limits (<30 µg PO_4^{-3} liter^{-1}) using ion chromatography.

During the June-July study, AFDW of the epilithon ranged from 0.21 to 2.63 mg per cm^2, lower than the 1.84 to 4.98 mg per cm^2 observed during the September-October study (Fig. 1). At the end of both studies AFDW was highest in streams dosed with 1.0 mg Zn $liter^{-1}$. This was true during the June-July study even though AFDW by the 5th day in the high zinc streams was significantly lower than other treatments. This change in AFDW in the high zinc streams represented a 10-fold increase in biomass over a 25-day period.

Protein and carbohydrate concentrations during the June-July study (Fig. 2A and 2B) correlated well with AFDW (Fig. 1A). The similarity of these figures suggested the merit of protein and carbohydrate as additional indicators of biomass. The lowest mean protein and carbohydrate concentrations were observed on the 5th day of the June-July study, coincident with the lowest mean AFDW. By the 30th day, when AFDW was highest in streams dosed with 1.0 mg Zn $liter^{-1}$, protein and carbohydrate concentrations also peaked. Again, by the end of the September-October study, these three measurements indicated higher epilithic biomass concentrations in the streams dosed with 1.0 mg Zn $liter^{-1}$ (Fig. 1B, 2C, and 2D); however, higher variability was noted in these measurements during most of the second study.

Although both epilithic protein and carbohydrate ultimately increased in the high dosed streams, the relative rates of increase for these variables differed. For example, in the epilithon dosed with 1.0 mg Zn $liter^{-1}$ from the 5th to the 30th day during the June-July study, the carbohydrate concentration increased a total of 0.86 mg per cm^2 while the protein concentration only increased by 0.54 mg per cm^2. Thus, during this period of biomass accrual, the epilithon in these streams maintained a lower protein-to-carbohydrate ratio than the epilithon in the control streams (Fig. 4A). By the 10th day of the summer study, the protein-to-carbohydrate ratio in epilithon from unamended streams was greater than 1.0 and significantly higher than the ratios obtained for the epilithon in the dosed systems. Although the control and 0.05 mg Zn $liter^{-1}$ treatments were different only on the 30th day of the fall study (Fig. 4B), differences between the control and 1.0 mg Zn $liter^{-1}$ dosed epilithon was evident from the 2nd day to the end of the study. A rapid decrease of the ratio (by the 2nd day) in the epilithon dosed with 1.0 mg Zn $liter^{-1}$ occurred primarily due to the great increase in carbohydrate at that time. By the 30th day, all treatments were significantly different from each other.

The respiratory rate for glucose per cm^2 was similar for all treatments throughout the June-July study except on the first day when streams dosed with 0.05 mg Zn $liter^{-1}$ had higher

rates than other streams (Fig. 3A). During September-October, epilithon from streams dosed with 1.0 mg Zn liter^{-1} showed elevated glucose respiration rates per cm^2 from the 2nd day to the end of the study with the highest rates occurring on the 5th day (Fig. 3B). These high rates of respiration during the late summer study correspond to periods when treatments were indistinguishable on the basis of biomass measurements.

The respiratory rate for glucose per unit protein on the 5th day of both studies was approximately three times greater in streams dosed with 1.0 mg Zn liter^{-1} than in other treatments (Fig. 5). Similar results were obtained for glucose respiration per unit AFDW.

A protected ANOVA, used to determine the effect of zinc on a variable during the entire study (except the initial day), found that the amount of protein per cm^2 (Fig. 2A and C) was not affected by zinc. A similar response was observed for AFDW (Fig. 1A) and glucose respiration per unit area (Fig. 4A) both during the June-July study. Although these variables do not appear to differ when the entire study is considered, the figures illustrate significant differences on isolated days. All of the other variables considered exhibited a significant effect due to zinc over the entire study during both studies.

DISCUSSION

Although calculations for zinc addition and adjustment of river water dilution rates were repeated periodically during the studies, the measured total zinc concentrations in the water were frequently below the desired levels. The explanation for this inability to maintain the target concentrations of zinc lies in the ability of the epilithon to bioconcentrate zinc. After two days of dosing, significant differences for zinc concentration in epilithon samples was evident among all three treatments (Genter, 1986). During the summer study the maximum concentration of zinc (μg Zn g^{-1} dry weight) in the epilithon dosed with 0.00, 0.05, and 1.00 mg Zn $liter^{-1}$ was 830, 3600, and 42000, respectively. During the fall study the maximum concentration of zinc in the epilithon dosed with 0.00, 0.05, and 1.00 mg Zn $liter^{-1}$ was 530, 4600, and 61000, respectively. The capacity of the epilithon for sequestering this metal continued through each study, representing a major way in which zinc was removed from the water column.

Possibly, the addition of zinc and the corresponding decrease in pH caused the observed changes in the epilithon. However, studies of the biological effect of decreases in pH with and without added zinc determined that at the concentrations used in the artificial streams, the metal is more important in

determining the growth rates of selected aquatic bacteria (S. Hornor, personal communication).

Visual comparison of the streams and nontaxonomic, structural measurements such as AFDW, protein, and carbohydrate indicated that zinc treated streams quickly developed distinct epilithon communities. Although all streams appeared indistinguishable during the predosing colonization period, visual differences between the low dose treatment and streams dosed with 1.0 mg Zn $liter^{-1}$ were evident as early as the 2nd day in both studies, the high dose streams having lost the brown coloration characteristic of the communities before dosing. By the end of both studies the three treatments were easily distinguishable from each other. Control streams were colonized by a brown floc typical of diatoms. This was in contrast to the streams dosed with 0.05 mg Zn $liter^{-1}$ which appeared greenish-brown, and those dosed with 1.0 mg Zn $liter^{-1}$, which contained dark green, loosely attached material. By the 5th day in each study, snails present in the high zinc streams had stopped grazing. Inactive snails were not removed and by the 10th day these snails had died (Farris, 1986).

Changes in the appearance of the streams and epilithic structural measurements were confirmed by analysis of algal species composition (Genter, 1986). The accrual of more

AFDW, protein, and carbohydrate in streams dosed with 1.0 mg Zn liter^{-1} compared to controls may be due to an increase in total numbers of organisms or may reflect algal taxonomic differences; green algae or cyanobacteria were most abundant in the high zinc streams and diatoms dominated the undosed systems (Genter, 1986). Williams and Mount (1965) observed similar changes in algal community composition as a result of zinc dosing. The cell walls of the green algae and cyanobacteria found in the epilithon receiving 1.0 mg Zn liter^{-1} were organic and might have caused the observed increase in AFDW without an increase in cell numbers or biovolume. The inorganic silicaceous cell walls of diatoms which dominated the control streams would not cause an increase in biomass estimates.

The inhibitory effect of the metal on snails in the high zinc streams might have contributed to the surplus of biomass in this treatment by reducing grazing activity. A previous study designed to distinguish the effect of snails and zinc (Appendix 1) consisted of four treatments: 1) no zinc added, snails absent; 2) no zinc added, snails present; 3) 0.50 mg Zn liter^{-1}, snails absent; and 4) 0.5 mg Zn liter^{-1}, snails present. A significant effect of snails on epilithic AFDW was evident in these experiments. Both snails and zinc caused decreases in epilithic biomass during the first 10 days of the study. Due to an acute high dose of zinc on the

13th day, the experiment was unable to determine whether snail death due to zinc was crucial to the ultimate accrual of biomass in the dosed systems. Although an increase in epilithic AFDW occurred by the 30th day in the control streams with snails, the lower temperatures after the 10th day probably decreased grazing activity and allowed accrual of epilithic material even during late November. Alimov (1975) indicates that lower temperatures directly contribute to lower metabolic rates in freshwater mollusks.

An early increase in glucose respiratory rates per unit biomass in epilithon dosed with 1.0 mg Zn $liter^{-1}$ was noted during both of the studies. During the September-October study, the peak in respiratory activity was evident on an areal basis. Although this response was not evident during the June-July period, respiratory rates (on an areal basis) in the streams treated with 1.0 mg Zn $liter^{-1}$ were comparable to other treatments despite significantly lower biomass in the high zinc streams early in the study. During both studies, the heterotrophs remaining active in the epilithon dosed with 1.0 mg Zn $liter^{-1}$ exhibited a transient (5th day) increase in capacity to cycle organic carbon (as measured by glucose respiration). This is different from the decrease in heterotrophic activity noted in planktonic microflora after 3 days in the presence of 0.05 mg Zn $liter^{-1}$ (Albright and Wilson, 1974).

If the epilithon was zinc-limited, then the addition of zinc may have caused the rapid stimulation of activity. Zinc may be limiting in some aquatic environments (Martin et al., 1980), but this is thought to occur in oceanic waters where trace element concentrations may be extremely low. Limitation of growth of a marine diatom was shown at concentrations of 0.65 ng Zn liter^{-1} (10^{-11} M) (Anderson et al., 1978). These concentrations are considerably below the most recent estimates of 0.65 to 65.00 µg dissolved Zn liter^{-1} (10^{-8} to 10^{-6} M) in industrialized freshwaters (Shiller and Boyle, 1985). Thus, it is unlikely that even low concentrations of zinc in the undosed streams would be limiting to epilithon activity or growth.

It is perhaps more likely that 1.0 mg Zn liter^{-1} indirectly caused greater respiratory activity in zinc-tolerant heterotrophs due to the loss of zinc-sensitive species. A zinc-induced decrease in diversity would release zinc-tolerant heterotrophs from competition with organisms intolerant to the higher metal concentration. Also, the zinc-sensitive epilithon may release cellular material as senescence occurs, thereby providing a source of organic energy to support the activity of surviving heterotrophs. This response of metal-tolerant heterotrophs to the decline of metal-sensitive species was suggested by studies of copper-dosed marine mesocosms (Thomas et al., 1977; Vaccaro et al.,

1977). Observation of bacterioplankton and phytoplankton activities and biomass indicated that heterotrophic activities peak following metal addition and this coincides with a general decrease in chlorophyll a concentrations and primary productivity. Simultaneously, more ^{14}C organic matter is released into the system during primary productivity experiments suggesting a loss of cell contents from metal-sensitive species.

It is improbable that the decaying snail biomass contributed to the observed peak in glucose respiration in the epilithon receiving the high zinc dosing. Snail death did not occur until the 10th day (Farris, 1986); 5 days after the apparent peak in glucose respiration. Also, the total mean snail biomass during these studies was approximately 0.23 g AFDW per stream; <50% of the lowest mean biomass estimates for epilithon on the glass rods alone (≥0.51 g AFDW per stream). Thus, the decaying epilithon communities would provide greater quantities of available carbon to the zinc-tolerant heterotrophs than the snail population.

The protein-to-carbohydrate ratio has been used as a determinant of the physiological health of phytoplankton populations (Haug et al., 1973), but it may also be considered as a relative gauge of the carbon-to-nitrogen ratio. Tenore et al. (1979) observed that supplementing a labile organic

carbon source with organic nitrogen significantly increases the secondary production of a detritivore. Higher fecundity and growth rates occur in freshwater snails feeding on epilithon composed of lower carbon-to-nitrogen ratios (McMahon et al., 1974). These studies infer that the nutritive value of the epilithon for grazers or detritivores is directly related to the relative concentration of protein. Although epilithon dosed with 1.0 mg Zn liter^{-1} had higher total protein and carbohydrate concentrations than control epilithon, grazers depending on this source of food would have to consume more total biomass to acquire the protein necessary for survival. Bioconcentrated zinc in the epilithon would, therefore, be consumed in greater quantities by grazing organisms as a result of higher feeding rates.

The accrual of biomass after 30 days of dosing with 1.0 mg Zn liter^{-1} complements the results of a parallel study of algal community taxonomy (Genter, 1986) indicating an adaptation of the epilithic systems to zinc stress. The loss of grazers due to dosing with zinc may contribute to the high epilithic biomass values in these streams. The protein-to-carbohydrate ratios during these two studies indicated significantly lower fractions of protein relative to carbohydrate in the epilithon receiving 1.0 mg Zn liter^{-1} compared to the controls and thus suggests a lower nutritive value of the dosed epilithon. The transient peak in glucose

respiration exhibited by epilithon exposed to 1.0 mg Zn liter^{-1} is a significant factor in the processing of organic carbon. This may be a response of zinc-tolerant organisms to the loss and senescence of zinc-sensitive cells which is consistent with a hypothesis developed from copper studies in marine systems that are fundamentally different than our artificial streams (Thomas et al., 1977; Vaccaro et al., 1977).

LITERATURE CITED

Albright, L.J. and E.M. Wilson. 1974. Sub-lethal effects of several metallic salts - organic compounds combinations upon the heterotrophic microflora of a natural water. Water Res. 8:101-105.

Alimov, A.F. 1975. The rate of metabolism in freshwater bivalve mollusks. Sov. J. Ecol. 6:10-20.

American Public Health Association. 1980. Standard methods for the examination of water and wastewater, 16th ed. American Water Works Association, Water Pollution Control Federation, Washington, D.C.

Anderson, M.A., F.M.M. Morel, and R.R.L. Guillard. 1978. Growth limitation of a coastal diatom by low zinc ion activity. Nature. 276:70-71.

Farris, J.L. 1986. Cellulolytic responses to heavy metal accumulation in Corbicula fluminea and Mudalia dilatata. Ph.D. Dissertation. Virginia Polytechnic Institute and State University. 170 pp.

Filip, D.S., T. Peters, V.D. Adams, and E.J. Middlebrooks. 1979. Residual heavy metal removal by an algae-intermittent sand filtration system. Water Res. 13:305-313.

Genter, R.B. 1986. Population and community response of attached algae to Zn stress alone and in combination with selected environmental variables. Ph.D. Dissertation. Virginia Polytechnic Institute and State University. 100 pp.

Haug, A., S. Myklestad, and E. Sakshaug. 1973. Studies on the phytoplankton ecology of Trondheimsfjord. I. The chemical composition of phytoplankton populations. J. Exp. Mar. Biol. Ecol. 11: 15-26.

Herbert, D., P.J. Phipps, and R.E. Strange. 1971. Chemical analysis of microbial cells, p. 209-344. In, J.R. Norris and D.W. Ribbons (ed.), Methods in microbiology, vol. 5B. Academic Press, London.

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

Itzhaki, R.F. and D.M. Gill. 1964. A micro-biuret method for estimating proteins. Anal. Biochem. 9:401-410.

Lehninger, A.L. 1982. Principals of biochemistry. Worth Publishers, Inc., New York.

Lock, M.A., R.R. Wallace, J.W. Costerton, R.M. Ventullo and S.E. Charlton. 1984. River epilithon: toward a structural-functional model. Oikos. 42:10-22.

Martin, J.H., G.A. Knauer and A.R. Flegal. 1980. Distribution of zinc in natural waters, p. 193-197. In, J.O. Nriagu (ed.), Zinc in the environment, Part I: Ecological cycling. John Wiley and Sons, New York.

McMahon, R.F., R.D. Hunter and W.D. Russell-Hunter. 1974. Variation in aufwuchs at six freshwater habitats in terms of carbon biomass and carbon : nitrogen ratio. Hydrobiologia. 45: 391-404.

Milliken, G.A. and D.E. Johnson. 1984. Analysis of messy data. Vol. 1: Designed experments. Wadsworth, Inc. Belmont, California.

Patrick, F.M. and M.W. Loutit. 1976. Passage of metals in effluents through bacteria to higher organisms. Water Res. 10:333-335.

Rausch, T. 1981. The estimation of micro-algal protein content and its meaning to the evaluation of algal biomass. 1. Comparison of methods for extracting protein. Hydrobiologia. 78:237-251.

Shiller, A.M. and E. Boyle. 1985. Dissolved zinc in rivers. Nature. 317:49-52.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry, 2nd ed. W.H. Freeman and Company, New York.

Spear, P.A. 1981. Distribution, p. 33-46. In, Zinc in the aquatic environment: chemistry, distribution and toxicology. National Research Council of Canada, Ottawa.

Sterritt, R.M. and J.N. Lester. 1979. The microbiological control of mine waste pollution. Minerals and the Environment. 1:45-47.

Tabatabai, M.A. and W.A. Dick. 1983. Simultaneous determination of nitrate, chloride, sulfate and phosphate in natural waters by ion chromatography. J. Environ. Qual. 12:209-213.

Tenore, K.R., R.B. Hanson, B.E. Dornseif, and C.N. Wiederhold. 1979. The effect of organic nitrogen supplement

on the utilization of different sources of detritus. Limnol. Oceanogr. 24: 350-355.

Thomas, W.H., O. Holm-Hansen, D.L.R. Seibert, F. Azam, R. Hodson, and M. Takahashi. 1977. Effects of copper on phytoplankton standing crop and productivity: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:34-43.

United States Environmental Prtection Agency. 1980. Ambient water quality criteria for zinc. EPA 440/5-80-079. Washington D.C.

Vaccaro, R.F., F. Azam, and R. Hodson. 1977. Response of natural marine bacterial populations to copper: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:17-22.

Weatherley, A.H., P.S. Lake, and S.C. Rogers. 1980. Zinc pollution and the ecology of the freshwater environment, p. 337-418. In, J.O. Nriagu (ed.), Zinc in the environment, Part I: Ecological cycling. John Wiley and Sons, New York.

Williams, L.G. and D.I. Mount. 1965. Influences of zinc on periphytic communities. Am. J. Bot. 52:26-34.

Williams, P.J.LeB., and C. Askew. 1968. A method of measuring the mineralization by micro-organisms of organic compounds in seawater. Deep-Sea Res. 15:365-375.

Table 1. Mean water chemistry measurements during June-July and September-October, 1984 studies, ± 1 standard error. n = 18, except where otherwise noted.

Study	Desired Zn conc (mg/L)	Total[a] Zn conc (mg/L)	Temp (°C)	pH[b]	Alkalinity[b] (mg $CaCO_3$/L)	Hardness[b] (mg $CaCO_3$/L)	Conductivity[b] (μmhos/cm)	NH_3^+-N[b] (mg/L)	NO_3^--N[c] (mg/L)	SO_4^--S[c] (mg/L)
June-July	0.00	0.02 ±0.01	25.5 ±0.5	8.39 ±0.07	49.8 ±1.7	70.3 ±3.1	122.8 ±6.0	N.D.	4.5 ±0.9	13.3 ±1.2
	0.05	0.04 ±0.00	-	8.31 ±0.09	49.5 ±1.4	70.7 ±3.4	124.5 ±5.4	N.D.	4.8 ±0.4	12.0 ±0.7
	1.00	1.17 ±0.27	-	8.06 ±0.06	49.8 ±1.4	72.3 ±2.5	128.6 ±4.9	N.D.	4.9 ±0.4	14.6 ±0.9
Sept-Oct	0.00	0.03 ±0.01	20.6 ±1.0	8.31 ±0.12	56.3 ±0.7	88.8 ±2.3	164.3 ±4.0	0.14 ±0.04	2.4 ±0.5	16.0 ±0.4
	0.05	0.08 ±0.03	-	8.27 ±0.10	55.6 ±0.8	88.3 ±2.6	160.7 ±3.8	0.13 ±0.04	2.5 ±0.5	18.0 ±0.2
	1.00	0.98 ±0.08	-	8.14 ±0.02	56.3 ±0.7	88.3 ±2.3	167.2 ±4.1	0.14 ±0.04	2.9 ±0.6	18.0 ±0.2

a. June-July: n = 15.

b. September-October: n = 12.

c. June-July: n = 12; September-October: n = 6.

N.D. = Not determined.

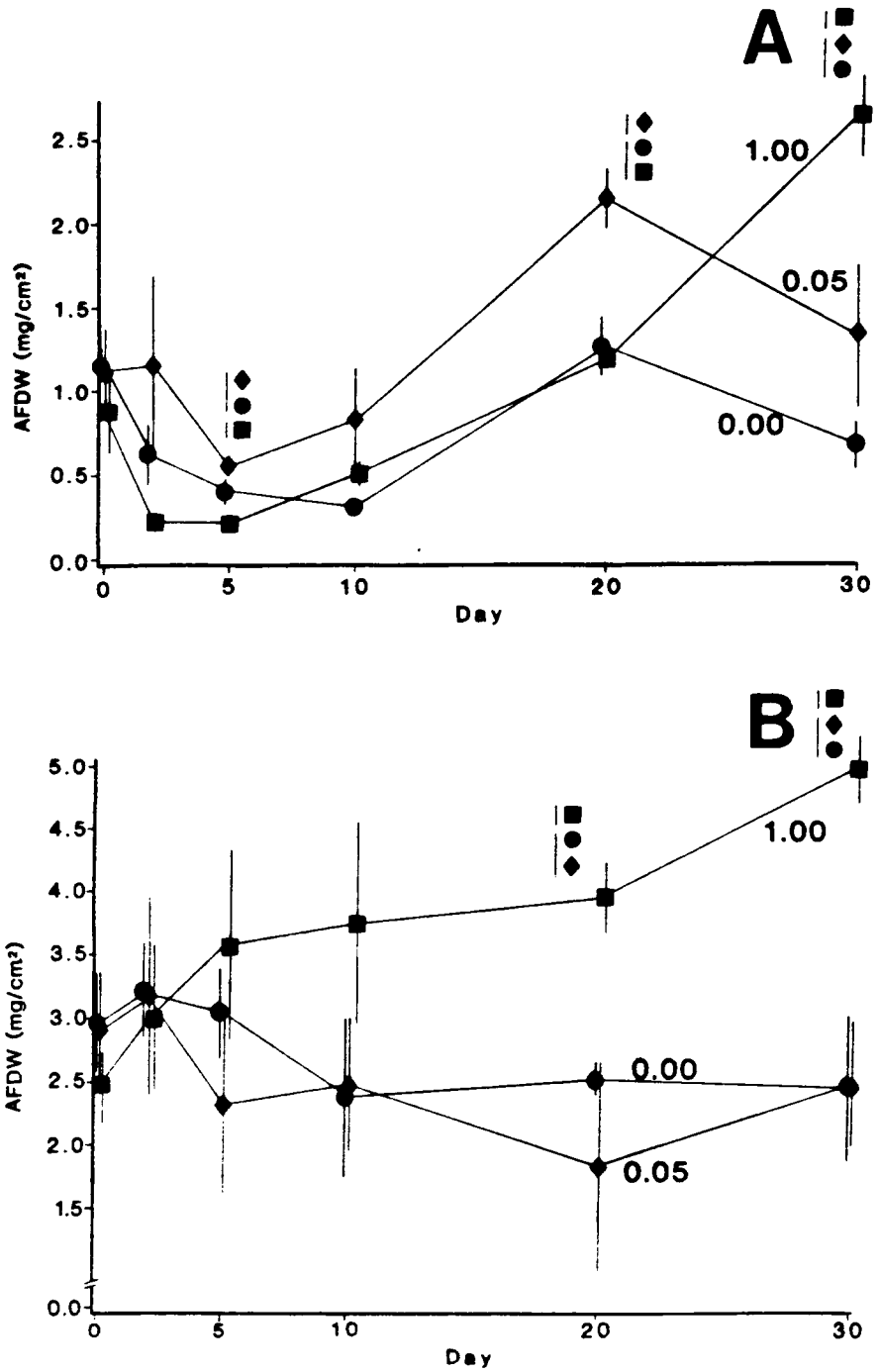

Figure 1. Ash-free dry weight (mg/cm^2) for the June-July (A) and September-October, 1984 (B) study periods. Points represent the mean of three replicates ± standard error. The results of Duncan's multiple range tests among treatments coincide with days when significant differences (α=0.05) were determined. Symbols that represent the different treatments are not significantly different when connected by a vertical bar.

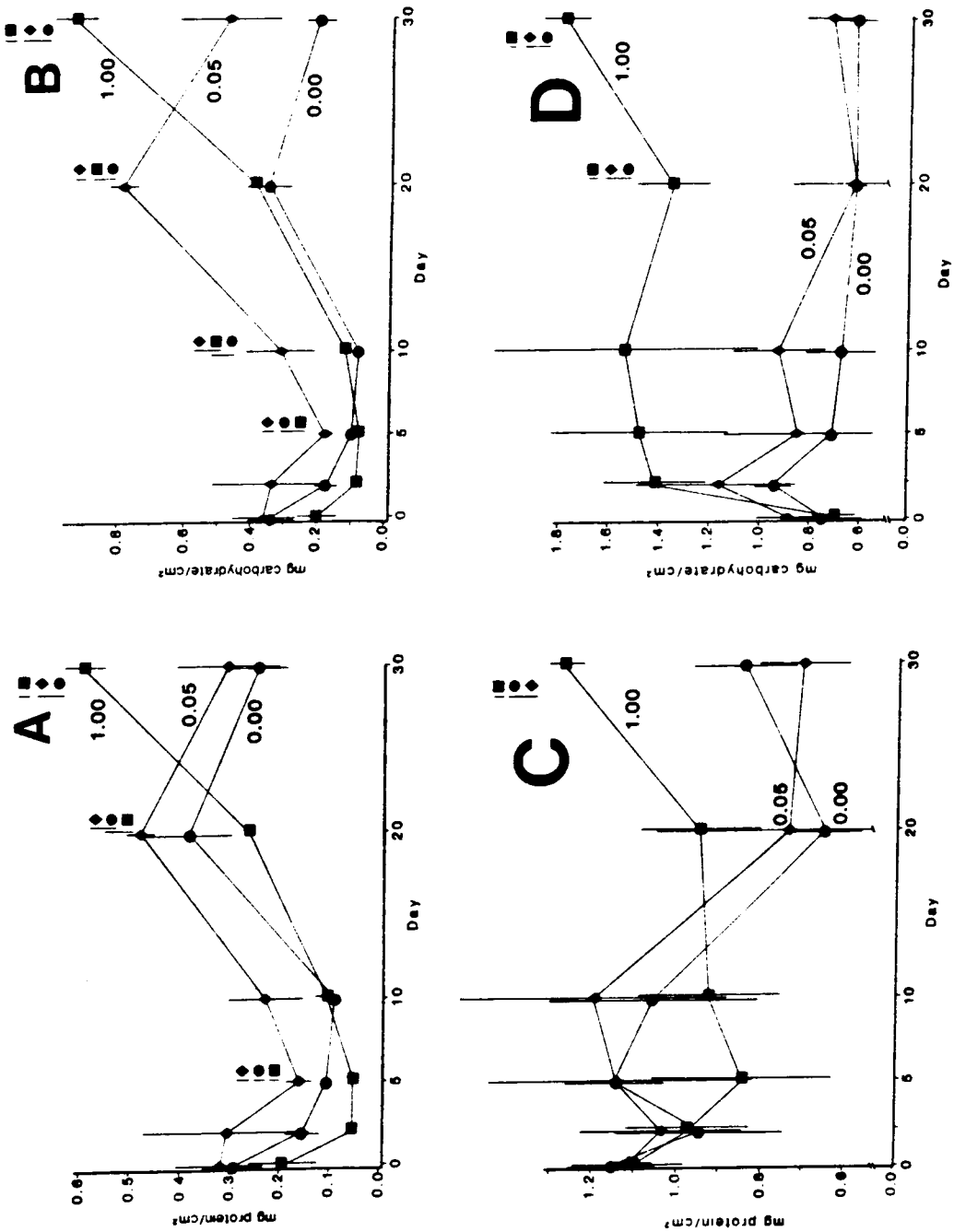

Figure 2. Epilithic protein (A) and carbohydrate (B) for June-July and protein (C) and carbohydrate (D) for September-October, 1984 studies (mg/cm^2). Points represent the mean of three replicates ± standard error. The results of Duncan's multiple range tests among treatments coincide with days when significant differences (α=0.05) were determined. Symbols that represent the different treatments are not significantly different when connected by a vertical bar.

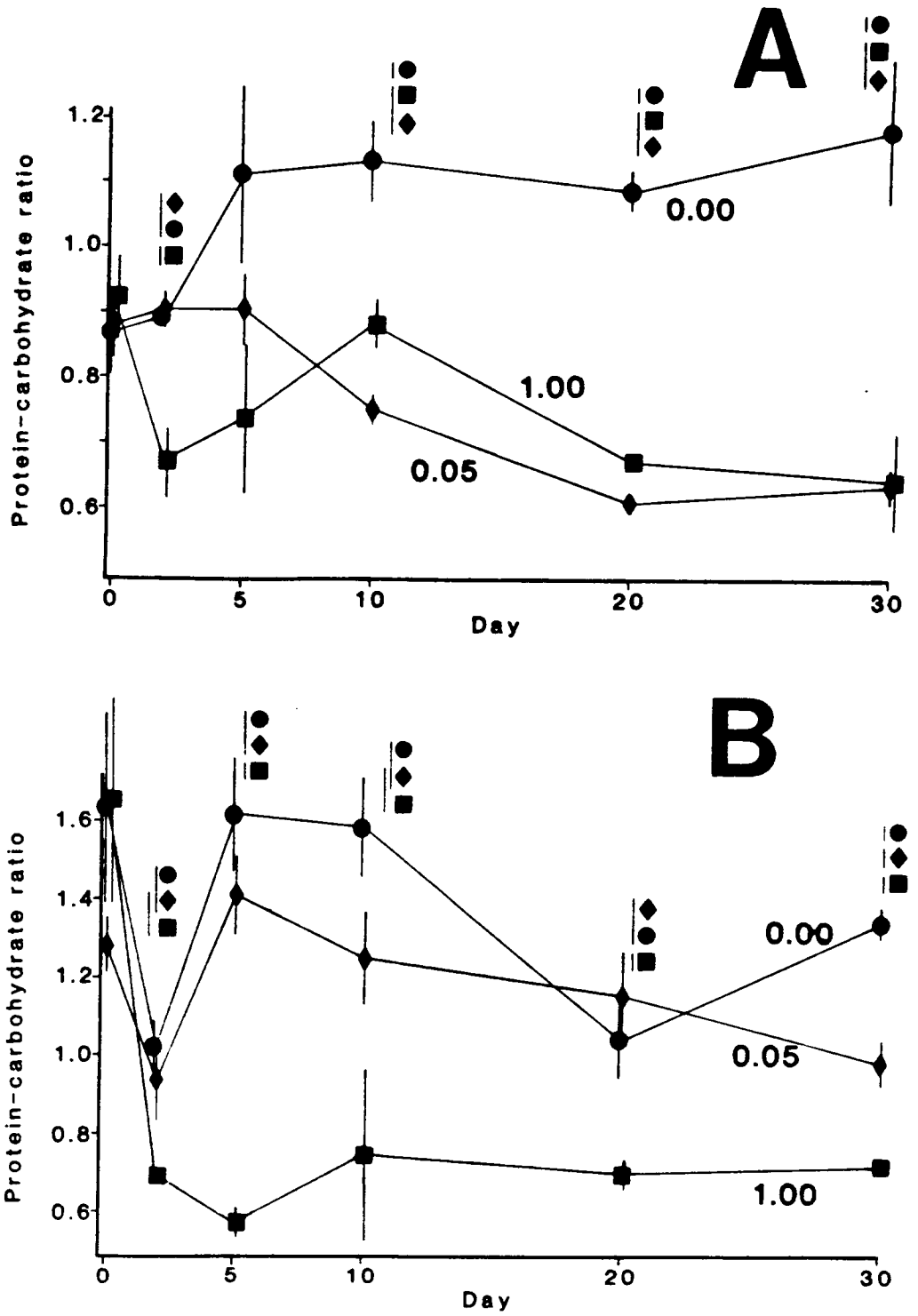

Figure 3. Epilithic protein-to-carbohydrate ratios for June-July (A) and September-October (B), 1984 studies. Points represent the mean of three replicates ± standard error. The results of Duncan's multiple range tests among treatments coincide with days when significant differences (α=0.05) were determined. Symbols that represent the different treatments are not significantly different when connected by a vertical bar.

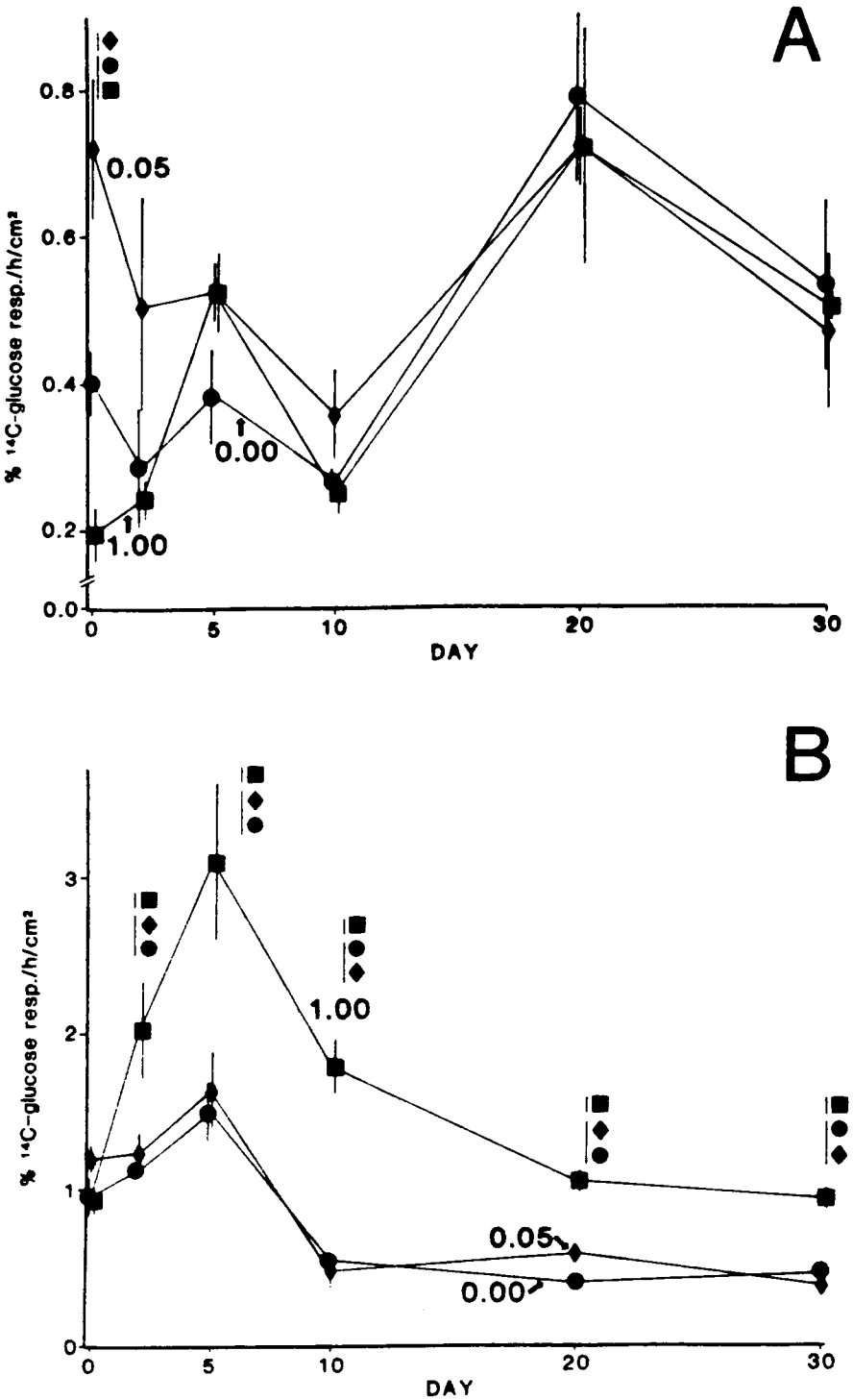

Figure 4. Glucose respiration rate (% ^{14}C-glucose respired/h/cm^2) of epilithon for the June-July (A) and September-October (B), 1984 studies. Points represent the mean of three replicates ± standard error. The results of Duncan's multiple range tests among treatments coincide with days when significant differences (α=0.05) were determined. Symbols that represent the different treatments are not significantly different when connected by a vertical bar.

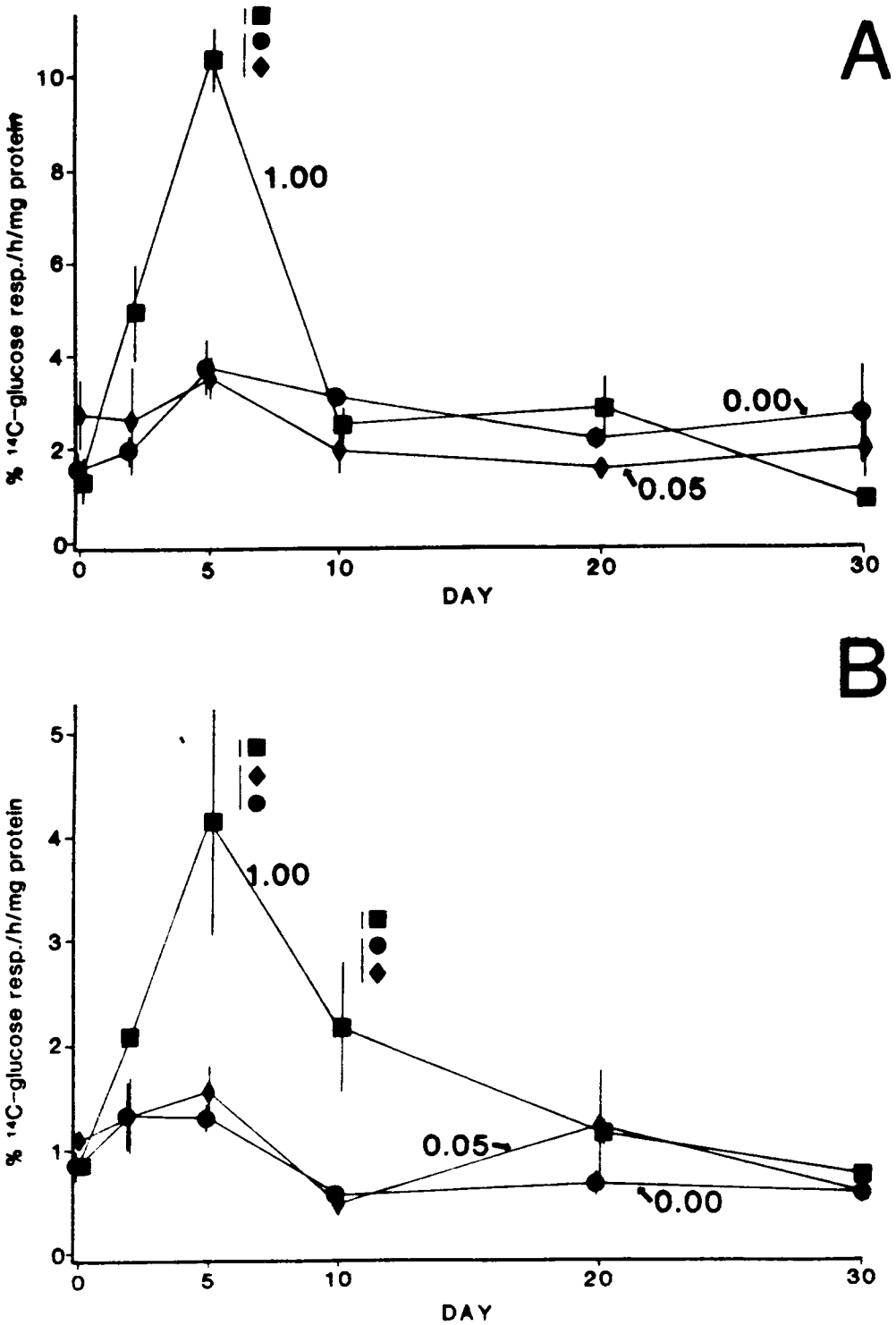

Figure 5. Glucose respiration rate per mg protein (% ^{14}C-glucose respired/h/mg protein) for the June-July (A) and September-October (B), 1984 studies. Points represent the mean of three replicates ± standard error. The results of Duncan's multiple range tests among treatments coincide with days when significant differences (α=0.05) were determined. Symbols that represent the different treatments are not significantly different when connected by a vertical bar.

CHAPTER II

EPILITHON EXPOSURE TO ZINC:
2. EVIDENCE OF STRUCTURAL AND FUNCTIONAL ADAPTATION

ABSTRACT

During summer and fall, 1984, dosing artificial streams with 1.0 mg Zn liter^{-1} caused a peak in glucose respiration and changes in epilithic total organic matter, protein, and carbohydrate concentrations (Chapter I). Additional experiments were conducted during summer and fall, 1985, to more completely characterize the respiratory response and structural changes in dosed communities. Artificial streams were continuously dosed with 0.0 and 1.0 mg Zn liter^{-1} and the following analyses were performed 0, 5, 10, and 20 days after starting zinc additions: 1) rates of respiration of ^{14}C-glucose and ^{14}C-glutamate by epilithon, 2) rates of O_2 and CO_2 flux, 3) ash-free dry weight (AFDW), protein, and photosynthetic pigment concentrations. Glucose respiration in dosed epilithon was greatest on the 10th day and coincided with decreases in the structural measurements of AFDW, protein, and algal pigment concentrations. Respiration of glutamate did not show a significant response to dosing

(α=0.05). By the 20th day, structural measurements and glucose respiration rates in dosed systems were indistinguishable from control values. Plate counts of epilithon using zinc amended and unamended media demonstrated more total and zinc-tolerant colony forming units in dosed than in control epilithon after 20 days of dosing. Dosed epilithon exhibited lower rates of O_2 and CO_2 flux at the end of the study. High glucose respiration rates and low biomass after 10 days of dosing complement previous observations and suggest the presence of zinc-tolerant heterotrophs that selectively respire glucose. After 20 days of dosing, zinc-tolerant heterotrophic communities were present with lower rates of respiratory gas flux than undosed epilithon, thus providing evidence for epilithic colonization with organisms capable of existence in these metal stressed conditions.

INTRODUCTION

Epilithic communities in rivers facilitate nutrient cycling, energy flow, biodegradative capacities, and provide the basis for secondary production in aquatic systems. Due to the fundamental activities and the increasing prevalence of toxic materials in freshwater, the influence of heavy metals and organic compounds on the epilithon has been considered in various studies (Clark et al., 1982; Leland and Carter, 1984; Yount and Richter, 1986). In a previous study (Chapter I), glucose respiration rates peaked in epilithon from artificial streams after 5 days of dosing with 1.0 mg Zn $liter^{-1}$. After 30 days of zinc dosing, glucose respiration rates resembled values in undosed epilithon; however, ash-free dry weight (AFDW), protein, and carbohydrate reflected changes in the total organic matter and macromolecular composition of the dosed communities. The changes in epilithon glucose respiration may be caused by a response of zinc-tolerant heterotrophs to the inactivity and senescence of zinc-sensitive species, followed by habituation to the metal and recolonization of the substratum by a zinc-tolerant community. A similar hypothesis was suggested by Thomas et al., (1977) to explain the effects of copper on marine planktonic systems.

Haack and McFeters (1982) noted that the activity of attached heterotrophs in epilithic mat communities appeared to be affected by the breakdown of the associated algal populations. More specifically, estimates of glucose-to-glutamate utilization ratios in marine plankton decrease with increasing depth (Gillespie et al., 1976) and transition of seasons (Griffiths et al., 1982). These authors suggested that healthy phototrophic communities release proportionately more glucose than amino acids, but when these communities decline due to decreasing light they release relatively more amino acids (e.g. glutamate). An abrupt decrease in the glucose-to-glutamate utilization ratio coinciding with a decline in primary productivity suggests that planktonic heterotrophs respond rapidly to changes in the quality of available dissolved organic carbon. Thus, the glucose-to-glutamate utilization ratio may be used to indicate the "health" of an algal community.

Another indicator of the physiological condition of algal communities is the chlorophyll a-to-pheophytin a pigment ratio (American Public Health Association, 1985). Stressed algae exhibit lower ratios than healthy algal populations. Both the chlorophyll a-to-pheophytin a ratio and the glucose-to-glutamate utilization ratio were selected to test the effect of zinc on the epilithic community in artificial streams.

The goal of this study was to measure epilithic respiration, and specifically the mineralization of glucose and glutamate, to understand community changes due to extended dosing with 1.0 mg Zn liter^{-1}. Further, I wished to determine whether zinc-tolerant microorganisms become established in these artificial streams during the study period and whether specific structural indicators of the algal community (i.e. photosynthetic pigment concentrations and ratios) respond to continuous zinc additions.

MATERIALS AND METHODS

Study site and treatment design. Glass rods (5.4 cm^2 surface area) separated by ≥2.2 cm were incubated in an attached, vertical position in river-fed artificial streams along the New River at Glen Lyn, Virginia, U.S.A. Design of the artificial streams was presented by Farris (1986). Six artificial streams were used, representing three replicates of each of two zinc treatments (0.0 and 1.0 mg Zn liter^{-1} additions as zinc sulfate). The rods were colonized with epilithon in the streams for two weeks before starting zinc dosing. One day before dosing with zinc, snails from the New River were placed in the streams (approximately 80-110 snails per m^2) to maintain conditions similar to the 1984 studies (Chapter I). One 20-day dosing period was started on August 22, 1985

(August-September study). After cleaning and recolonization, on September 29, 1985 (September-October study), a second 20-day study was started. Shorter (20 day) dosing periods were used during the present study since the critical effect of zinc on the epilithon was noted soon after starting dosing. Rods colonized by epilithon were measured for respiratory capacities and biochemical composition 0, 5, 10, and 20 days after starting zinc dosing.

Glucose and glutamate respiration. Respiratory rates for glucose and glutamate were determined during the August-September study using a modification of the method of Williams and Askew (1968). Rods with intact epilithon were removed randomly from two zones in each stream and placed into 250-ml glass jars containing 60 ml of water from the corresponding stream. Trace amounts of [U-^{14}C] glucose (specific activity: 345 µCi per mmole) and [U-^{14}C] glutamate (specific activity: 290 µCi per mmole) were added to separate jars containing the glass rods to achieve final concentrations of 0.44 and 0.21 µg liter^{-1}, respectively. The contents were mixed and the jars sealed and incubated in the dark with gentle shaking for 15 min so that < 10% of the labeled substrate would be respired. Biological activity was stopped by the injection of 2 ml of 6N H_2SO_4. Acid-killed controls were used to correct for abiotic production of

$^{14}CO_2$. A dry filter paper in each jar was saturated with 0.15 ml of phenylethylamine allowing absorption of $^{14}CO_2$ which occurred during 1.5 h of gentle shaking. Subsequently, filter papers were placed into 10 ml of cocktail (4 g PPO and 0.1 g POPOP liter^{-1} toluene) and counted using a Beckman LS-3150 scintillation counter. The sample channels ratio method was used to correct for quench.

Respiratory gas analyses. On each study day during the August-September study, O_2 and CO_2 concentrations were measured in the headspace of Hungate tubes containing a single epilithon-colonized glass rod and 8 ml of water from the corresponding artificial stream. Samples were transported on ice to the laboratory and analyses started within 3 h of collection. Measurements were made before and after 6 to 8 h shaking incubations in the dark at 20° C, allowing an estimate of the rate of consumption of O_2 and production of CO_2 during the shortest period necessary to obtain a response. Gases were analyzed using a Varian Model 920 gas chromatograph equipped with a thermal conductivity detector and parallel, 6 foot, stainless steel columns containing Porapak-Q and Molecular Sieve 5-A (45/60 mesh) packings (Supelco, Bellefonte, PA). A 1.0-ml Hamilton gas-tight syringe was used to remove 300 µl of headspace gas after which 250 µl was injected for analysis. The carrier gas was

helium and the injector, column, and detector temperatures were 110, 40, and 140° C, respectively. A Fisher Recordall Series 5000 chart recorder was used to measure the output signal from the thermal conductivity detector. Symmetric peaks allowed quantitation of gases using peak height measurements and a custom mixture of gases containing 73.6% nitrogen, 20.4% oxygen, 4.3% carbon dioxide, and 1.7% methane was used as a standard.

Ash-free dry weight, protein, and pigment determinations. Epilithon was scraped from 3-10 glass rods from each stream and homogenized in a Waring blendor. The resulting slurry was stored at -20° C for subsequent measurement. AFDW was determined by subtracting the combusted weight (1 h at 500° C) from the dry weight (dried to constant weight at 105° C) for samples from the replicated treatments (American Public Health Association, 1985). Chlorophyll a was estimated by measuring the optical density (O.D.) of an acetone extracted epilithon sample at 663 nm (American Public Health Association, 1985). Subsequently, the sample was acidified and measured at O.D. 665 nm to derive pheophytin a. The ratio of O.D. 663 nm before acidification-to-O.D.665 nm after acidification provided an estimate of the chlorophyll a-to-pheophytin a ratio. Protein was extracted from the slurries at 80° C in 0.5 N NaOH for 10 min, repeating the procedure

three times per sample (Rausch, 1981). Protein was analyzed using the micro-biuret method with bovine serum albumin as a standard (Itzhaki and Gill, 1964). Recovery of bovine serum albumin was 90% after the extraction process.

Heterotrophic counts. Total heterotrophic counts were determined for the initial and 20th days during the August-September study using the method of Hornor and Hilt (1985). An appropriate dilution of epilithon slurry from each stream was spread onto triplicate plates containing 1/4 strength Nutrient Agar (Difco) and 0.0, 1.0, or 10.0 mg Zn $liter^{-1}$. Plates were incubated in the dark at 20° C. Plates containing 30-300 colonies were counted after 5 days and expressed as colony forming units (CFU) per mg AFDW.

Water chemistry. On each sampling day of both studies, temperature, pH, alkalinity, conductivity, hardness, ammonia, and total zinc in each stream were measured by standard methods (American Public Health Association, 1985). At the beginning and end of each study, nitrate, sulfate, and phosphate concentrations were measured using ion chromatography (Dionex) (Tabatabai and Dick, 1983). Dissolved organic carbon (DOC) from each stream was measured in filtered water samples (Gelman glass fiber filters, type A/E,

0.3 μm pore size) on each sampling day during the August-September study using a Model 700 Total Organic Carbon Analyzer-O.I. Corporation (Menzel and Vaccaro, 1964).

Statistical analysis. A repeated measures or split plot design (without replication or blocking) was used to analyze the experiment (Milliken and Johnson, 1984). Water chemistry measurements from each sample day and heterotrophic plate counts (August-September study) were compared using analysis of variance (ANOVA). The non-parametric Mann-Whitney test which utilizes a rank-sum protocol (Sokal and Rohlf, 1981) was used to distinguish sample distributions on each study day for mean glucose and glutamate respiration rates, rates of O_2 and CO_2 flux, as well as AFDW, protein, chlorophyll a, and pheophytin a measurements. For each variable, differences were considered significant at the $\alpha=0.05$ level.

RESULTS

Temperature, which did not vary significantly between replicate streams or treatments, increased from 20 to 25° C during the first 20-day study (Table 1). Except for a low reading on the 10th day, temperatures during the later study were consistently between 17 and 18° C.

Among the other water chemistry measurements, only the total zinc concentration and pH were significantly different between treatments on a given study day (Table 1). Total zinc concentrations in the dosed streams ranged from below detectable limits at the start of both studies to high values of 0.89 and 0.87 mg Zn $liter^{-1}$ for the August-September and September-October studies, respectively. Total zinc concentrations for the control streams were often below detecton (<0.025 mg $liter^{-1}$). pH ranged from 8.93 to 7.41 during the studies, with lower values frequently noted in the zinc-dosed streams. Phosphate concentrations were always below detectable limits (<30 µg PO_4^{-3}-P $liter^{-1}$) using ion chromatography. DOC was not significantly different between treatments on any of the study days. DOC ranged from 2.5 to 4.3 mg carbon $liter^{-1}$ during the study.

After similar rates of glucose respiration were observed in both treatments during the first two study days (Fig. 1A), there was a peak in glucose respiration in the dosed epilithon on the 10th day of the study equal to 3-4 times that observed concurrently in the controls. This was followed by a decrease such that on the 20th day the glucose respiration rates in streams receiving zinc were again indistinguishable from rates in the control stream epilithon.

Glutamate respiration rates during the same study were more variable (Fig. 1B). No significant differences were evident during the 20-day dosing period. Epilithon from reference streams showed a pattern of glutamate respiration that mimicked glucose respiration although relatively more glutamate was respired per unit AFDW than glucose on each study day. This trend is also apparent in the glucose-to-glutamate respiration ratio (Fig. 1C). While this ratio remained stable (ca. 0.3) for measurements made on epilithon from control streams, significantly higher ratios were evident in the zinc-dosed epilithon on the 5th and 10th days. Subsequently, the glucose-to-glutamate respiration ratio of dosed epilithon appeared to approach control values by the 20th day.

By the end of the August-September study, epilithon from control streams had significantly higher rates of CO_2 production and O_2 consumption than epilithon from zinc-dosed systems (Fig. 2). This contrasted with O_2 and CO_2 measurements made on the earlier sampling days when differences between treatments were usually not significant.

A decrease in epilithic AFDW, protein, chlorophyll a, and pheophytin a concentrations occurred in the dosed streams relative to controls within the first 5 to 10 days of each study (Tables 2 and 3). During this period, the AFDW of zinc-dosed epilithon ranged from 35 to 45% of control values.

Although epilithic AFDW in control streams generally decreased by the 20th day during both studies, it increased in zinc-treated epilithon from the 10th to the 20th days. Protein, chlorophyll a, and pheophytin a concentrations also increased between the 10th and 20th days in the zinc-dosed epilithon. The ratio of chlorophyll a-to-pheophytin a ranged from 1.6 to 1.7 in both treatments throughout each study and did not indicate any effect of zinc dosing.

Before beginning dosing for the August-September study, epilithic heterotrophs from the control and pre-dosed streams did not differ significantly with regard to total CFU or CFU tolerant to zinc in 1/4 strength Nutrient Agar (Fig. 3). Epilithon from each stream demonstrated progressively fewer CFU as the concentration of zinc in the media increased. On the 20th day, epilithon from streams that had received continuous dosing had significantly more total CFU than epilithon from control streams and also significantly more zinc-tolerant CFU. By the end of the study, colony counts from the dosed epilithon on plates containing 10 mg Zn $liter^{-1}$ were not significantly different than counts from plates lacking any added zinc. Additionally, the ANOVA demonstrated a significant effect of day on CFU on unamended and 10 mg Zn $liter^{-1}$ amended plates.

DISCUSSION

As discussed in a previous report of similar studies (Chapter I), the inability to achieve total zinc concentrations equal to the desired (target) concentration was due to the ability of the epilithon to bioconcentrate this metal. Although target concentrations were not always achieved, a mean total zinc concentration approaching 1.0 mg Zn $liter^{-1}$ was achieved and significant differences in total zinc concentrations between dosed and control streams indicate that the treatment was effectively applied. The lower pH values in the zinc-treated systems were a direct result of the addition of zinc sulfate since the metal ion exists as a hydrate in solution and increases the acidity of the coordinated water (Stumm and Morgan, 1981). There were no measurable dose-associated increases in conductivity or sulfate concentrations.

The peak in glucose respiration rates that was observed on the 10th day of the August-September study was noted in studies during summer and fall, 1984 (Chapter I), except that during 1984 the highest glucose respiration rates occurred on the 5th day. The present study also indicated that by the 20th day glucose respiration in epilithon from dosed streams had returned to values similar to the controls, as seen in 1984. The zinc-dependent changes in glucose respiration during the 1984 and 1985 studies were very similar, and con-

firm this response of the epilithon to dosing with 1.0 mg Zn $liter^{-1}$.

The respiration of glutamate by epilithic communities dosed with zinc did not increase significantly. This is reflected in higher glucose-to-glutamate ratios which accentuate the peak in glucose respiration. The ratio derived from epilithon from control streams remained constant indicating that fluctuations in glucose respiration were matched by fluctuations in glutamate respiration throughout the study.

The trend towards a higher glucose-to-glutamate respiration ratio in zinc-dosed epilithon is not in accord with the expected result as extrapolated from marine studies (Gillespie et al., 1976; Griffiths et al., 1982). In the marine studies phytoplankton blooms coincided with high glucose-to-glutamate utilization ratios in the associated planktonic heterotrophs. However, as the health of the algal populations declined, the ratio decreased, indicating a physiological adjustment of the heterotrophs towards scavenging higher concentrations of amino acids present due to algal senescence. The pelagic marine systems considered in these studies are similar to large batch cultures in that algal exudates remain in the system allowing heterotrophic adaptation to this newly available organic carbon. In contrast, the artificial streams resemble continuous cultures in which

zinc-sensitive epilithic species and organic carbon from these organisms are washed out of the system.

The chlorophyll a-to-pheophytin a ratio is a sensitive indicator of algal stress (American Public Health Association, 1985). A sample with a ratio of 1.7 has essentially no pheophytin a (physiologically healthy) whereas a ratio of 1.0 indicates pure pheophytin a (stressed or senescing algae). Since all of the ratios calculated during these studies were between 1.7 and 1.6 and not correlated with dosing, senescence due to zinc did not actually occur *in situ*. It is more likely that the significant loss of biomass during the 5th through 10th days represents the wash out of zinc-sensitive species in the dosed systems.

The absence of increased glutamate respiration in zinc-dosed epilithon during the 5th through 10th days may simply reflect a loss of total or excreted organic carbon from the streams before the zinc-tolerant heterotrophs could adapt to utilize this source of energy. Zinc-dosed epilithon at this time also did not exhibit higher rates of O_2 and CO_2 flux as might be expected in a community metabolizing large amounts of organic matter. The loss of epilithic biomass also implies a loss of the extracellular matrix, which functions as a trap for colloidal and dissolved organic matter (Lock *et al.*, 1984; Lock and Ford, 1985). Peaks in glucose respiration

during this period may indicate an enhanced ability of the zinc-tolerant heterotrophs to respire glucose under conditions favoring stream recolonization and low competition. Later (20th day) when glucose respiration rates in the dosed epilithon decreased, these streams showed increased biomass and probably greater competition.

It has been suggested that measurement of O_2 consumption rates may estimate carbon mineralization processes more accurately than experiments using ^{14}C-labelled substrates (Sepers et al., 1982). On the 10th day, when rates of glucose respiration peaked and epilithon structural measurements had declined in the zinc-dosed streams, there was no clear difference in O_2 consumption or CO_2 production between treatments. However, by the 20th day, when taxonomically different algal communities had developed between the treatments (Genter, 1986) and plate counts indicated the establishment of zinc-tolerant heterotrophs in the dosed streams, measurements of overall respiration were strikingly different between treatments. Lower fluxes of O_2 and CO_2 may be inherent to these zinc-adapted communities due to physiological differences or possibly decreased diffusion of gases out of the epilithon.

During both studies AFDW, protein, chlorophyll a, and pheophytin a concentrations decreased by the 10th day and

sometimes as early as the 5th day in the dosed epilithon. Subsequently, an increase in these measurements by the 20th day suggests that the stressful effect of zinc involves recolonization of the substrate by components of the epilithon that were capable of existing in this altered habitat. These results correspond to those obtained during summer, 1984 (Chapter I).

Approximately equal numbers of CFU from dosed epilithon appeared on zinc amended and unamended media at the end of the August-September study indicating the presence of zinc-tolerant heterotrophic populations. The effect of day in determining the number of CFU was especially evident on plates without added zinc. This indicated that during the study, changes in the epilithon from control streams occurred such that by the 20th day these communities had fewer total CFU per mg AFDW than at the start of the study. This may identify a real trend in heterotrophic numbers in these evolving communities or simply indicate that a large number of the heterotrophs from these epilithon samples are incapable of growth or develop as single colonies on 1/4 strength Nutrient Agar. A correlation between zinc-tolerant heterotrophs and zinc concentration has been noted previously in stream sediments (Hornor and Hilt, 1985). In studies of the effect of mercury on marine planktonic bacteria, viable bacterial counts decrease upon addition of the metal but

rapidly recover and remain at least equal to control values throughout the next 10 days of the study, indicating a development of tolerance to mercury similar to that found in the epilithic communities dosed with zinc (Azam et al., 1977). Studies of the effect of copper on marine bacterioplankton suggest a similar development of tolerance to that metal (Vaccaro et al., 1977).

It is evident that epilithon in these artificial streams responded to 1.0 mg Zn liter^{-1} with increased rates of glucose respiration coincident with decreases in AFDW, protein, chlorophyll a, and pheophytin a concentrations. While peaks in glucose respiration occurred, glutamate respiration remained unaffected suggesting that amino acids and other compounds excreted from zinc-sensitive epilithon were washed out of the system before zinc-tolerant heterotrophs could respond. Since chlorophyll a-to-pheophytin a ratios remained high during the period of biomass reduction, the epilithon was not senescing in situ, but washing-out of the systems and thus limiting the use of the glucose-to-glutamate respiration ratio for detecting an altered physiological response by the zinc-tolerant epilithon. Epilithic O_2 and CO_2 fluxes were not affected by zinc dosing until distinctive zinc-tolerant heterotrophs, as determined by zinc-tolerant CFU, had developed. By the 20th day, these communities maintained lower fluxes of O_2 and CO_2 than comparable control epilithon. De-

creases in epilithic AFDW, protein, and photosynthetic pigment concentrations after 5 to 10 days of dosing were reversed by the 20th day. After 20 days, more total and zinc-tolerant CFU were evident in dosed than in control epilithon, providing evidence that these epilithic assemblages adapted to the stressful conditions exerted by the zinc. Although dosing with 1.0 mg Zn $liter^{-1}$ caused an initial reduction in total epilithic biomass, continued zinc stress resulted in the development of epilithic communities that maintained functional processes (e.g. organic carbon cycling) similar to undosed epilithon.

LITERATURE CITED

American Public Health Association. 1985. Standard methods for the examination of water and wastewater, 16th ed. American Water Works Association, Water Pollution Control Federation, Washington, D.C.

Azam, F., R.F. Vaccaro, P.A. Gillespie, E.I. Moussalli, and R.E. Hodson. 1977. Controlled ecosystem pollution experiment: Effect of mercury on enclosed water columns. II. Marine bacterioplankton. Mar. Sci. Communs. 3:313-329.

Clark, J.R., D.S. Cherry, and J. Cairns. 1982. Food quality of aufwuchs from artificial streams receiving low levels of perturbations. Water Res. Bull. 18:761-767.

Farris, J.L. 1986. Cellulolytic responses to heavy metal accumulation in Corbicula fluminea and Mudalia dilatata. Ph.D. Dissertation. Virginia Polytechnic Institute and State University. 170 pp.

Genter, R.B. 1986. Population and community response of attached-algae to Zn stress alone and in combination with selected environmental variables. Ph.D. Dissertation. Virginia Polytechnic Institute and State University. 100 pp.

Gillespie, P.A., R.Y. Morita, and L.P. Jones. 1976. The hetero- trophic activity for amino acids, glucose , and acetate in Antarctic waters. J. Oceanogr. Soc. Japan. 32:74-82.

Griffiths, R.P., B.A. Caldwell, and R.Y. Morita. 1982. Seasonal changes in microbial heterotrophic activity in subarctic marine waters as related to phytoplankton primary productivity. Mar. Biol. 71:121-127.

Haack, T.K. and G.A. McFeters. 1982. Microbial dynamics of an epilithic mat community in a high alpine stream. Appl. Environ. Microbiol. 43:702-707.

Hornor, S.G. and B.A. Hilt. 1985. Distribution of zinc-tolerant bacteria in stream sediments. Hydrobiologia. 128:155-160.

Itzhaki, R.F. and D.M. Gill. 1964. A micro-biuret method for estimating proteins. Anal. Biochem. 9:401-410.

Leland, H.V. and J.L. Carter. 1984. Effects of copper on species composition of periphyton in a Sierra Nevada, California, stream. Freshwat. Biol. 14:281-296.

Lock, M.A. and T.E. Ford. 1985. Microcalorimetric approach to determine relationships between energy supply and metabolism in river epilithon. Appl. Environ. Microbiol. 49:408-412.

Lock, M.A., R.R. Wallace, J.W. Costerton, R.M. Ventullo, and S.E. Charlton. 1984. River epilithon: toward a structural-functional model. Oikos. 42:10-22.

Menzel, D.W. and R.F. Vaccaro. 1964. The measurement of dissolved organic and particulate carbon in seawater. Limnol. Oceanogr. 9: 138-142.

Milliken, G.A. and D.E. Johnson. 1984. Analysis of messy data. Vol. I: Designed experiments. Wadsworth Inc., Belmont, California.

Rausch, T. 1981. The estimation of micro-algal protein content and its meaning to the evaluation of algal biomass. 1. Comparison of methods for extracting protein. Hydrobiologia. 78:237-251.

Sepers, A.B.J., G. Cahet, and H. Goossens. 1982. Comparison between the carbon-14 and oxygen consumption method for determination of the activity of heterotrophic bacterial populations. Mar. Biol. 66:237-242.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry, 2nd ed. W.H. Freeman and Company, New York.

Stumm, W. and J.J. Morgan. 1981. Aquatic chemistry, 2nd ed. John wiley and Sons, Inc., New York.

Tabatabai, M.A. and W.A. Dick. 1983. Simultaneous determination of nitrate, chloride, sulfate and phosphate in natural waters by ion chromatography. J. Environ. Qual. 12:209-213.

Thomas, W.H., O. Holm-Hansen, D.L.R. Seibert, F. Azam, R. Hodson, and M. Takahashi. 1977. Effects of copper on phytoplankton standing crop and productivity: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:34-43.

Vaccaro, R.F., F. Azam, and R. Hodson. 1977. Response of natural marine bacterial populations to copper: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:17-22.

Williams, P.J.LeB. and C. Askew. 1968. A method of measuring the mineralization by micro-organisms of organic compounds in seawater. Deep-Sea Res. 15:365-375.

Yount, J.D. and J.E. Richter. 1986. Effects of pentachlorophenol on periphyton communities in outdoor ex-

perimental streams. Arch. Environ. Contam. Toxicol. 15:51-60.

Table 1. Mean water chemistry measurements during August-September and September-October, 1985 studies, ± standard error. n = 12, except where otherwise noted.

Study	Desired Zn conc (mg/L)	Total Zn conc (mg/L)	Temp (°C)	pH	Alkalinity (mg $CaCO_3$/L)	Hardness (mg $CaCO_3$/L)	Conductivity (μmhos/cm)	NH_3^+-N (mg/L)	NO_3^--N[a] (mg/L)	SO_4^--S[a] (mg/L)	DOC[b] (mg C/L)
Aug-Sept	0.0	0.01 ±0.01	22.4 ±0.9	8.76 ±0.05	43.5 ±1.2	71.3 ±3.6	115.8 ±9.2	0.04 ±0.01	7.0 ±1.2	17.8 ±2.7	2.97 ±0.12
	1.0	0.69[c] ±0.07	-	7.95[c] ±0.21	42.7 ±1.07	72.9 ±2.7	122.9 ±8.3	0.06 ±0.02	6.2 ±1.5	15.7 ±3.8	2.93 ±0.07
Sept-Oct[d]	0.0	B.D.L.	17 ±0.5	7.81 ±0.08	50.0 ±0.6	88.8 ±3.6	148.4 ±4.5	0.04 ±0.02	7.9 ±1.3	25.7 ±3.3	N.D.
	1.0	0.86[c] ±0.02	-	7.77[c] ±0.05	49.5 ±0.7	91.3 ±2.8	147.9 ±4.6	0.02 ±0.01	7.4 ±1.3	23.0 ±2.5	N.D.

a. Measured on first and last days only (n = 6).

b. n = 15.

c. Reported for measurements after starting zinc dosing (n = 9).

B.D.L. = Below detectable limits.

N.D. = Not determined.

Table 2. Means for epilithon AFDW, protein, chlorophyll a, and pheophytin a during the August-September, 1985 20-day study; n = 3.

Day	Target Zn conc. (mg/L)	AFDW (mg/cm^2)	Protein conc. (mg/cm^2)	Chlorophyll a conc. ($\mu g/cm^2$)	Pheophytin a conc. ($\mu g/cm^2$)
0	0.0	1.41	0.68	34.7	2.97
	1.0	1.51	0.73	40.7	3.39
5	0.0	1.21	0.59	31.6*	2.09*
	1.0	1.41	0.35	13.7*	1.07*
10	0.0	1.37*	0.70*	34.7*	2.21*
	1.0	0.48*	0.23*	10.0*	0.50*
20	0.0	0.84	0.44	18.5	1.39
	1.0	1.45	0.48	20.9	0.65

* - Means representing sample distributions on a given study day that are significantly different (P = 0.05) using the Mann-Whitney, U-test.

Table 3. Means for epilithon AFDW, protein, chlorophyll a, and pheophytin a during the September-October, 1985 20-day study; n = 3.

Day	Target Zn conc. (mg/L)	AFDW (mg/cm^2)	Protein conc. (mg/cm^2)	Chlorophyll a conc. ($\mu g/cm^2$)	Pheophytin a conc. ($\mu g/cm^2$)
0	0.0	2.04	0.82	35.1	1.14
	1.0	3.18	1.26	60.2	2.50
5	0.0	3.28*	1.17*	63.2*	4.55*
	1.0	1.49*	0.45*	19.4*	0.41*
10	0.0	4.15*	1.66*	66.6*	6.98*
	1.0	1.17*	0.51*	21.8*	0.36*
20	0.0	1.63	0.55	23.4	2.23
	1.0	1.59	0.63	24.7	0.61

* - Means representing sample distributions on a given study day that are significantly different (P=0.05) using the Mann-Whitney, U-test.

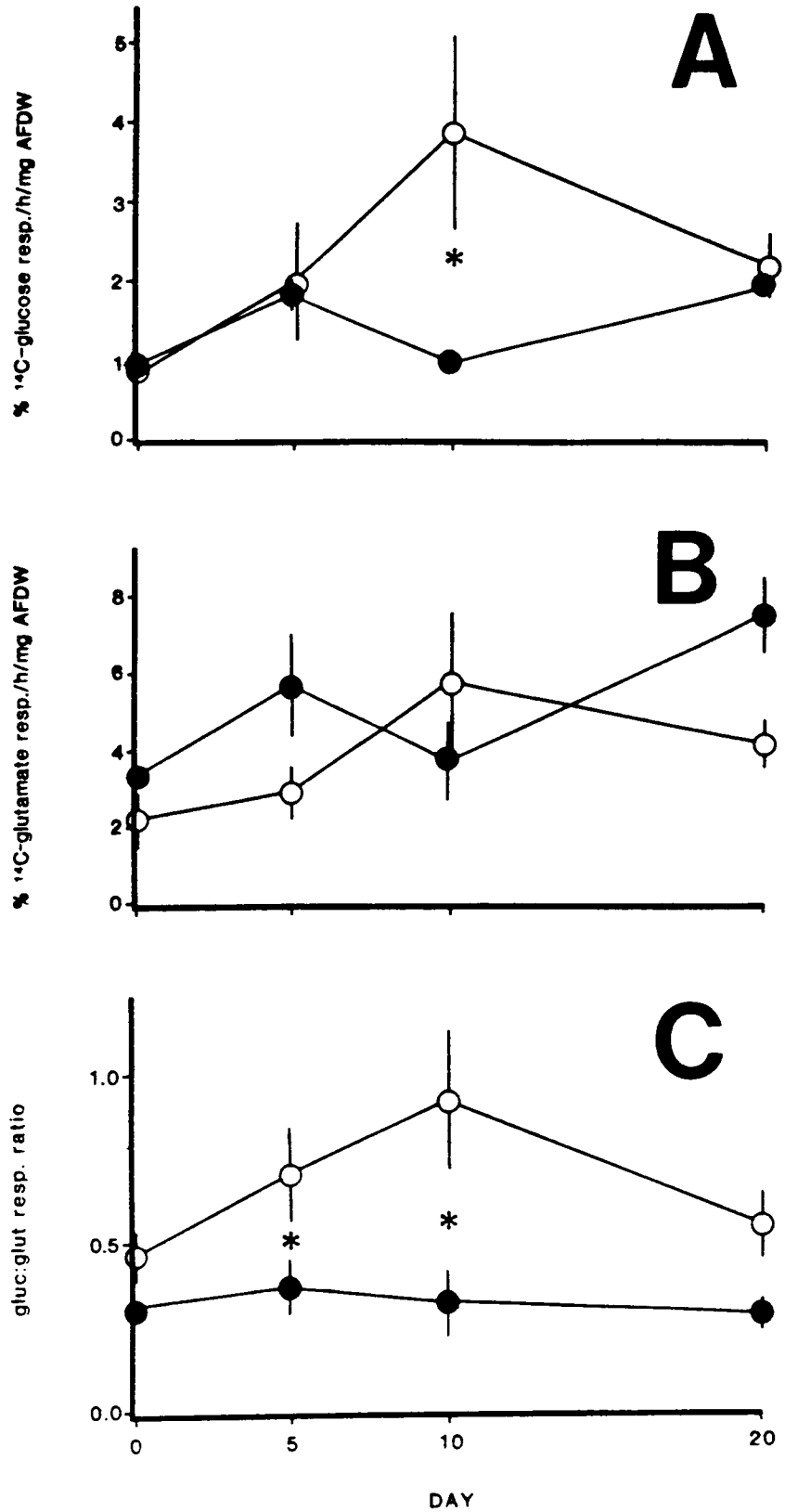

Figure 1. Glucose respiration (A), glutamate respiration (B), and glucose-to-glutamate respiration ratio (C) for epilithon samples during the August-September, 1985 study. Points represent the mean of three replicates ± standard error. * indicates significant differences at $\bar{P}$=0.05 level. Open circles = zinc treatment; closed circles = controls.

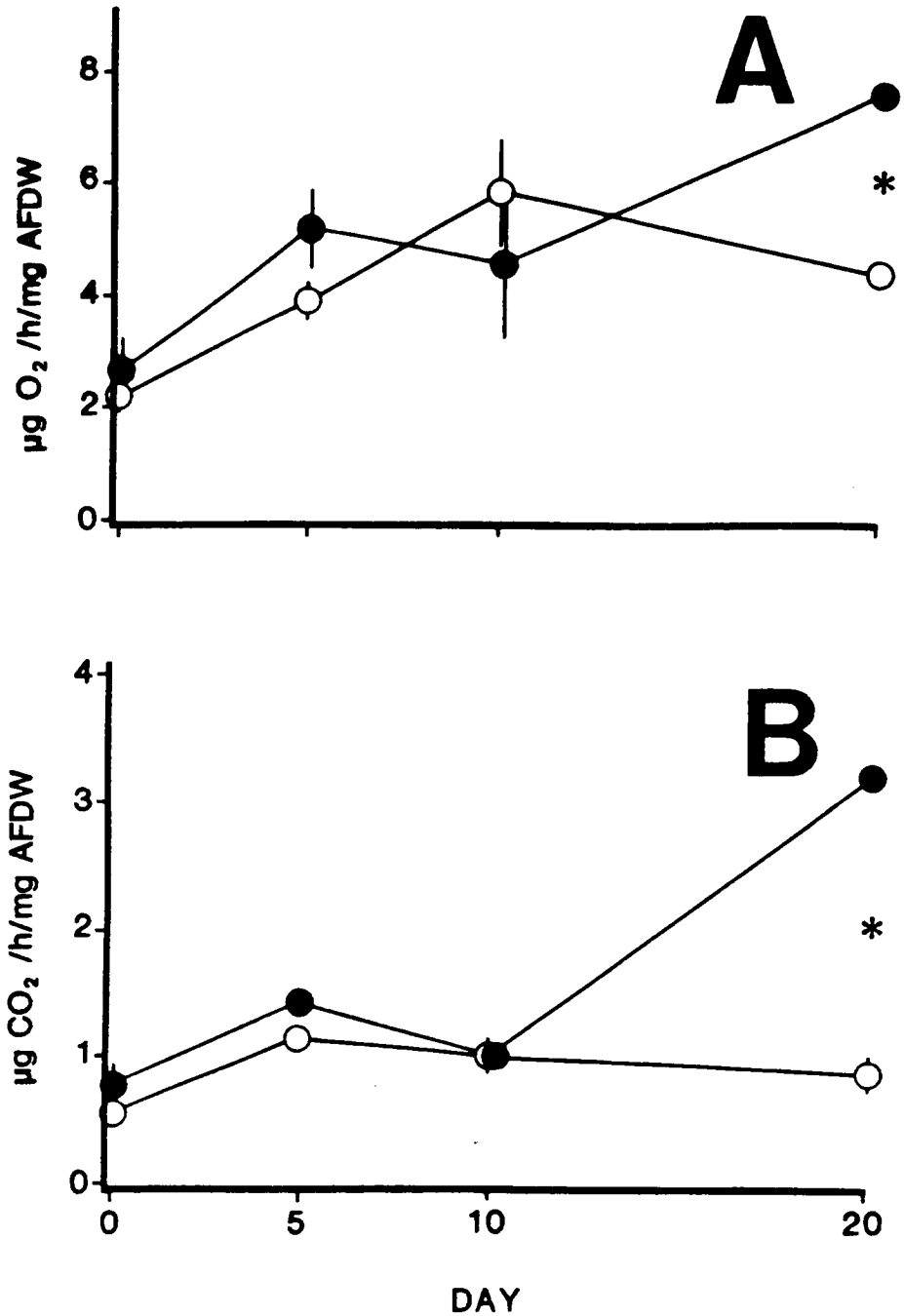

Figure 2. O_2 and CO_2 flux occurring in epilithon during August-September, 1984 study as measured by the rate of O_2 consumption (A) and the rate of CO_2 production (B). Points represent the mean of three replicates ± standard error. * indicates significant differences at the P=0.05 level. Open circles = zinc treatment; closed circles = controls.

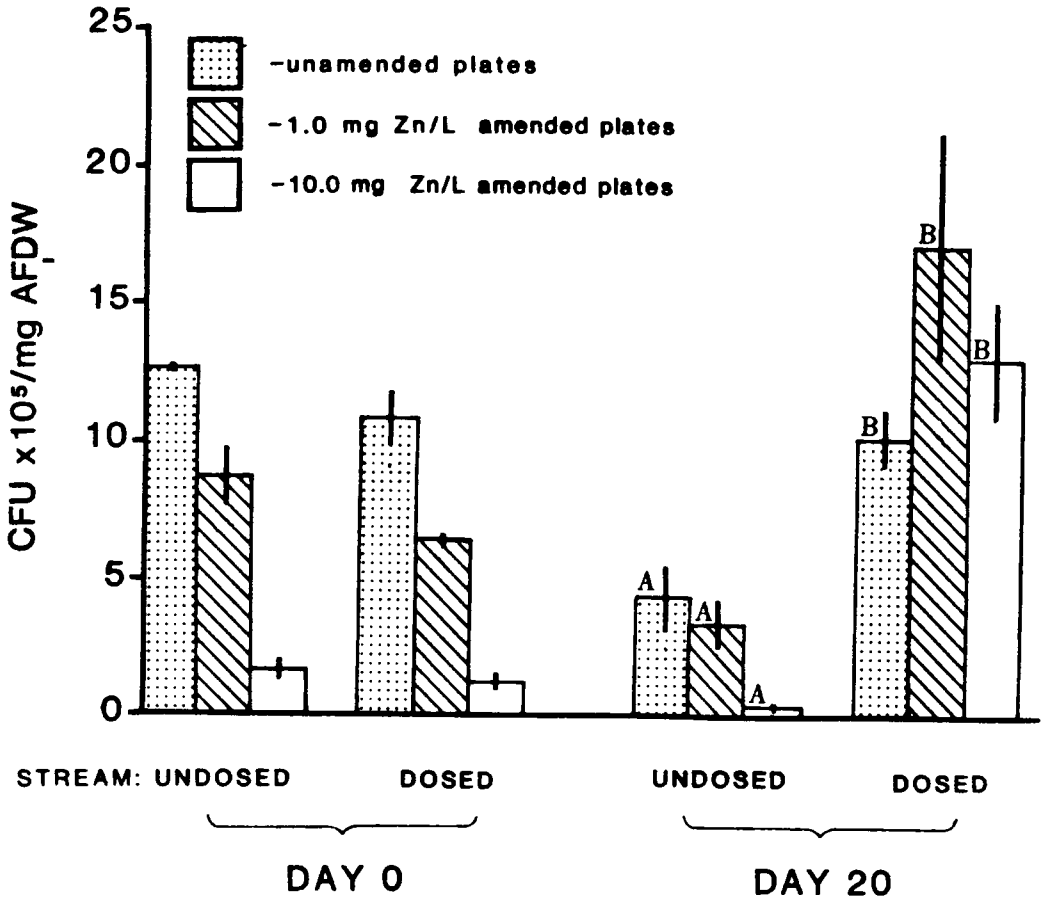

Figure 3. Mean epilithic colony forming units (x 10^5) per mg ash-free dry weight from control and zinc-exposed streams at the beginning and end of the August-September, 1985 study (n=3). Values represent the mean CFU obtained on triplicate plates containing 0.0, 1.0, or 10.0 mg Zn $liter^{-1}$ $\pm$ standard error. Different letters above bars indicate significantly different values (α=0.05) between treatments on the designated media-type and day. The absence of letters indicates that no significant differences occurred between the treatments on that day.

SUMMARY AND CONCLUSIONS

These experiments have determined significant changes in epilithic biomass quality and quantity and organic carbon cycling due to long term dosing with moderate concentrations of zinc. The 1984 studies (Chapter I), provided a basis for further inquiry during summer and fall, 1985, by demonstrating a transient peak in glucose respiration rates per unit protein in epilithon communities soon after starting continuous dosing with 1.0 mg Zn $liter^{-1}$. Higher AFDW, protein, and carbohydrate were evident after 30 days of dosing with 1.0 mg Zn $liter^{-1}$ indicating an eventual accrual of biomass in these systems. Fundamental changes in the quality of epilithic biomass were apparent as lower protein-to-carbohydrate ratios in epilithon receiving 1.0 mg Zn $liter^{-1}$. Dosing with 0.05 mg Zn $liter^{-1}$, approximately 25% of the U.S. EPA maximum recommended concentration, caused some change in epilithon structural characteristics and practically no change in the measured functional attributes.

During the 1985 studies (Chapter II), peaks in the glucose respiration rate agreed with 1984 observations in epilithon dosed with 1.0 mg Zn $liter^{-1}$. This activity corresponded to the period of low values of AFDW, protein, and algal pigment concentrations as noted during the summer, 1984 study. By

the 20th day, these structural measurements were again similar to values obtained from reference stream epilithon which suggested adaptation to the stressful effect of zinc. Despite similar glucose respiration rates and biomass measurements that were indistinguishable by treatment on the 20th day, the epilithon from dosed streams exhibited lower rates of O_2 consumption and CO_2 production and a zinc-tolerant heterotrophic population relative to the epilithon from control streams.

Marine studies suggest that heterotrophs in copper dosed mesocosms exhibit higher numbers and activites than in undosed systems due to a compromised algal population (Thomas et al., 1977; Vaccaro et al., 1977). The results from the 1985 season provided insight into the differences between marine and artificial stream studies. Measurement of the glucose-to-glutamate respiration ratio as an indicator of algal physiological health (Griffiths et al., 1982) provided evidence of stimulated glucose respiration but no significant differences between treatments for glutamate respiration. The glucose-to-glutamate utilization ratio decreases in planktonic populations when the algal population declines. The absence of a similar response in the artificial streams suggested that glutamate (and other compounds released from zinc-sensitive organisms) was washed out of the streams and therefore unavailable to the zinc-tolerant heterotrophs.

Algal pigment concentrations decreased in epilithon dosed with 1.0 mg Zn liter^{-1}, yet chlorophyll a-to-pheophytin a ratios indicated that the algal populations did not senesce *in situ* but were probably washed out of the streams as they died. Significant quantities of biomass were lost from the dosed streams in three of the four studies. This also involved the loss of the matrix which is necessary for sequestering ions and dissolved and particulate organic carbon (Lock *et al.*, 1984). The nature of small artificial streams as continuous culture apparatuses limits the analysis of community level changes dependent on the material that is lost downstream. However, the observed increase in glucose respiration rates occurred despite the loss of biomass from the systems and, therefore, must represent a characteristic of zinc-tolerant heterotrophs in these streams at an early stage of recolonization. According to the results of Lock and Ford (1985) epilithic communities devoid of algae maintain substantial metabolic activities that derive their major energy source from riverborne organic matter.

AFDW, protein, carbohydrate, and algal pigments were consistent indicators of change in the epilithic biomass and valuable in providing evidence of a decrease in biomass in the streams dosed with 1.0 mg Zn liter^{-1} and a subsequent recovery of epilithon. After 30 days (Chapter I), the epilithon dosed with 1.0 mg Zn liter^{-1} had acquired more

AFDW, protein, and carbohydrate than control streams, indicating significant structural differences in the dosed epilithon relative to the undosed communities. However, higher carbohydrate concentrations relative to protein represented a decrease in the nutritive value of the epilithon dosed with 1.0 mg Zn $liter^{-1}$.

The development of a zinc-tolerant community was evident in higher total and zinc-tolerant CFU in epilithon dosed with 1.0 mg Zn $liter^{-1}$ for 20 days compared to the undosed epilithon. Epilithon from reference streams showed low tolerance to increasing concentrations of zinc in 1/4 strength Nutrient Agar.

During the August-September, 1985 study, the rate of flux of O_2 and CO_2 did not differ between treatments until the 20th day when dosed epilithon consumed less O_2 and produced less CO_2 than the undosed systems. These measurements represent significant functional distinctions between the unique communities that developed by the 20th day of zinc dosing.

Zinc represents a common metal in aquatic systems and its importance is related to the adverse effects that high concentrations of the metal may have on the processes and composition of the epilithon. These studies represent the first characterization of the long-term effects of zinc on

epilithic microbial structure and function. Zinc (1.0 mg liter^{-1}) caused a pronounced decrease in epilithic biomass after 5-10 days and high rates of glucose respiration during this time that were characteristic of the surviving, zinc-tolerant heterotrophs. Subsequently, a community became established that was distinct from control epilithon in the degree of zinc tolerance and rates of flux of O_2 and CO_2. After 30 days, more total organic material was present in the streams dosed with 1.0 mg Zn liter^{-1} than in the controls. However, a shift in the relative abundance of epilithic protein and carbohydrate in the dosed systems reflected a decrease in the nutritional quality of the epilithon for higher trophic level organisms. These experiments have stressed the importance of the organic glycocalyx in which the epilithon exists as a matrix which regulates the exchange of organic and inorganic material between the cells of the epilithon and the surrounding medium.

ADDITIONAL LITERATURE CITED

Albright, L.J. and E.M. Wilson. 1974. Sub-lethal effects of several metallic salts - organic compounds combinations upon the heterotrophic microflora of a natural water. Water Res. 8:101-105.

American Public Health Association. 1985. Standard methods for the examination of water and wastewater, 16th ed. American Water Works Association, Water Pollution Control Federation, Washington, D.C.

Babich, H. and G. Stotsky. 1980. Environmental factors that influence the toxicity of heavy metals and gaseous pollutants to microorganisms. CRC Crit. Rev. Microbiol. :99-145.

Berkuen, M. 1982. Effects of inorganic metal toxicity on BOD - I. Methods for estimation of BOD parameters. Wat. Res. 16:559-564.

Bott, T.L. and T.D. Brock. 1970. Growth and metabolism of periphytic bacteria: methodology. Limnol. Oceanogr. 15:333-342.

Brock, T.D. and M.L. Brock. 1967. The measurement of chlorophyll, primary productivity, photophosphorylation and

macromolecules in benthic algal mats. Limnol. Oceanogr. 12:600-605.

Clark, J.R., D.S. Cherry, and J. Cairns. 1982. Food quality of aufwuchs from artificial streams receiving low levels of perturbations. Water Res. Bull. 18:761-767.

Cuhel, R.L., H.W. Jannasch, C.D. Taylor, and D.R.S. Lean. 1983. Microbial growth and macromolecular synthesis in the northwestern Atlantic Ocean. Limnol. Oceanogr. 28:1-18.

Farris, J.L. 1986. Cellulolytic responses to heavy metal accumulation in Corbicula fluminea and Mudalia dilatata. Ph.D. Dissertation. Virginia Polytechnic Institute and State University. 170 pp.

Forstner, U. and G.T.W. Wittmann. 1981. Metal pollution in the aquatic environment. 2nd ed. Springer Verlag, Berlin.

Foster, P.L. 1982a. Species associations and metal contents of algae from rivers polluted by heavy metals. Freshwater Biol. 12:17-40.

Foster, P.L. 1982b. Metal resistances of Chlorophyta from rivers polluted by heavy metals. Freshwat. Biol. 12:41-61.

Gadd, G.M. and A.J. Griffiths. 1978. Microorganisms and heavy metal toxicity. Microb. Ecol. 4:303-317.

Gillespie, P.A., R.Y. Morita, and L.P. Jones. 1976. The heterotrophic activity for amino acids, glucose , and acetate in Antarctic waters. J. Oceanogr. Soc. Japan. 32:74-82.

Gocke, K. 1977. Comparison of methods for determining the turnover times of dissolved organic compounds. Mar. Biol. 42:131-141.

Griffiths, R.P., B.A. Caldwell, and R.Y. Morita. 1982. Seasonal changes in microbial heterotrophic activity in subarctic marine waters as related to phytoplankton primary productivity. Mar. Biol. 71:121-127.

Haug, A., S. Myklestad, and E. Sakshaug. 1973. Studies on the phytoplankton ecology of the Trondheimsfjord. I. The chemical composition of phytoplankton populations. J. Exp. Mar. Biol. Ecol. 11:15-26.

Herbert, D., P.J. Phipps, and R.E. Strange. 1971. Chemical analysis of microbial cells, p. 209-344. In, J.R. Norris and D.W. Ribbons (ed.), Methods in microbiology, vol. 5B. Academic Press, London.

Hitchcock, G.L. 1982. A comparative study of the size-dependent organic composition of marine diatoms and dinoflagellates. J. Plankt. Res. 4:363-377.

Holm-Hansen, O. and B. Riemann. 1978. Chlorophyll a determinations: improvements in methodology. Oikos. 30:438-447.

Hornor, S.G. and B.A. Hilt. 1985. Distribution of zinc-tolerant bacteria in stream sediments. Hydrobiologia. 128:155-160.

Hornor, S.G. and M.J. Mitchell. 1981. Effect of the earthworm, Eisenia foetida (Oligochaeta), on fluxes of volatile carbon and sulfur compounds from sewage sludge. Soil Biol. Biochem. 13:367-372.

Itzhaki, R.F. and D.M. Gill. 1964. A micro-biuret method for estimating proteins. Anal. Biochem. 9:401-410.

Lehninger, A.L. 1982. Principals of biochemistry. Worth Publishers, Inc., New York.

Lock, M.A. and T.E. Ford. 1985. Microcalorimetric approach to determine relationships between energy supply and metabolism in river epilithon. Appl. Environ. Microbiol. 49:408-412.

Lock, M.A., R.R. Wallace, J.W. Costerton, R.M. Ventullo, and S.E. Charlton. 1984. River epilithon: toward a structural-functional model. Oikos. 42:10-22.

McMahon, R.F., R.D. Hunter, and W.D. Russell-Hunter. 1974. Variation in aufwuchs at six freshwater habitats in terms of carbon biomass and carbon : nitrogen ratio. Hydrobiologia. 45: 391-404.

Mills, A.L. and R.R. Colwell. 1977. Microbiological effects of metal ions in Chesapeake Bay water and sediment. Bull. Environ. Contam. Toxicol. 18:99-103.

Rausch, T. 1981. The estimation of micro-algal protein content and its meaning to the evaluation of algal biomass. 1. Comparison of methods for extracting protein. Hydrobiologia. 78:237-251.

Sayler, G.S., L.C. Lund, M.P. Shiaris, T.W. Sherrill, and R.E. Perkins. 1979. Comparative effects of Aroclor 1254 (polychlorinated biphenyls) and phenanthrene on glucose uptake by freshwater microbial populations. Appl. Environ. Microbiol. 37:878-885.

Shriner, C. and T. Gregory. 1984. Use of artificial streams for toxicologial research. CRC Crit. Rev. Toxicol. 13:253-
.

Singleton, F.L. and R.K. Guthrie. 1977. Aquatic bacterial populations and heavy metals. I. Composition of aquatic bacteria in the presence of copper and mercury salts. Wat. Res. 11:639-642.

Spear, P.A. 1981. Distribution, p. 33-46. In, Zinc in the aquatic environment: chemistry, distribution and toxicology. National Research Council of Canada, Ottawa.

Strickland, J.D.H. and T.R. Parsons. 1968. Pigment analysis, p. 185-206. In, A practical handbook of seawater analysis. Fisheries Research Board of Canada, Bulletin 167.

Stringfellow, W.T. 1984. Effect of phenol on oxygen consumption, carbon dioxide production, methane production, phosphatase enzyme activity, and glucose respiration in freshwater sediments. M.S. Thesis. Virginia Polytechnic Institute and State University. 90 pp.

Thomas, W.H., O. Holm-Hansen, D.L.R. Seibert, F. Azam, R. Hodson, and M. Takahashi. 1977. Effects of copper on

phytoplankton standing crop and productivity: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:34-43.

United States Environmental Prtection Agency. 1980. Ambient water quality criteria for zinc. EPA 440/5-80-079. Washington D.C.

Vaccaro, R.F., F. Azam, and R. Hodson. 1977. Response of natural marine bacterial populations to copper: Controlled ecosystem pollution experiment. Bull. Mar. Sci. 27:17-22.

Weatherley, A.H., P.S. Lake, and S.C. Rogers. 1980. Zinc pollution and the ecology of the freshwater environment, p. 337-418. In, J.O. Nriagu (ed.), Zinc in the environment, Part I: Ecological cycling. John Wiley and Sons, New York.

Whitton, B.A. 1980. Zinc and plants in rivers and streams, p. 363-400. In, J.O. Nriagu (ed.), Zinc in the environment. Part II: Health effects. John Wiley and Sons, New York.

Williams, L.G. and D.I. Mount. 1965. Influences of zinc on periphytic communities. Am. J. Bot. 52:26-34.

Williams, P.J.LeB. and C. Askew. 1968. A method of measuring the mineralization by micro-organisms of organic compounds in seawater. Deep-Sea Res. 15:365-375.

Wright, R.T. 1978. Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. Appl. Environ. Microbiol. 36: 297-305.

Wright, R.T. and J.E. Hobbie. 1966. Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology. 47:447-464.

APPENDIX A. 1984 ZINC-SNAIL STUDY

On October 26th, 1984, a 30-day study was started to distinguish the effects of zinc and snails on the epilithon colonizing the glass rods. The artificial streams were used as previously reported (Chapters I and II), except that 0.5 mg Zn liter^{-1} was used instead of 1.0 mg Zn liter^{-1}. On the 13th day a mechanical problem caused an acute high dose of zinc (≤48 mg total Zn liter^{-1}). After correcting the problem, dosing was continued at 0.5 mg Zn liter^{-1}. Epilithic glucose respiration and ash-free dry weights (AFDW) were measured (as reported in Chapters I and II) on 0, 2, 5, 10, 20, and 30 days after starting zinc dosing. Analysis of variance and Duncan's multiple range tests were used to determine significant differences among treatments (Sokal and Rohlf, 1981).

Undosed streams without snails exhibited an increase in AFDW during the 30-day study (Fig. 1). Final weights were similar to those observed during September-October, 1984 (Chapter I). AFDW in undosed streams with snails decreased until the 10th day. Subsequently, AFDW in the two undosed treatments were indistinguishable. The AFDW in epilithon receiving 0.5 mg Zn liter^{-1} (both with and without snails) decreased until the

10th day and then remained low for the last 20 days of the study.

Unlike the summer and early fall 1984 study periods, the control streams had higher biomass values than the zinc-dosed systems at the end of the study, possibly because recovery of biomass in the zinc-dosed streams was delayed by seasonal changes (e.g. lower light and temperature). Increases in AFDW noted in control streams with snails present may have occurred due to lower temperatures and consequently lower grazing rates after the 10th day ($\leq 10^{\circ}$ C). Lower mollusk metabolic rates have been correlated with lower temperatures (Alimov, 1975). Although high zinc concentrations occurred on the 13th day, by the 10th day zinc-amended systems had already reached the low level of AFDW at which they would remain. Bioconcentration factors (Genter, 1986) and zinc chemistry indicated that by the 20th day the dosed streams had returned to levels of zinc similar to those before the high dosage event (mean total zinc concentration on the 20th day was 1.46 mg liter^{-1}). Presumably, after this episode, when dosing returned to its target concentration, the zinc-dosed streams would have had an opportunity to accumulate biomass. Since these streams maintained significantly lower AFDW than the control streams to the end of the study, the metal may have had a greater effect on epilithon quantity during this season compared to summer and fall.

After a peak in glucose respiratory activity per unit area on the 2nd day in the zinc-dosed streams without snails, epilithic glucose respiration declined to low levels in these streams (Fig. 2A). The other three treatments exhibited various rates of decline of this variable early in the dosing period and subsequently remained at lower levels than at the beginning of the study. Rates of glucose respiration were comparable to those observed during the September-October, 1984 study. The effect of season was noted by the decrease in glucose respiration rates in all treatments; however, results from the 20th and 30th days indicated that the effect of zinc was to decrease glucose respiratory activity in the metal-dosed epilithon communities. This may have been a lingering effect of the spike in zinc concentration on the 13th day.

A peak in glucose respiration per unit AFDW was evident in the zinc-dosed epilithon on the 5th day (Fig. 2B). On the 10th day, when the epilithon in control streams without snails had significantly greater biomass than other treatments, these streams also had lower glucose respiration rates per unit biomass. On the last two study days, the dosed epilithon had higher glucose respiration rates per unit AFDW than the undosed communities, reflecting higher relative activities regardless of the snail treatment.

The high rates of glucose respiration on a biomass basis on the 10th day in any stream with snails, zinc or both factors, indicated that both zinc and snails determined the relative rates of glucose respiration at this time. Higher glucose respiration rates per unit biomass in streams by the end of the study suggested that zinc-dosed epilithon maintained higher relative activities than the controls. This was true in spite of the high concentration of zinc reached on the 13th day, apparently indicating some residual activity or recolonization after this acute dosing period. It is possible that snail death and decay due to the high zinc levels may have maintained high epilithon respiration rates at the end of the study. Unlike previous 1984 studies, when epilithon dosed with 1.0 mg Zn $liter^{-1}$ showed peak respiratory activities on the 5th day, this study indicated that epilithon dosed with half that concentration had higher glucose respiration rates per unit biomass late in the study.

If AFDW and glucose respiration rates are compared among treatments using an analysis of variance excluding days 0, 20, and 30 (before or after relevant dosing) then it is clear that both zinc and snails contribute to changes in these variables. Temperatures during the first 10 days were similar to those during the fall study (Chapter I), therefore snail activities were probably comparable between these studies. It is significant to note that in this study the

zinc concentration was half that shown to elicit a response during the summer and fall study periods (0.5 versus 1.0 mg liter^{-1}), and, at the higher concentrations, the effect of zinc is probably more pronounced.

APPENDIX B. ZINC EFFECT ON PHOSPHATE UPTAKE

As with organic substrate uptake, phosphate turnover time may be measured using a radioisotope experiment. The method is best known as a limnological technique (Lean and Nalewajko, 1979; Cembella *et al.*, 1983); however, recently Mulholland *et al.*, (1984) have applied the assay in stream studies to detect phosphate uptake on leaf disks. As stated by Cembella *et al.*, (1983) the effect of trace elements, such as zinc, on phosphate uptake, has not been studied. Additionally, the recent development of this method for use in streams dictates that it has not been applied in perturbation studies. Mulholland *et al.*, (1984) noted that phosphate uptake rates increased considerably with the development of microflora on the leaf disks used in their study. Decreases in microbial biomass and activity based on zinc stress should decrease the efficiency of phosphate uptake. Higher microbial biomass should increase this cycling efficiency.

This experiment was performed during the summer and fall, 1984, periods. Rods with intact epilithon were randomly removed from three zones in each stream and placed into 250-ml glass jars containing 60 ml of water from the corresponding stream. Phosphate turnover time was measured by a method similar to that used by Lean and Nalewajko (1979) and Richey

(1979). A trace quantity of carrer-free ^{32}P-phosphate (<0.25 µg liter^{-1}) was added to the jars and 1-ml samples of overlying water were removed initially and again after 5, 10, and 20 min. Abiotic uptake was estimated using a formaldehyde-killed control for each treatment. Incubations were done in the dark at stream temperature with gentle shaking. Samples containing ^{32}P-phosphate were measured using Cerenkov counting on a Beckman LS-3150 T liquid scintillation counter.

During the June-July study a high rate of uptake of phosphate occurred on the 5th day in streams dosed with 1.0 mg Zn liter^{-1} (Table 1). This contrasted with lower phosphate uptake rates on the 20th and 30th days in the same streams. Due to lower AFDW and protein concentrations in streams dosed with 1.0 mg Zn liter^{-1} on the 5th day, phosphate uptake per unit AFDW or protein indicated greater differences between the high zinc and other treatments during this period.

The high uptake rate of phosphate noted in systems dosed with 1.0 mg Zn liter^{-1} early in the June-July study did not occur during the September-October study (Table 2). Instead, the epilithon present in these high zinc streams took up significantly less ^{32}P-phosphate than other treatments on the 5th day. During the rest of the study, phosphate uptake was significantly lower in streams receiving 1.0 mg Zn liter^{-1}

than in other streams. This pattern was unchanged when phosphate uptake rate considered per unit AFDW or protein.

During June-July, there was an apparent correlation between the stimulation of glucose respiration and ^{32}P-phosphate uptake in the epilithon dosed with 1.0 mg Zn liter^{-1}, both measurements peaking on the 5th day of the study. The microbial community in these streams would be expected to have high phosphate uptake rates associated with the high activities and low but accumulating biomass. Recent studies of planktonic bacteria and algae indicate that bacteria are responsible for uptake of ortho-phosphate and subsequent release of organic phosphorus which is available for algal hydrolysis (Currie and Kalff, 1984).

It is difficult to explain the low phosphate uptake rates which coincided with the greatest glucose respiration in the high zinc streams during the fall study. Seasonal differences between the summer and fall studies may account for taxonomic and/or physiological changes in the epilithon. Microorganisms responsible for the previous peak in phosphate uptake rates may have been absent or incapable of such activities during the second study period.

The polyanionic nature of the glycocalyx of attached microbial communities (Costerton et al., 1978) may provide

partial explanation of the different results obtained in the phosphate uptake experiments. As the epilithic biomass increases, diffusion of anions into this matrix should decrease. This suggests an inverse relationship between the amount of biomass and diffusion, and therefore uptake, of anions such as phosphate. On the 5th day of the June-July period, streams dosed with 1.0 mg Zn $liter^{-1}$ had the lowest biomass recorded during the study. This correlated with high rates of phosphate uptake. Subsequently, as biomass increased in these streams, phosphate uptake rates decreased.

APPENDIX C. ZINC EFFECT ON CARBOHYDRATE FRACTION OF AFDW

Zinc dosing had an effect on the macromolecular composition of the epilithon. The fraction of the AFDW made up of carbohydrate during the September-October study is shown in Table 2. Treatments of 0.05 and 1.0 mg Zn liter^{-1} caused an increase in the relative amount of carbohydrate present in the epilithon. Although differences among treatments were apparent on the first day, the zinc dosing accentuated the effect; control streams always showed less than 30% carbohydrate and zinc dosed epilithon usually showed 34% or more carbohydrate. Similar, though less distinct results were noted for the June-July study, with epilithon on the 10th and 20th days having significantly lower fractions of carbohydrate in control streams than in streams receiving 0.05 mg Zn liter^{-1}.

Amendment of streams with zinc caused an increase in the fraction of AFDW composed of carbohydrate. This paralleled previous work that indicated an increase in the concentration of carbohydrate in zinc amended cultures of an aquatic bacterium. A change in the physiological capacity of the persistent microflora or the introduction of species which normally produce greater quantities of carbohydrate may account for these observations. The green algae and

cyanobacteria that colonized the high zinc systems are surrounded by a mucilaginous sheath which may serve as a protective barrier to the penetration of toxic metal ions (Foster, 1982; Say et al., 1977).

LITERATURE CITED (APPENDIX)

Alimov, A.F. 1975. The rate of metabolism in freshwater bivalve mollusks. Sov. J. Ecol. 6:10-20.

Cembella, A.D., N.J. Antia, and P.J. Harrison. 1983. The utilization of inorganic and organic phosphorus compounds as nutrients by eucaryotic microalgae: a multidisciplinary perspective: Part 1. CRC Crit. Rev. Microbiol. 10 317-391.

Costerton, J.W., G.G. Geesey and K.J. Cheng. 1978. How bacteria stick. Scientific Am. 238:86-95.

Currie, D.J. and J. Kalff. 1984. The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in freshwater. Limnol. Oceanogr. 29:311-321.

Foster, P.L. 1982. Species associations and metal contents of algae from rivers polluted by heavy metals. Freshwater Biol. 12:17-40.

Genter, R.B. 1986. Population and community response of attached algae to Zn stress alone and in combination with selected environmental variables. Ph.D. Dissertation. Virginia Polytechnic Institute and State University. 100 pp.

Lean, D.R.S. and C. Nalewajko. 1979. Phosphorus turnover time and phosphorus demand in large and small lakes. Arch. Hydrobiol. Beih. Ergebn. Limnol. 13:120-132.

Mulholland, P.J., J.W. Elwood, J.D. Newbold, J.R. Webster, L.A. Ferren, and R.P. Perkins. 1984. Phosphorus uptake by decomposing leaf detritus: Effect of microbial biomass and activity. Verh. Internat. Verein. Limnol. 22: 1899-1905.

Richey, J.E. 1979. Patterns of phosphorus supply and utilization in Lake Washington and Findley Lake. Limnol. Oceanogr. 24: 906-916.

Say, P.J., B.M. Diaz and B.A. Whitton. 1977. Influence of zinc on lotic plants: I. Tolerance of Hormidium species to zinc. Freshwater Biol. 7:357-376.

Sokal, R.R. and F.J. Rohlf. 1981. Biometry, 2nd ed. W.H. Freeman and Company, New York.

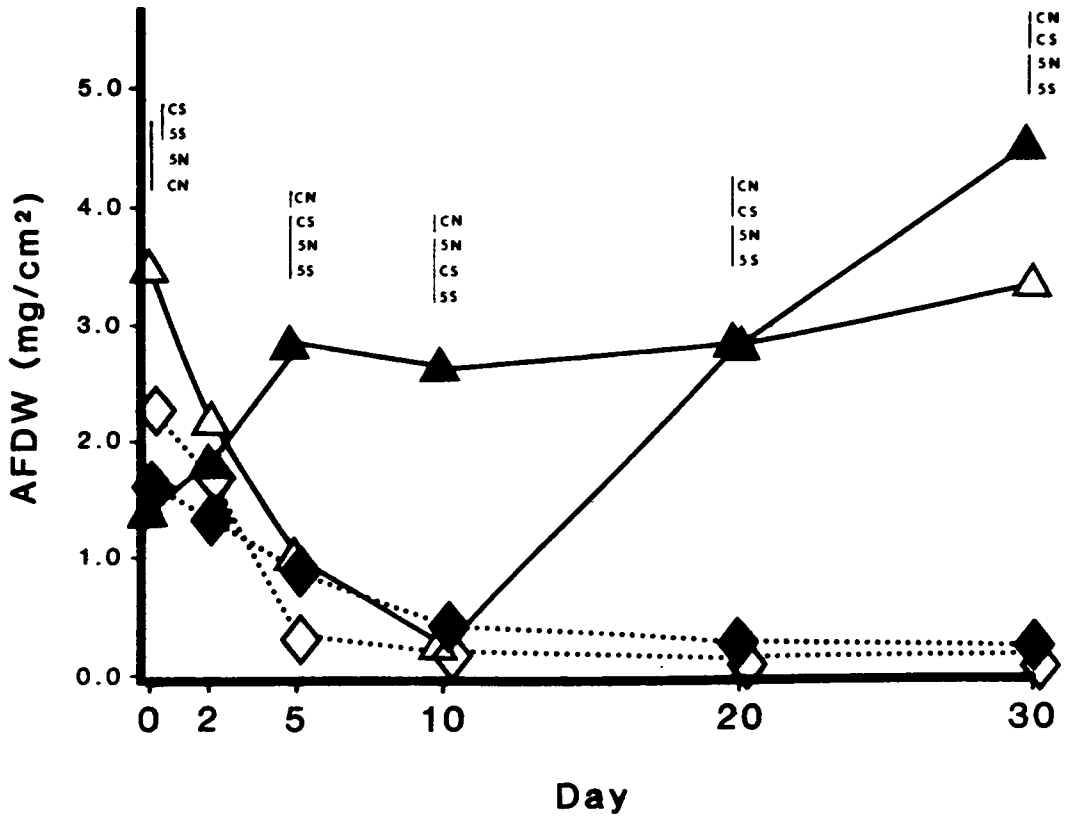

Figure 1. Ash-free dry weight (mg/cm^2) for the October-November, 1984 study. Points represent the mean of three replicates ± standard error. Open triangles (CS) = control, with snails; closed triangles (CN) = control, without snails; open diamonds (5S) = zinc-dosed, with snails; closed diamonds (5N) = zinc-dosed, without snails. The results of Duncan's multiple range tests among treatments coincide with days when significant differences (α=0.05) were determined. Designations for different treatments are not significantly different when connected by a vertical bar.

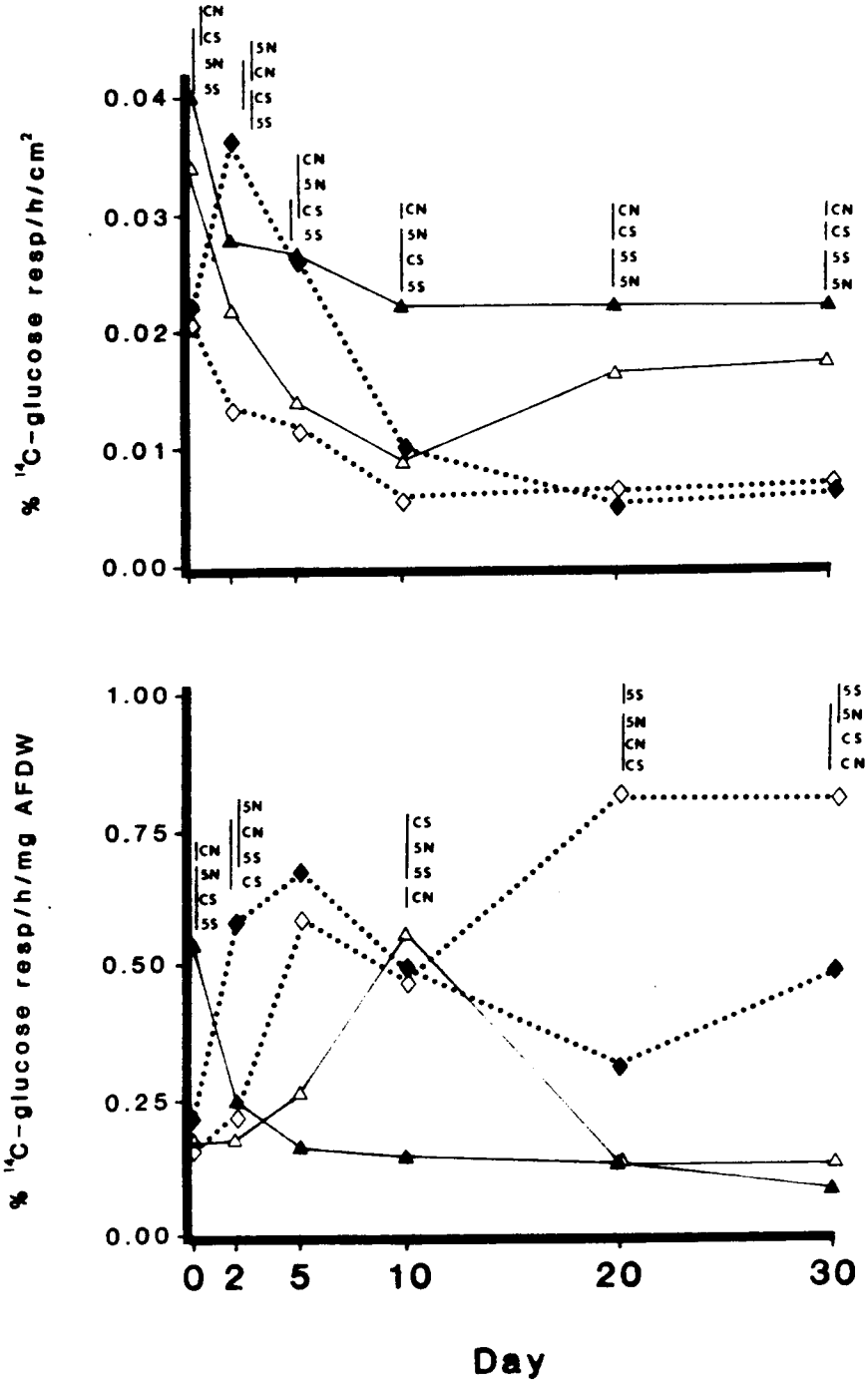

Figure 2. ^{14}C-glucose respiration per unit area (top) and per unit biomass (bottom) for the October-November, 1984 study. Points represent the mean of three replicates ± standard error. Open triangles (CS) = control, with snails; closed triangles (CN) = control, without snails; open diamonds (5S) = zinc-dosed, with snails; closed diamonds (5N) = zinc-dosed, without snails. The results of Duncan's multiple range tests among treatments coincide with days when significant differences (α=0.05) were determined. Designations for different treatments are not significantly different when connected by a vertical bar.

Table 1. Water chemistry data for October-November, 1984 snail-zinc study. Values represent the mean of three replicates ± standard error.

Day (date)	Temp (°C)	pH	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)
0 (10-26-84)	19	N.D.[a.]	N.D.	N.D.
2 (10-28-84)	20	8.79 ±0.09	70	63.8 ±0.72
5 (10-31-84)	18	8.62 ±0.02	80	72.5 ±0.36
10 (11-5-84)	14	8.77 ±0.05	83.3 ±3.33	71.5 ±0.21
16 (11-11-84)	N.D.	8.41 ±0.04	80	63.8 ±3.75
20 (11-15-84)	10	9.32 ±0.22	70	63.3 ±0.42
30 (11-25-84)	6	8.70 ±0.01	80	72.5 ±0.72

a. N.D. = Not determined.

Table 2. Zinc concentrations for October-November, 1984 snail-zinc study. Values represent means for pooled data for each treatment obtained throughout the study ±standard error.

Stream Treatment			
Target Zn conc (mg/L)	Snail presence[a.]	Dissolved Zn[b.] (mg/L)	Total Zn[b.] (mg/L)
0.0	N	0.041 ±0.01	0.06 ±0.01
0.0	S	0.021 ±0.01	0.11 ±0.06
0.5	N	1.98 ±0.96	1.74 ±0.52
0.5	S	1.44 ±0.72	1.76 ±0.85

a. S = snails present; N = snails not present.

b. Means for zinc-dosed streams include data from the 16th day. If this data is not included in the analysis then means for 0.5 N streams are 0.44 ±0.04 and 1.05 ±0.33 for dissolved and total zinc, respectively. For 0.5 N streams, means are 0.45 ±0.04 and 0.50 ±0.04 for dissolved and total zinc, respectively.

Table 3. Effect of zinc on the percent ^{32}P-phosphate uptake per min[a.] during the June-July and September-October, 1984 study periods.

Study period	Day	zinc addition rate (mg liter^{-1})		
		0.00	0.05	1.00
June-July, 1984	0	1.4^{b}	1.0^{b}	0.8^{b}
	2	0.3^{b}	0.9^{b}	0.7^{b}
	5	0.4^{b}	0.2^{b}	1.7^{c}
	10	0.5^{b}	0.2^{b}	0.2^{b}
	20	1.2^{b}	0.8^{bc}	0.2^{c}
	30	1.2^{b}	1.0^{b}	0.6^{b}
Sept.-Oct., 1984	0	2.6^{c}	3.9^{b}	1.7^{d}
	2	2.9^{b}	2.9^{b}	2.4^{b}
	5	4.4^{b}	2.2^{c}	0.1^{d}
	10	1.7^{bc}	2.9^{b}	0.9^{c}
	20	1.3^{c}	2.5^{b}	0.8^{c}
	30	2.3^{c}	3.5^{b}	0.7^{d}

a. Values are the means of three replicates; means followed by the same letter for each day are not significantly different (α=0.05) by the Duncan's multiple range test.

Table 4. Effect of zinc on the percent of combusted weight composed of carbohydrate[a.] in epilithon slurries obtained during the September - October, 1984, study period.

Study period	Day	zinc addition rate (mg liter^{-1})		
		0.00	0.05	1.00
Sept.-Oct., 1984	0	24.6^{b}	30.7^{c}	28.1^{bc}
	2	28.6^{b}	36.7^{bc}	48.1^{c}
	5	24.6^{b}	35.7^{c}	40.7^{c}
	10	29.1^{b}	38.1^{b}	38.1^{b}
	20	24.9^{b}	34.1^{c}	34.6^{bc}
	30	27.0^{b}	30.0^{bc}	36.0^{c}

a. Values are the means of three replicates; means followed by the same letter for each day are not significantly different (α=0.05) by the Duncan's multiple range test.

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