An Assessment of the Environmental Effects of Coal Ash Effluents Using Structural and Functional Parameters of Aufwuchs Communities.

by

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Chapter I

INTRODUCTION

An increased national dependence upon coal for the production of electrical energy has the potential for severe environmental impact to aquatic ecosystems, the degree of which can only be grossly estimated utilizing current hazard evaluation methodologies. The magnitude of the problem of safely disposing of, or isolating, the end products of coal combustion to minimize environmental impacts is staggering. Theis and Richter (1979) estimated that over 40 million tons of fly ash are currently produced each year by the electrical power industry. The Council on Environmental Quality, and researchers at the Lawrence Livermore Laboratory recently projected that coal production will double by 1985, and increase fivefold by the year 2000, when over one billion tons of coal will be burned per year (Friedlander, 1978; Heit, 1978).

When coal is burned in the typical steam plant, two fractions of coal ash are produced. Heavy ash settles to the bottom of the boiler where it is pulverized and disposed of in land fills , or sluiced to settling basins. Subsequent to the development and deployment of electrostatic precipitators, up to 99.5 percent of the lighter fly ash, which

used to be exhausted to the atmosphere , is collected and typically sluiced to fly ash settling basins. As effluent from these basins is released into aquatic receiving systems there is potential for environmental impact due to particulates, heavy metals in solution, pH excursions, and thermal and nutrient loading (Cherry and Guthrie, 1977, 1978, ; Coutant et al, 1978; Guthrie et al, 1978b; Cairns et al, 1979).

Current U.S. Environmental Protection Agency water quality criteria for fly ash particulates establish a maximum discharge not to exceed 100 ppm as Total Suspended Solids (TSS) per day, with a 30 day average not to exceed 30 ppm. These quidelines were proposed even though no evidence was available indicating what is a safe level for aquatic biota, or if they were too protective or stringent.

Protocols for evaluating potential biological hazards of chemical species and introduced perturbants have been extensively developed (Cairns and Dickson, 1978; Cairns, 1980); however, there exists a real need for tests reliable enough to determine the potential environmental effects of a complex effluent such as fly ash to specific aquatic communities. Methodologies are required which advance beyond traditional single species tests, allowing predictions at higher levels of ecosystem organization, for example, enabling prediction concerning the maintenance of system integrity and response upon exposure to perturbants (Cairns, 1981).

Receiving system biota exist not merely as a collection of isolated species, but as a complex, interacting community, and therefore toxicity tests conducted with single or carefully selected organisms may fall short in their predictive ability at the community level. Additionally, since effluents often contain a variety of potentially toxic, and in some cases stimulatory compounds in a complex and dynamic chemical solution, it is extremely difficult to predict effects upon resident biota by examining the isolated toxicities of one, or even several individual components of the effluent system. Even if every chemical and physical specie could be isolated and tested individually, it is unlikely that actual release conditions could be modeled to an extent which would allow accurate predictions of the chemical behavior and overall environmental impacts of the whole effluent. Since the whole effluent is that which is encountered by resident biota, the importance of developing techniques for the evaluation of effluents as they will be released into the environment is evident.

The primary producing aufwuchs community, existing in intimate association with the physical and chemical characteristics of ambient water, exhibits rapid turnover rates, and thus functions as an integrator of environmental conditions. When specific and sensitive structural and functional mea-

surements are applied to this community, information concerning the relative health of the entire lotic ecosystem can be extracted and evaluated, providing a possible methodology for determining the degree of impact that an introduced perturbant may exert upon the whole system .

1.1 RESEARCH HYPOTHESIS

Changes in the composition, diversity, viability, and quantity of aufwuchs communities exposed to a complex effluent, such as that from a fly ash settling basin, can be evaluated utilizing specific non-taxonomic structural and functional parameters. Measurements of the dynamics of dry weight, ash free dry weight, chlorophylls, ATP, carbon fixation, and sulfate assimilation, when combined with physical and chemical analyses of ambient water conditions, reflect the status and relative health of aufwuchs communities in natural and perturbed systems. These parameters can be utilized to assess the environmental impact of a specific, or complex, effluent and allow for predictions of changes in important functions at the ecosystem level of organization.

1.2 OBJECTIVES :

1. To determine the effects of coal ash effluent upon aufwuchs communities inhabiting lotic receiving systems.
2. To identify and isolate the effects of individual constituents of ash basin effluents to aufwuchs communities.
3. To determine the efficacy of utilizing non-taxonomic structural and functional parameters of aufwuchs to assess the environmental impacts of a complex effluent.
4. To determine if current Environmental Protection Agency water quality criteria standards for coal ash effluents (100 ppm TSS maximum per day, 30 day average not to exceed 30 ppm TSS) are sufficient to protect the biota of aquatic receiving systems.

Chapter II

LITERATURE REVIEW

2.1 AUFWUCHS

One very important component of lotic ecosystems is the entire community comprised of those microorganisms which grow attached to various substrates. An indication of the importance and complexity of this community is demonstrated by the vast effort and quantity of manuscripts produced in an attempt merely to define in a logical manner the exact ecology, spatial occurence and individual taxa which combine to produce the microcommunity assemblage. Easily located, identified and observed in virtually every lotic system, the vast complexity and heterogenity demonstrated by this submerged surface community is reflected by the numerous terms that have been advanced to describe it.

Reviews of the history and development of these terms has been exhaustively addressed by numerous authors (Cooke, 1956; Sladeckova, 1962; Wetzel, 1964; Rodgers, 1977; Weitzel, 1979); only the salient terminology will be presented here. Perhaps the two most widely utilized and useful terms applied to the submerged attached microcommunity are periphyton most common in American scientific literature and the German "Aufwuchs", or "aufwuchs".

Apparently the word periphyton was first used by Russian investigators to refer to the assemblage of microorganisms colonizing artificial substrates placed in aquatic environments (Cooke, 1956; Sladeckova, 1962; Weitzel, 1979). Although the literal translation refers to organisms which grow around plants, O.W. Young expanded this term in 1945 to include :

> . . . that assemblage of organisms growing upon free surfaces of submerged objects in water, and covering them with a slimy coat. It is that slippery brown or green layer usually found adhering to the surfaces of water plants, wood, stones, or certain other objects immersed in water and may gradually develop from a few gelatinous plants to culminate in a wooly, felted coat that may be slippery or crusty with contained marl or sand (Weitzel, 1979).

Wetzel and Westlake (1974) suggest that the term include all of the plant organisms, excluding rooted macrophytes, which grow on submerged materials such as sediments, rocks, debris and living organisms. Most authors, however, also include epizoic forms (Sladeckova, 1962), bacteria, algae, fungi, protozoans, and microinvertebrates when describing the periphyton community (Wetzel, 1964; Rodgers, 1977).

The original definition of the German term Aufwuchs is . . " alle einer festen Unterlage anhaffenden, aber . . . nicht in diese eindringenden Organismem" (Ruttner, 1953). Which translates as . . ." all those organisms that are firmly attached to a substratum but do not penetrate into it."

Some authors use aufwuchs to refer to organisms including those more commonly described as benthic forms (i.e. those living on the bottom). The definition that will be utilized throughout the remainder of this paper is adapted from Ruttner (1953). Ruttner defined the community of aufwuchs as comprising all attached organisms except rooted macrophytes, including bryozoa and sponges, and various motile life forms living freely, within the entangled mat of attached sessile microorganisms (Weitzel, 1979). For convenience, the word aufwuchs will be presented subsequently in this manuscript in its non-capitalized form.

There is little doubt that the aufwuchs community is of great ecological importance to lotic ecosystems. McIntire (1968, 1973) has advanced the concept that the aufwuchs assemblage can be conveniently considered as a single functional unit without a quantitative concern for the dynamics and intricacies of its constituent species. Based loosely on this concept, a number of ecological studies have been conducted by numerous investigators upon the whole aufwuchs community. Some of the more salient lines of investigation will be reviewed here.

Since there is no true phytoplankton community in lotic systems, except for the reproductive cells of mosses, liverworts, and some vascular plants (McIntire, 1973), which

function as primary producers (Reid and Wood, 1976), there is general agreement among investigators that what primary productivity does occur in the lotic system is directly attributable to the aufwuchs community (McIntire, 1966a, 1973; Minshall, 1978; Mason and Bryant, 1975; Caltaneo and Ghiltori, 1975; Cushing, 1967; Clark et al. 1979; Pryfogle and Lowe, 1979; Rodgers, 1977).

The importance of the quantity of autotrophic primary production within lotic ecosystems directly attributable to aufwuchs communities has often been underestimated. Since actual biomass of this community is often small (less than 10 g per m) and is easily overlooked by the casual observer, it is often easy to conclude that consumer organisms present must be supported through allochthonous detrital pathways (McIntire, 1973). Minshall (1978) questions past investigations concerning the relative contributions of autochthonous and allochthonous inputs into lotic systems and suggests that due to the the rapid turnover rate of aufwuchs species, autotrophy may play a much larger role in stream ecosystem dynamics than previously considered. Rapid turnover rates are supported by 32-P uptake studies which demonstrate that recycling within the aufwuchs community occurs even in the presence of considerable grazing pressure (Elwood and Nelson, 1972).

Bacteria and fungi included within the aufwuchs community are able to utilize and decompose organic material, releasing inorganic nutrients into the ambient water (Clark, 1980). They also may assimilate and adsorb, thereby effectively removing various toxic elements and compounds, radionuclides, and nutrients from downstream communities. In addition, aufwuchs communities are important biological components for transforming energy in the lotic ecosystem. Cushing (1967), and Cushing and Rose (1971), have shown that the large surface to volume ratios, and autotrophic nature of aufwuchs communities provide a mechanism for substantial radionuclide accumulation. In studies of 32-P and 65-Zn uptake Rose and Cushing (1970), reported that although accumulation does occur, processes of assimilation through absorption appear to be secondary to adsorption mechanisms.

2.1.1 Grazing

Biomass produced by the aufwuchs community can be a significant food resource for consumers either through direct grazing of the attached microflora, or through filter feeding in downstream communities as upstream aufwuchs slough off the substrate. Kehde and Wilhm (1972) showed that grazing of aufwuchs by snails in laboratory streams created a slight reduction in standing crop, and an increase in mea-

sured chlorophyll a concentration, but no effect upon species diversity. Other investigators have demonstrated that grazing pressures do limit production rates by controlling the standing crop of aufwuchs communities (Elwood and Nelson, 1972); Mason and Bryant, 1975). Clark (1980) extensively investigated the utilization of aufwuchs by grazing organisms when the aufwuchs community was exposed to selected pollutants typical of coal ash effluents. Grazing of aufwuchs has been shown to modify the species compositon of organisms in artificial streams, but although biomass was reduced by 30%, no decrease in primary production rates was observed (Sumner and McIntire, 1982).

2.1.2 Pollution Assessment

Several unique aspects of aufwuchs communities make them a valuable tool for water quality assessments (Rodgers and Harvey, 1976; McIntire and Phinney, 1965). Microorganisms of the community exist in close juxtaposition with the ambient water conditions, and exhibit rapid turnover rates, short generation times, and are taxonomically and genetically complex. This ensures that even under extreme environmental stress at least some members of the community will survive. Aufwuchs also remained attached to the substrate, and are therefore useful for assessing water conditions of

the present and recent past (Patrick, 1973). Thus the aufwuchs community has the ability to biologically integrate information about ambient water quality. This information may be extracted by the application of various investigative techniques. Several methods have been advanced to monitor water quality changes through changes in the aufwuchs community. Taxonomic analysis based upon quantitative and qualitative observations of aufwuchs species composition have been used by several investigators (Economou-Amilli, 1980; Sladeckova, 1962; Cattaneo and Ghittori, 1975; Patrick, 1973). Others have examined specific non-taxonomic biomass parameters such as dry weight, chlorophyll pigments, and ATP, to examine whole aufwuchs communities (Rodgers, 1977; Vollenweider, 1974; Clark et al., 1979).

2.2 COAL ASH

An increased national dependence upon coal for the production of electrical energy may have the potential for serious environmental impacts, not the least of which is related to the introduction of trace elements into aquatic receiving systems. Table 1 presents some of the toxic and potentially toxic substances that may enter the aquatic environment due to the combustion of coal. Of an estimated 3.5 x 10 8 tons of coal burned in 1974 (Klein et al. 1975),

approximately 50 million tons of fly ash are produced by electrostatic precipitators which remove up to 69.5% of particulate emissions (Fisher et al., 1978; Kubitschek and Venta, 1979). This ash is typically sluiced to ash settling basins using large quantities of water. Chu et al. (1978) estimated that up to 168,000 liters of water per metric ton of fly ash (40,000 gal/ton) are required. When added to water consumed during bottom ash slucing, 12 TVA coal-fired stream plants utilized an average total of 11.5 million gallons per 1000 MW of electrical power produced.

Table 1. Toxic and Potentially Toxic Substances Released Into the Aquatic Environment during Coal Combustion (From Torrey, 1978).

Group	Examples	
1. Acids and anhydrides	Maleic anhydride	Sulfuric acid
	Benzoic acid	Nitric acid
	Hydrochloric acid	
2. Amines	Ammonia	Aliphatic amines
	Aniline	Benzidine
	Methylaniline	
	- and -naphthylamines	
3. Inorganic salts	Chromium chloride	
	Chromium sulfide	
4. Carbonyl compounds	Formaldehyde	
	Acetaldehyde	
5. Heterocyclics	Pyridines	
	Quinolines	
6. Hydrocarbons	Benzene	Toluene
	Xylene	Aliphatics
	Olefins	
7. Phenols	Phenols	Cresols
	Xylenols	
8. Polycyclic aromatic hydrocarbons	Chrysene	Carbazoles
	Pyrenes	Biphenyl
	Fluorene	Anthracene
	Indenopyrenes	Perylene
	Benzo(a)pyrene	Dibenzofluorene
	Dibenzoanthracene	Benzoanthracenes
	Benzo(a)anthrone	Methylchrysenes
	Benzocarbazoles	Acenaphthene
	Acenaphthylene	Alkylanthracenes
	Alkylphenanthracenes	
	Dimethylbenzoanthracene	
	Benzoperylene	Coronene

Table 1. (Continued). Toxic and Potentially Toxic Substances Released Into the Aquatic Environment during Coal Combustion (From Torrey, 1978).

Group	Examples	
9. Sulfur compounds	Hydrogen sulfide	Thiophenes
	Mercaptans	Carbon disulfide
	Methylthiophene	Carbonyl sulfide
10. Trace Elements	Antimony	Arsenic
	Barium	Beryllium
	Bismuth	Cadmium
	Chromium	Cobalt
	Copper	Fluorine
	Gallium	Lead
	Lead	Manganese
	Mercury	Molybdenum
	Nickel	Selenium
	Silver	Tellurium
	Thallium	Tin
	Uranium	Vanadium
	Zinc	
11. Organometallics	Nickel carbonyl	
	Tetraethyl lead	
12. Fine particulates	Sulfur particulates	
	Respirable coal dusts	
	Tar	
	Soot	
13. Gases	CO	SO_2
	SO_3	NO^2
14. Cyanides	Hydrogen cyanide	Naphthyl cyanide
	Ammonium cyanide	
	Ammonium thiocyanate	

Fly ash effluent varies considerably in its composition due to variability in parent coal, boiler characteristic and the physical and chemical properties of sluicing water. Although a portion of some of the metals released during the combustion of coal volatilize (Hg, As) and are released into the atmosphere, the more refractory metals condense as oxides onto the particulate fly ash (Theis et al., 1978). The dominant metal oxides include SiO_2, CaO, MgO, Fe_2O_3, and Al_2O_3, and smaller quantities of SO_3, P_2O_5, and carbon residuals, as well as numerous trace elements (Chu et al., 1978). Since a large portion of these metals have been shown to exist as a surface-sorbed form they must be considered mobile enough to be released into aquatic receiving systems under proper conditions. This phenomenon has the potential for environmental impacts of toxic elements from ash basin effluents, (Cherry and Guthrie, 1977) and under certain circumstances, biological hazard from uncontrolled seepage of rainwater and slurry water through containment dams (Coutant et al., 1978). Trace metals may also enter groundwater tables in significant quantities to raise the question of the efficacy of providing impermeable chemical or physical linings for proposed ash basins (Theis et al., 1978).

Few toxicological studies have been conducted using whole ash or whole ash effluents. Kubitschek and Venta (1979) found that in contrast to results obtained by Chrisp et al, (1978) of mutagenic activity in stack-collected fly ash, ash collected from electrostatic precipitators was not significantly mutagenic as detected using the Ames Salmonella assay system.

Bioassay investigations performed by Birge (1977) to determine effects of aqueous leachates of fly ash on the eggs of the goldfish (Lepomis microlophus), leopard frog (Rana pipiens), and Fowler's toad (Bufo fowleri), produced rapid and complete mortality in the frog and toad eggs with undiluted effluent, and 43% mortality in goldfish eggs during exposures of one to three days.

2.2.1 Coal Ash Field Studies

By far the most extensive field investigations on the influence of coal ash to natural communities have been the investigations of Drs. Cherry, Guthrie, Davis, Harvey, and others on the ash basin drainage system in the 400D area of the Savannah River Project, Aiken, South Carolina. These investigators have examined the impacts of increased turbidity, the toxicity and environmental transport of associated heavy metals, and thermal discharges upon abiotic and biotic

components at several trophic levels within the swamp ash basin drainage system (Cherry and Guthrie,1977,1978,1979a; Cherry et al, 1976, 1979a, 1979b; Guthrie and Cherry,1976,1979a; Guthrie et al,1978b; Rodgers et al,1978) Their work has provided a detailed account of the environmental stresses imposed throughout various trophic levels due to ash basin effluents.

Cherry et al, (1976) examined the response of the resistant mosquitofish (Gambusia affinis) to ash and thermal effluents and determined that several elements (Ca,Cl,Se,Zn,Br) were bioaccumulated in this species to concentrations well above ambient water concentrations. A decrease in diversity of members of higher trophic levels was observed at the most perturbed sampling stations, which was postulated to have a serious impact upon the elemental cycling functions of the food web within this ecosystem (Guthrie and Cherry, 1976; Cherry and Guthrie, 1978). Bioaccumulation of 10 toxic elements (Ti,Mn,Cu,Cr,Zn,As,Se,Co,Cd,Hg) was found in all biota sampled within the system including aquatic plants, bacteria, macroinvertebrates, and vertebrates (Cherry and Guthrie, 1977 ; Guthrie et al, 1977; Guthrie and Cherry,1979b). Data was also collected which demonstrated a reduction in bacterial diversity and percent chromagenic colonies with a corresponding increase in total

culturable colonies in response to the ash basin effluents (Guthrie et al, 1976; 1978a). Lowered pH values within the drainage system due to the ash effluent were implicated in the alteration of composition, and the rearrangement of the bacterial community structure (Guthrie et al,1978b). Primary producers sampled within the swamp drainage system including the algae Oscillatoria, and Hydrodicton, cattail (Typha latifolia), broomsedge (Andropogon virginicus),nut grass (Cyperus rectofractus), bald cypress (Taxodiun distichum), and duckweed (Lemna perpusilla) demonstrated the ability to bioconcentrate 15 chemical elements above ambient water concentrations, and two elements,(Hg and Zn) above measured sediment levels. Of these primary producers, Hydrodicton, Pontederia, Typha latifolia, and Lemna were identified as the most efficient biomagnifiers (Guthrie and Cherry,1979a; Cherry and Guthrie,1979a,1979b). These findings agree with those of Clark et al (1981), who examined the uptake and depuration of heavy metals from duckweed (Lemna perpusilla) obtained from a heavy ash settling basin. Cherry et al (1979a; 1979b) found that in response to heavy ash siltation rates, decreasing pH levels, and coal ash associated toxic elemental concentrations, populations of macrobenthic invertebrates and mosquitofish were markedly reduced. The diverse modes of elemental dissipation observed

from the ash basin drainage system underscores the need for maintenance of biotic diversity to accomplish efficient and complete cycling and removal of toxic chemical elements (Cherry and Guthrie,1978). Guthrie and Cherry (1976) suggest that systems exhibiting characteristics similar to the one studied might demonstrate increased elemental cycling and removal if tolerant consumer organisms were introduced. A recovery of population densities to reference station levels was observed following the construction and implementation of a more efficient ash settling basin which substantially lowered the effluent concentrations of particulates and toxic chemical elements within the swamp basin drainage system (Cherry et al, 1979a,1979b; Guthrie and Cherry,1979a,1979b; Guthrie et al,1982).

2.3 ENVIRONMENTAL IMPACTS ON AUFWUCHS

There are numerous natural and anthropogenic substances which may affect and alter the structure and function of the aufwuchs community. Heavy metals have been studied extensively for their impact upon the aquatic environment; what is reviewed here are the relevant studies of investigations with heavy metals utilizing members of the aufwuchs community. A brief review of some studies involving thermal and nutrient enrichment is also presented.

2.3.1 Heavy Metals

Although numerous heavy metals reach the aquatic environment, either singly or in combination, there have been relatively few studies integrating environmental effects of heavy metals on entire communities, specifically the aufwuchs. It is well known that under most field conditions various members of the primary producing community, especially diatoms, exhibit differing patterns of abundance in direct correlation to ambient heavy metal concentrations (Rushforth et al,1981). Depressions in population numbers, shifts in species dominance, reproductive impairment, and bioaccumulation by members of the primary producing community in response to abnormally high environmental concentrations of various heavy metals have been extensively documented (Biesinger and Christensen, 1972; Gibson, 1972; Stokes et al,1973; Trollope and Evans, 1976; Rushforth et al, 1981; Sanders et al, 1981; Shehata and Whitton, 1981).; Foster, 1982; Prasad and Prasad, 1982). Clark et al (1981) investigated the bioconcentration and depuration of eight heavy metals (Cd,Cu,Fe,Mn,Zn,Cr,Pb,Ni) in another primary producer, duckweed (Lemna perpusilla), and Rodgers et al,(1978) documented the cycling of various heavy elements in Lemna in a swamp drainage system receiving coal ash effluents.

Most of the literature dealing with primary producer-heavy metal interactions has examined either physiological changes or uptake mechanisms and kinetics within specific organisms that occur as a result of exposure to heavy metals. Numerous authors have attempted to delineate ranges of toxic exposure levels to individual species of isolated heavy metals.

Several investigators have examined the effects of assorted heavy metals to marine algae and phytoplankton species. Sanders et al (1981) noticed a decrease in centric diatom species with a concurrent shift to dominance of microflagellates in response to additions of Cu to natural marine phytoplankton communities cultured in 1000-liter cylindrical tanks. Hardstedt-Romeo and Gnassia-Barelli (1980) examined the growth rate and accumulation in the marine haptophyceae, Cricosphaera elongata, when exposed to Cd and Cu. They utilized bioassays which included natural metal complexing phytoplankton exudates. Eide and Myklestad (1980) investigated the uptake and release of Zn, Pb, and Hg in the brown alga, Ascophllum nodosum. The most extensive work with marine phytoplankton and a complex mixture of heavy metals is that of Thomas et al (1980), and Hollibaugh et al (1980). These authors utilized in vivo chlorophyll fluorescence and direct microscopic cell counts to determine the acute toxicities of 10 metals

(Cu,Hg,Pb,Cd,Zn,Ni,Cr,Se,Sb,As) to natural estuarine populations and laboratory cultures of Thalassiosira aestivalis. They demonstrated that the laboratory growth inhibition observed due to Hg and Cu occurred at levels 5 to 10 times that expected to occur in a moderately polluted estuary.

Many authors have examined the toxic effects of selected metals upon individual freshwater algae such as Chlorella vulgaris, Chlorella ellipsoidea, Scenedesmus quadricauda, Haematococcus, Chlamydomonas, Ankistrodesmus falcatus, Scenedesmus obliquus, Chlorococcum spp., the protozoans Tetrahymena pyriformis and Vorticella convallaria, and the diatom Skeletonema costatum (Stokes et al,1973; Hutchinson, 1973; Klass et al, 1974; Rachlin and Farran, 1974; Sartory and Lloyd,1976; Berland et al,1977; Rosko and Rachlin, 1977; Toledo et al, 1979; Dunlop and Chapman,1981; Prasad and Prasad, 1982). Most of these authors measured cell growth and division to determine metal toxicity, but some examined changes in chlorophyll levels (Lue-Kim et al, 1980; Rosko and Rachlin, 1977). Berland et al, (1977) measured mean cell volume, particulate carbon and nitrogen, and the uptake of 14-C bicarbonate in laboratory cultures to determine heavy metal effects. Dunlop and Chapman (1981) utilized electron microscopy and Electron Probe X-Ray microanalysis

to characterize ultrastructure abnormalities of a freshwater ciliate protozoan resulting from exposures to Zn and Cd. Gibson (1972) noted that low concentrations of Cu effectively removed the bluegreen alga Anabaena flos-aqua, in a freshwater reservoir, which was replaced by Scenedesmus quadricauda Shehata and Whitton (1981) demonstrated in field and laboratory studies that blue-green algal species were more resistent to zinc when isolated from areas high in zinc concentration, than were those from areas of low zinc concentration. It has been shown that the influence of Cu and Pb causes a depression in total numbers of algal species, although it appears that the degree, rather than the type of pollution determines which species will be present in a polluted environment (Foster, 1982). In a study of a freshwater reservoir and model ecosystem, Kartasheva and Lebedeva (1981) noted a reduction in total abundance and an alteration of species composition in response to elevated levels of Zn and Cr.

Studies of the effects of two heavy metals (Cr and Cu) upon aufwuchs communities inhabiting artificial streams have been conducted utilizing selected non-taxonomic structural and functional parameters including: dry weight, ash free dry weight, chlorophyll a, ATP, and 14-Carbon (14-C) uptake and 35-Sulfate (35-S) assimilation rates (Rodgers, 1977;

Rodgers et al, 1979; Clark, 1980). Cd effects and uptake kinetics in aufwuchs within artificial streams were investigated by Geisey et al (1979). These authors demonstrated effects upon the community structure as measured by standing crop, species composition, photosynthetic pigments, and upon functional parameters including; primary productivity, metabolism and nutrient export. These findings indicate that even at low, environmentally realistic levels of dosed heavy metals, that the aufwuchs community can function as a sensitive indicator of environmental stress.

2.3.2 Thermal and Nutrient Loading

Responses of aufwuchs communities to either thermal or nutrient loading has been virtually ignored by aquatic investigators, particularily the effects of low level chronic exposures under controlled conditions. Cherry and Guthrie (1975) examined the effects of thermal loading on bacteria in a laboratory stream using seed water from an outdoor artificial pond. They noticed a shift in microbial population structure to favor the occurrence of Flavobacterium lutescens with a decrease in Escherichia coli and Streptococcus faecalis. Cherry and Guthrie (1975), and Guthrie et al (1977; 1979) found that the percentage of chromogenic bacteria increased with elevations in ambient temperature, alt-

hough total microbial populations decreased when temperatures reached 21 C in an ash basin drainage system. Stockner (1968) and Naiman (1976) examined primary productivity of aufwuchs in natural thermal streams, and reported higher than normal levels of productivity, as expressed by biomass accrual, on a yearly basis. Significant productivity occurred even during the winter months due to the elevated temperatures. Other investigators have demonstrated an increase in community respiration of aufwuchs grown in artificial streams following a short term increase in temperature from 6 to 21 C (Phinney and McIntire, 1965).

Investigations of nutrient loading are few and must be interpreted with caution. In one study, glucose was added to 60-ton sea water enclosures, which stimulated heterotrophic production and caused a corresponding depression in photosynthetic algal production (Parsons et al, 1980). Cole (1973) noticed an increase in stream primary productivity in a system receiving nutrient salts from an upstream waste-water treatment facility. Discharge from a reservoir containing elevated ammonia levels increased the primary productivity of aufwuchs colonizing glass slide substrates, and created a shift in the species dominance pattern of diatoms (Marcus, 1980). Rodgers (1977) and Rodgers et al (1979) examined the response of aufwuchs communities to the additions

of sucrose, phosphate salts and dextrose in an artificial stream system. Increases in heterotrophic production and sulfate assimilation were reported, as well as the accumulation of material on artificial substrates, particularily in artificial streams receiving sucrose additions. Sumner and McIntire (1982) investigated the relationships between light intensity and nitrate enrichment of aufwuchs communities in artificial streams and report a higher rate of primary productivity in nutrient enriched streams.

2.4 STRUCTURE AND FUNCTION OF AUFWUCHS

The aquatic ecosystem, composed of living organisms closely and inseparably interacting with abiotic physical and chemical environmental parameters , is a dynamic system which is inherently capable of self- maintenance, internal regulation, and sustained homeostasis through energy flow and nutrient cycling. An ecosystem demonstrates the ability to resist perturbation from environmental fluxes, and to some extent from anthropogenic influences, and thus remains in a state of equlibrium. Useful areas of observation to delineate in the study of ecosystems and their development are: 1) community energetics, 2) community structure, 3) life history, 4) nutrient cycling, 5) selection pressure, and 6) overall homeostasis (Odum, 1969). Two important

characteristics of the aquatic ecosystem which regulate and influence the above areas are its structure and function. Odum (1962) defined the terms "structure" and "function" in a detailed manner:

> By structure we mean: (1) The composition of the biological community including species, numbers, biomass, life history and distribution in space of populations; (2) the quantity and distribution of the abiotic (non-living) materials such as nutrients, water, etc. ; (3) the range, or gradient, of conditions of existence such as temperature, light, etc.
>
> By function, we mean: (1) The rate of biological energy flow through the ecosystem, that is, the rates of production and the rates of respiration of the populations and community; (2) the rate of material or nutrient cycling, that is the biogeochemical cycles; (3) biological or ecological regulation including both regulation of organisms by environment (as, for example, in photoperiodism) and regulation of environment by organisms (as, for example, in nitrogen fixation by microorganisms).

The structure of a community of organisms is usually represented by some quantifiable expression of composition, either at the taxonomic, trophic or biochemical level. At any instant in time a particular community will exhibit a unique structure; the dynamic process of change in structure of a community through time is termed succession (McIntire, 1975). Function defines any of the rate processes exhibited by the ecosystem. The two basic metabolic processes, which when measured integrate the most system information, are

photosynthesis and respiration (Odum, 1977; Rodgers et al, 1979). Table 2 presents examples of the analyses of structural and functional properties of aufwuchs.

Table 2. Definitions, categories and examples of analyses of structural and functional properties of lotic aquatic ecosystems.

STRUCTURE	definition	measurement of abiotic or biotic characteristics at a point in time.	
	TAXONOMIC	definition	organism or species-level analyses.
		examples	species lists, diversity indices, distributional pattern, density, indicator species.
	NONTAXONOMIC	definition	chemical, physical, biochemical or biological analyses not requiring identification of organisms composing the community.
		examples	biomass, chlorophyll, carotenenoids, adenosine triphosphate, deoxyribonucleic acid.
FUNCTION	definition	measurement of any rate process of the ecosystem.	
	TAXONOMIC	definition	organism or species-level analysis.
		examples	species colonization rate, rate of recovery to equilibrium number of species following disturbance.
	NONTAXONOMIC	definition	chemical, physical, biochemical or biological rate processes in the ecosystem.
		examples	primary productivity, respiration rate, assimilatory sulfate reduction rate.

Analysis of structural parameters provides information about the community of organisms inhabiting the aquatic ecosystem. Functional measurements reveal characteristics of the entire ecosystem. It is apparent that ecosystem structure and its corresponding functional attributes are not directly or simply related, and equally apparent that methodologies for determining qualities and quantities of the degree of relatedness or independence of these two categories are inadequate. Since protection of the structural components of a lotic ecosystem may not provide for the maintenance of important functional properties required by downstream biota, and perturbation of structural integrity does not necessarily affect the functioning of the whole ecosystem, a logical approach to the study of aquatic ecosystems is one that integrates investigations of both structure and function (Rodgers, 1977; Rodgers et al, 1979).

Since methodologies for the determination of structure and function differ in their expense, effort, and quantity and quality of useful information extracted from a given community, when assessing the impact of a specific perturbant, the researcher is faced with myriad choices of investigative paths to follow. From a regulatory and ecological standpoint it may prove more realistic and feasible to attempt to protect the overall functions of a specific commu-

nity, such as primary productivity and respiration rates, nutrient and energy cycling, and assimilative capacity, rather than the individual species which comprise that community.

2.4.1 ATP:

One parameter that appears to offer a reliable method for estimating the biomass of natural aufwuchs samples is the measurement of adenosine- 5'-triphosphate (ATP). ATP is a ubiquitous component of all living cells and is the driving force of most bioenergetic reactions. As a primary phosphorylating agent, ATP serves as the major energy source in cellular metabolism(Tifft and Speigel, 1976).

To accurately reflect total biomass , three assumptions must be met: 1) The quantity of measured ATP must be proportional to some cellular entity such as total carbon (Patterson et al, 1970 ; Holm-Hansen , 1969); 2) ATP must have an extremely short survival time subsequent to cell death to prevent erroneously large estimates of cellular biomass during assay procedures; and 3) A simple and sensitive analytical procedure must be available for determination of ATP concentration from biological samples which is based upon the luceferine-luciferin-luciferase system of the firefly Photinus pyralis. The key reactions are:

$$LH + ATP + E \rightarrow E - LH2 - AMP + PPi \quad (1)$$

$$E - LH2 - AMP + O2 \rightarrow E - L - O^* + Product \quad (2)$$

$$E - L - O^* \rightarrow E - L - O^- + hv \quad (3)$$

Where:

LH2 = luciferin

E = luciferase

PPi = inorganic pyrophosphate

The initial reaction which produces light is luciferase catalyzed (1). One product, an enzyme-luciferin-adenosine monophosphate complex (luciferyl-adenate) is rapidly oxidized to oxyluciferyl- adenate (2), which is followed by the release of a quantum of light for each ATP molecule present (McElroy et al, 1969; Patterson et al, 1970). The assay procedure involves the measurement of this emitted light with a scintillation counting device, or a commercial ATP photometer. From the early pioneering work of Holm-Hansen and Booth (1966) who examined ATP concentrations in open water, numerous investigators have determined that the above assumptions are sound, and that ATP is a valid parameter to examine for biomass determinations. Holm-Hansen (1969) and Hamilton and Holm-Hansen (1967) found ATP measurements to be in excellent agreement with chlorophyll data and direct microscope measurements of microbial biomass in ocean samples.

Brezonik et al (1975) determined that a correlation existed between ATP quantities ,dry weight,and chlorophyll contents of laboratory cultures of Selenastrum capricornutum. ATP measurements have been applied to microbial biomass determinations of lake water (Rudd and Hamilton, 1973; Tobin et al, 1978), small streams (Geesey et al,1978), marine sediments (Bancroft et al, 1976; Karl and LaRock, 1975; Yingst, 1978), and activated sludge (Patterson et al, 1970). ATP measurements have also been utilized to monitor the effectiveness of disinfectants in sewage treatment plants (Tifft and Spiegel, 1976).

A few investigators have utilized the ATP assay as an indicator of toxicity from pH changes, chloroform, acetone, acrolein, and mercuric chloride in natural pond water samples (Kennicutt, 1980). Both Kennicutt and Brezonik et al (1975), who monitored nutrient addition effects on cellular ATP pools and the toxic effects of mercury, indicate that the ATP assay demonstrates definite potential for monitoring the toxicity of environmental contaminants. Brezonik et al (1975) notes that changes observed in measured ATP quantities results from changes in cellular ATP pools rather than changes in viable cell populations. He also noted that sublethal doses of mercury induced a reduction in cellular ATP pools; therefore, ATP assays may provide a useful tool for

monitoring the physiological state of natural microbial populations in response to environmental stress. Stephens and Shultz (1982) note however, that variability in ATP data from a given sampling site may make ATP analyses of limited applicability as a response bioassay indicator.

2.4.2 Primary Production of Lotic Systems

Of all measurements that can be applied to natural aufwuchs communities those that pertain to the primary productivity function are perhaps of most ecological importance. Aufwuchs are responsible for the majority of primary production in lotic ecosystems, and since the accrual of biomass by this community determines downstream water chemistry and limits food resources of grazing organisms which form the intricate interconnections of the aquatic food web, an indication of the potential rate of biomass production is a valuable parameter to measure when assessing and characterizing the potential of the entire system. Close monitoring of this change in potential due to environmental and anthropogenic influences may prove to be a useful technique for determining the ecosystem impact of a specific compound or discharge and provide a mechanism for establishing ecologically responsible water quality criteria standards.

The familiar equation for the primary production process in aerobic photoautotrophs can be expressed as follows:

$$6CO_2 + 6H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6O_2$$

It can be seen that valid approximations of primary productivity can be arrived at by measurements of carbon uptake, oxygen production, the formation of organic compounds, the gain of chemical energy of the cellular system, or changes in the internal redox system (Vollenweider, 1978). The practical experimental measurements of these cellular changes are based on several conceptually different, yet equally valid approaches. Isolated portions of the natural community of interest may be fixed or re-suspended within their natural environment, (" in situ " measurements), or removed to a laboratory situation where natural conditions are simulated. Alternatively, entire non-isolated communities can be assessed by the experimental measurement of certain gross metabolic activities, such as oxygen production, pH shifts, and conductivity changes. Indirect estimation methods such as the measurement of nutrient uptake, biomass accumulation, or carbon dioxide accumulation can also be used. Entire communities may be utilized if environmental conditions allow and accuracy is not essential (Vollenweider, 1978; Rodgers,1979). McIntire (1975) examined the accumulation of dry weight and ash free dry weight to assess

primary productivity in an artificial stream system; King and Ball (1966) estimated primary productivity through measurements of extracted phytopigments, dry weight and ash free dry weight; and Kevern and Ball (1965) examined pH and CO_2 changes and rates of biomass accumulation on artificial substrates. Goryunova and Vorob'yeva (1981) report that measurements of minute changes in pH values demonstrate a relationship to primary productivity measurements obtained by 14 C methodology, however they present no evidence to explain the mechanism of the pH shift.

Two techniques are commonly applied towards the measurements of primary productivity rates in natural lotic systems. The first involves the measurement of dissolved oxygen, either at sampling stations above and below the lotic community of interest, or following an incubation time of an enclosed portion of the community. Although some investigations have used the open stream gas measurement (Kevern and Ball, 1965), Bott et al, (1978) and Lingeman and Vermij, (1980) agree that measurements of entire open systems, although allowing characterization of whole community respiration, generally result in unrealistic over or under estimates of primary productivity. This is due to problems of gaseous diffusion and the degree of CO_2 saturation. To overcome this problem many investigators have developed cham-

bers of various sizes and complexity in which communities can be isolated and the dynamics of dissolved gases empirically measured (Rodgers,1977). Several investigators have utilized enclosed chambers and changes in dissolved oxygen as measured by the Winkler titration method to assess the primary productivity of aufwuchs communities (McIntire et al,1964,1966a,1968b; Thomas and O'Connell, 1966; Ertl and Tomajka, 1973; Williams, 1969; Pfeifer and McDiffett, 1975; Hansmann, 1971) in both natural and artificial stream systems. Despite an improved titration method to allow for more sensitive measurements of dissolved oxygen concentrations(to 0.05 ug/l; Bryan et al, 1976) the dissolved gas method has proven to be somewhat limiting, particularily when attempts have been made to measure the productivity of a system over a short time period. To overcome this shortcoming a method utilizing the incorporation and measurement of 14-C uptake into cellular components has been advanced and refined (Steeman Nielsen, 1951,1952). Although this method is approximately 50 times more sensitive than other methods, and has been used extensively by oceanographers in open ocean systems, it has not been widely utilized for measurements in lotic systems (Wetzel, 1963,1964,1965; Schindler et al, 1973; Rodgers and Harvey, 1976; Rodgers, 1977, Rodgers et al, 1978; Malone, 1982). Detailed comparisons of

the two methods(dissolved oxygen vs. 14-C uptake) by Anderson and Sand-Jensen(1980) , Bott et al, (1978) Hunding and Hargrave, (1973) and Fogg ,(1974) indicate that since each method measures different enzyme systems of the photosynthetic process, results from each should be compared and interpreted with caution. Neither one is free from experimental artifacts and assumptions, and at low light intensities the dissolved oxygen method may overestimate gross photosynthesis. However, 14-C measurements, due to a possible reassimilation of respired CO_2 by the community when overall CO_2 flux into the actively photosynthesizing cell is small, tend to underestimate gross primary productivity. In general, under most conditions the 14-C uptake method emerges as a more sensitive and applicable technique for measurements of the primary production rates of lotic communities. However, Fitzwater et al (1982) caution that trace metal contamination may seriously affect both the accuracy and precision of the measurement technique.

Measurements of aufwuchs primary productivity have been applied towards the determination of various environmental effects upon the community. McIntire (1966a,1968a,1975) investigated effects of light intensities, water velocity, dissolved oxygen levels, temperature, seasonal changes, and grazing pressures on aufwuchs inhabiting artificial streams.

All parameters investigated produced shifts in the community composition and functional abilities of the aufwuchs community. Rodgers and Harvey (1976) examined the effect of current velocities on aufwuchs communities using 14-carbon methodology, and Pfeifer and McDiffett (1975) related algal density, light intensity ,and current velocity to changes in stream riffle community primary productivity. A few authors have even applied techniques of primary productivity measurement towards the assessment of the effects of toxic substances, including Kraft mill effluents, a lampricide (TFM; 3-trifluoromethyl-4- nitrophenol), and chlorine, copper, and chromium (Williams, 1969; Maki and Johnson, 1976; Rodgers, 1977,1978; Rodgers et al, 1979; Malone, 1982).

2.4.3 Sulfate Assimilation

With the recognition that planktonic and periphytic microbial communities demonstrate complex ecological functions beyond the metabolic activities coupled to photosynthetic processes, ecologists have searched for a method to directly assess the contribution of heterotrophic organisms in natural systems. What is needed is a methodology that will encompass all microbial biomass functions not associated with photosynthesis. When combined with measurements of primary productivity, the activity and metabolic health of the en-

tire community can be evaluated. If the community can be partitioned into autotrophic and heterotrophic fractions, information concerning specific characteristics of the community such as the availability of nutrients, diversity, and stability when confronted by environmental perturbations can be obtained. One strategy used to measure heterotrophic productivity is to introduce labeled carbon-14 compounds such as glucose and acetate into appropriate incubation vessels, and quantitatively measure the activity incorporated into cellular constituents. However, as Jassby (1975) points out, many organic compounds present in natural waters are capable of maintaining heterotrophic activity, and it would be impractical to identify, test, and partition all of the possible chemical species with this method. Furthermore, although of limited use in a laboratory condition, this method is of no value in a field situation.

Monheimer (1974,1975a,1975b) suggested that a measure of the fixation of the essential element sulfur to a particulate, and hence filterable form, might provide a useful yardstick of aerobic bacterial production. All organisms require, and can transport sulfate, and the incorporation of sulfur into cellular components appears closely linked to carbon uptake (Monheimer, 1975a,1975b). As a constituent of the amino acids cysteine and methionine, and the cofactors

biotin, thiamin, coenzyme A, and lipoic acid, sulfur is an essential element for the structure and function of all living cells. Sulfur contents of biota range from 0.05 to almost 5.0 percent (Wetzel, 1975a, 1975b). Jassby (1975) reports a ratio of sulfur to dry weight in microbes of about 0.011.

Although animals require pre-formed sulfur-containing amino acids and vitamins (with the exception of some ruminants), most plants and microorganisms can obtain sulfur from naturally occurring sulfate compounds. The processes involved in the reduction of sulfates to organic sulfur-containing cellular components is termed assimilatory reduction; that process producing sulfide ions with a corresponding energy yield, dissimilatory. Both form an important part of the biochemical sulfur cycle, but are mediated by distinctly different enzymes. Assimilatory sulfate reduction occurs under aerobic conditions , (and by an occasional anaerobe) in the majority of microorganisms and plants. This is demonstrated by their widespread ability to utilize ambient sulfate compounds as a sole sulfur source (Roy and Trudinger, 1970).

Schriff and Hodson (1973) have attempted to construct a picture of sulfate uptake and assimilatory reduction applicable to a wide range of microorganisms. In order to be

utilized in most biochemical reactions, sulfate, which is relatively unreactive, must be activated with ATP to form APS, a reaction catalyzed by ATP sulfurylase (Schiff and Hodson, 1973). Three mechanisms operate to offset the unfavorable equilibrium which results due to the relative strengths of sulfuric and phosphoric acids, and the free energies of the phosphate-sulfate anhydride bond: 1) removal of APS by another ATP to form adenosine 3'-phosphate 5'-phosphosulfate (PAPS); 2) APS kinase has a high affinity for APS; and 3) inorganic pyrophosphatase cleaves the pyrophosphate released by the sulfurylase reaction. Thus PAPS, the activated intermediate, will be accumulated. PAPS is the starting compound for the formation of sulfate esters in many biological processes, which are then incorporated into proteins and other cellular constituents.

Before sulfate can be utilized by organismal biochemical machinery, it must be concentrated from extra-cellular pools. Studies of bacteria, higher plants, and the green alga Chlorella, indicate that this is accomplished via a unidirectional carrier mediated active transport system (Wedding and Black,1960; Monheimer, 1975a). The rate of transport is determined by pH, temperature, ionic strength, sulfate concentration, and available metabolic energy (Schiff and Hodson, 1973; Monheimer,1974,1975a; Karbonowska

et al, 1977). Sulfate uptake in reponse to ambient concentrations in Chlorella resembles an adsorption isotherm (Wedding and Black,1960).

Monheimer (1975a, 1975b) examined the effects of sulfate, organic sulfur, and organic carbon concentrations on three aerobic bacteria; Pseudomonas fluorescens, Corynebacterium striatus, and Serratia marcescens. He established a metabolic link between the uptakes of sulfate and organic carbon, and although demonstrated that heterogeniety in uptake kenetics exists between bacterial species, implied that sulfate uptake rates might be utilized as an indicator of microbial biomass and heterotrophic production in freshwater ecosystems.

Since sulfate is rarely limiting in natural waters, and is available as a radio-labeled compound (Sulfur-35), a technique was pioneered by Monheimer (1974, 1975a, 1975b) utilizing the incorporation of filterable isotope as a direct measure of aerobic heterotrophic production. Preliminary culture work, (Monheimer, 1975b) was followed by an assessment of the natural communities of Lake St. Clair (Monheimer, 1975a). Jassby (1975) used a similar dark in-situ incubation technique to assess planktonic samples in Castle Lake, and Rodgers (1977), and Clark,(1980) applied this method to aufwuchs communities colonizing glass micro-

scope slides incubated in artificial streams. During the latter two experiments the following assumptions were made: 1) there is no luxury uptake of sulfur into algal or bacterial cells; 2) sources for sulfur in natural environments other than sulfate are negligible; 3) extracellular sulfur-containing products are not important; and 4) uptake of sulfate in the dark by autotrophic organisms is insignificant (Rodgers, 1977). However, Monheimer (1978) reports that since the dark bottle method assumes that only photosynthetic metabolic processes cease, while others continue, and that some algae may become heterotrophic in the dark, actively incorporating sulfate into cellular biomass, the use of dark sulfate uptake as a measure of heterotrophic production is probably invalid. Sulfate uptake based upon indirect calculations from photosynthetic carbon uptake, total community uptake, and an assumed carbon:sulfur ratio are valid only if there is evidence for direct metabolic coupling of the two uptake processes. Actually C and S uptake rates vary with physiological condition in algae (Monheimer, 1978), and among species. Campbell and Baker (1978a, 1978b) demonstrated dark uptake of sulfate in Nannochloris, Scenedesmus, Chlorella, and Navicula pelliculosa, and proposed that the ability to assimilate sulfate under dark conditions is more widespread than previously suspected. Although

these findings are at odds with the laboratory work conducted by Jassby (1975), who determined dark sulfate uptake in Scenedesmus obliquus and Chlorella vulgaris, the fact that some members of the aufwuchs community undoubtedly possess the ability to assimilate sulfate under dark conditions creates serious problems during the interpretation of such data. Sulfate uptake methodology would appear to be a valid technique for drawing general conclusions about heterotrophic productivity, however separating algal uptake from that of other members of the aufwuchs community is difficult and unreliable (Monheimer, 1981).

2.5 ARTIFICIAL STREAMS: AUFWUCHS INVESTIGATIONS

As a research tool, model or artificial streams have much to offer the lotic ecologist. In the laboratory, or with an outdoor artificial stream system, it is possible to simplify the lotic community to the desired level of complexity, and to isolate and control environmental factors that influence the structure and function of lotic communities. With an appropriate system, and careful manipulation of environmental conditions, much information can be extracted from artificial stream studies under experimental regimes that closely resemble "natural" stream systems, without the corresponding variability, complexity, and rigors of field

investigations. Various environmental parameters that have been successfully manipulated to investigate aspects of aufwuchs community structure and function include: light, current velocity, temperature, oxygen, carbon dioxide, seasonal photoperiod, and turbidity (McIntire et al, 1964; McIntire and Phinney, 1965; Kevern and Ball, 1965; McIntire, 1966a, 1968b, 1973, 1975).

Kevern and Ball (1965) examined the effect of various nutrients and EDTA upon primary production in artificial streams. Maki and Johnson (1976) evaluated the impact of a toxicant (lampricide) utilizing measurable parameters of lotic communities in a model stream. Kehde and Wilhm (1972) studied the effects of grazing by snails on the community structure of aufwuchs in laboratory streams, and Cushing and Rose, (1971) looked at the cycling of 65-Zinc in aufwuchs using a closed lotic microcosm. Rodgers (1977), and Rodgers et al (1979), used a series of outdoor artificial streams to investigate the effects of heavy metals (Cu and Cr), chlorine, and potential enrichments (phosphate and sucrose) upon selected non-taxonomic structural and functional parameters of aufwuchs communities colonizing glass microscope slides.

To accomplish these investigations, and others, artificial streams have been used which exhibit a variety of complexity; from the glass condenser tubes of Odum and Hoskin

(1957), to those of the Stroud Water Research Center which operate on a sophistocated once-through flow basis and include both pool and riffle habitats (McIntire, 1975). Table 3 summarizes some of the research conducted using aufwuchs communities inhabiting artificial stream ecosystems.

Table 3. A summary of some references concerned with artificial stream research on aufwuchs communities. The general type of stream, experimental orientation, and environmental factors or experimental variables investigated are included. "Closed", "partially-closed", and "open" refer respectively to 1) systems with no water exchange; 2) systems with some water exchanged; and 3) systems operating on a once-through flow basis.

Reference	Date	Artificial Stream Type	Experimental Orientation	Environmental Factors
Cushing and Rose	1971	glass tube; closed or open	^{65}Zn cycling	light
Cushing et al.	1975	glass tube; closed or open	^{65}Zn cycling modeling	none
Kedhe and Wilhm	1972	laboratory, wooden trough; partially-closed	primary production, grazing	none
Kevern and Ball	1965	laboratory, plastic trough; closed	primary production	temperature, light current velocity, EDTA
Kevern et al.	1966	laboratory, plastic trough; closed	methods of measuring primary production	none
Maki and Johnson	1976	laboratory, hatchery channels; open	metabolism, net community productivity, community respiration	lampricide TFM (3-trifluoromethyl-4-nitrophenol)

Table 3. (Continued). A summary of some references concerned with artificial stream research on aufwuchs communities. The general type of stream, experimental orientation, and environmental factors or experimental variables investigated are included. "Closed", "partially-closed", and "open" refer respectively to 1) systems with no water exchange; 2) systems with some water exchanged; and 3) systems operating on a once-through flow basis.

Reference	Date	Artificial Stream Type	Experimental Orientation	Environmental Factors
McIntire	1966a, 1966b 1968a, 1968b 1975	laboratory, wooden trough; partially-closed	primary production, community metabolism, community structure	light, current velocity, temperature, CO_2, oxygen
McIntire et al.	1964	laboratory, wooden trough; partially-closed	methods of measuring primary production	light
McIntire and Phinney	1965	laboratory, wooden trough; partially closed	primary production community structure	light, CO_2
Patrick et al.	1968	plastic box; open	community structure	pH, temperature manganese
Phinney and McIntire	1965	laboratory, wooden trough; partially-closed	primary production community respiration	temperature

Table 3. (Continued). A summary of some references concerned with artificial stream research on aufwuchs communities. The general type of stream, experimental orientation, and environmental factors or experimental variables investigated are included. "Closed", "partially-closed", and "open" refer respectively to 1) systems with no water exchange; 2) systems with some water exchanged; and 3) systems operating on a once-through flow basis.

Reference	Date	Artificial Stream Type	Experimental Orientation	Environmental Factors
Rodgers	1977	outdoor, hatchery channels; open	primary production community structure sulfate assimilation	heavy metal (Cr, Cu), chlorine, sucrose, PO_4
Rodgers and Harvey	1976	stainless steel-PVC trough; open	primary production	current velocity
Rose and McIntire	1970	laboratory, wooden trough; partially-closed	pesticide accumulation	dieldrin
Traaen et al.	1972	laboratory, polyethylene channel; open	nutrient consumption organic production	sucrose, NH_4, Cl, K_2HPO_4
Whitford	1960	laboratory, wooden trough; closed	algal growth	current velocity
Williams and Mount	1965	outdoor, polyethylene-lined channels; open	community structure primary production	zinc sulfate

Table 3, (Continued). A summary of some references concerned with artificial stream research on aufwuchs communities. The general type of stream, experimental orientation, and environmental factors or experimental variables investigated are included. "Closed", "partially-closed", and "open" refer respectively to 1) systems with no water exchange; 2) systems with some water exchanged; and 3) systems operating on a once-through flow basis.

Reference	Date	Artificial Stream Type	Experimental Orientation	Environmental Factors
Wuhrman	1964	outdoor, wooden trough; open	stream assimilation	sewage effluents

With all of these experiments the gain in control of various environmental parameters is at the expense of a realistic loss of ecological reality (Warren and Davis, 1971). McIntire (1975), warns that . . . "there is always a danger of becoming preoccupied with the elegance of a particular laboratory ecosystem, while losing sight of the natural system that the laboratory contrivance attempts to model."

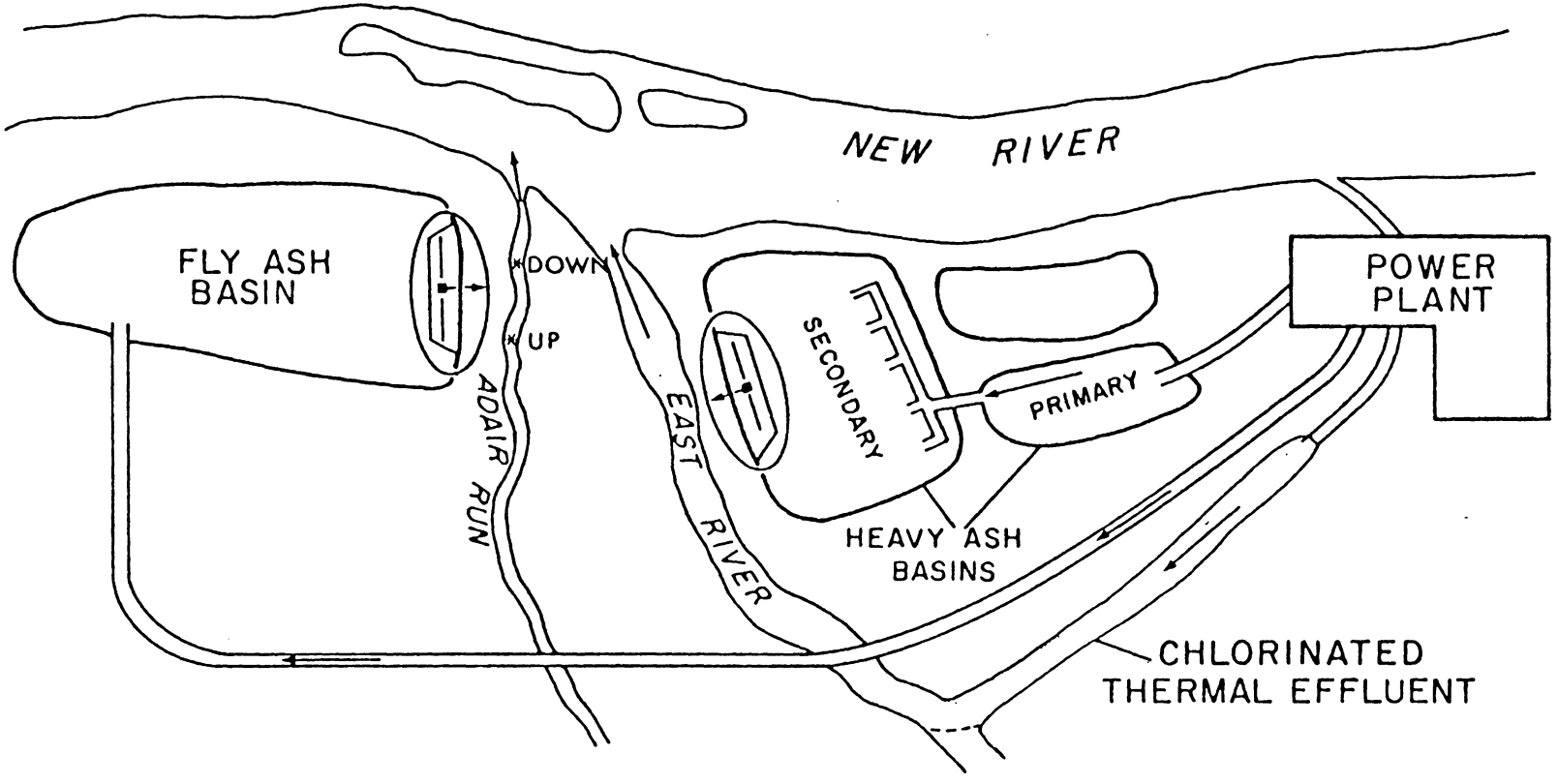
Chapter III

MATERIALS AND METHODS

3.1 FIELD STUDIES

Two field sampling stations were established at locations above and below the point of ash effluent discharge to investigate the effects of fly ash effluents upon the primary producing community of Adair Run (Figure 1). Both stations were similar concerning solar insolation, waterflow and depth. At each station Plexiglas diatometers containing 10, 75 X 35 mm pretreated glass microscope slides were placed immediately above the substrate and allowed to become colonized with native aufwuchs communities for a period of 10 to 21 days, depending upon the season and the observed appearance of the mature slides. The target quantity of colonization represented an intermediate point between maximum growth and the beginnings of sloughing-off of the attached periphytic community.

Figure 1. Map of the Glen Lyn power plant and ash basins, including the Adair Run field sampling stations.


NEW RIVER
FLY ASH
BASIN
DOWN
UP
ADAIR RUN
EAST RIVER
SECONDARY
PRIMARY
POWER
PLANT
HEAVY ASH
BASINS
CHLORINATED
THERMAL EFFLUENT

At predetermined intervals the populations colonizing the microscope slides were randomly sampled for the following non-taxonomic structural and functional parameters: Dry Weight, Ash Free Dry Weight, ATP, Chlorophyll a,Light Carbon Fixation, Dark Carbon Fixation, and Light and Dark Sulfate Assimilation. During each sampling period ambient water samples were obtained to determine pH, alkalinity, turbidity, and temperature. Nutrients (PO_4,SO_4,Nitrates), selected heavy metals, and Total Suspended Solid (TSS) were routinely monitored. All water chemistry parameters were determined following procedures presented in Standard Methods (APHA, 1975), (Table 4).

Table 4. Methods for analyses of physical-chemical parameters of water quality and Aufwuchs.

Parameter	Units	Method or Instrument	Reference
Alkalinity, Total	mg l^{-1} $CaCO_3$	titration, mixed indicator	APHA, 1975
Cadmium	mg l^{-1}	atomic absorption spectrophotometer (Perkin-Elmer 460)	APHA, 1975
Chlorine	mg l^{-1}	amperometric titrator (Fisher Porter)	APHA, 1975
Chromium	mg l^{-1}	atomic absorption spectrophotometer (Perkin-Elmer 460)	APHA, 1975
Copper	mg l^{-1}	atomic absorption spectrophotometer (Perkin-Elmer 460)	APHA, 1975
Lead	mg l^{-1}	atomic absorption spectrophotometer (Perkin-Elmer 460)	APHA, 1975
Nickel	mg l^{-1}	atomic absorption spectrophotometer (Perkin-Elmer 460)	APHA, 1975
Nitrate	mg l^{-1}	cadmium reduction	APHA, 1975
pH		glass electrode (Fisher portable meter)	APHA, 1975
Phosphate	mg l^{-1}	colorimetric	APHA, 1975

Table 4. (Continued). Methods for analyses of physical-chemical parameters of water quality and Aufwuchs.

Parameter	Units	Method or Instrumentation	Reference
Sodium	mg l^{-1}	atomic absorption spectrophotometer (Perkin-Elmer 460)	APHA, 1975
Sulfate	mg l^{-1}	turbidimetric	APHA, 1975
Temperature	^{o}C	mercury-filled thermometer (0.1 C divisions)	APHA, 1975
Total Suspended Solids (TSS)	ppm	glass fiber filter	APHA, 1975
Turbidity	Jackson Turbidity Units (JTU)	turbidimeter (Hach Chemical Co.)	APHA, 1975
Zinc	mg l^{-1}	atomic absorption spectrophotometer (Perkin-Elmer 460)	APHA, 1975

3.1.1 Dry Weight

To determine dry weights of aufwuchs communities the colonized material was scraped into pre-tared 15-ml porcelain crucibles with a single edged razor blade. The crucibles were placed on ice and transported to the Virginia Tech laboratory and dried for 24 hours at 55 C (Clark, 1980). For each station four slides were scraped and the mean value calculated as grams of dried material per square meter by dividing by 0.00375 (APHA, 1975).

3.1.2 Ash Free Dry Weight

Crucibles used to obtain dry weights were fired in a muffle furnace at 550 C for one hour, then to rehydrate the clay moiety, 3 drops of distilled water were added to each and the crucibles re-dried at 55 C for an additional 24 hours. Re-weighing provided a value for ash free dry weights which were also calculated on a weight in grams per square meter basis.

3.1.3 ATP

For ATP analysis, randomly selected slides were transported to the Glen Lyn laboratory, where they were placed into plastic microscope slide mailers containing 15 ml of preheated TRIS buffer (0.025 M TRIS adjusted to pH 7.5 with

acetic acid), and boiled for 10 minutes to extract cellular ATP. The samples were then placed on ice and transported to the Virginia Tech laboratory where they were frozen at -5 C until assayed. The assay procedure utilized the firefly luciferin-luciferase system (Sigma FLE 50), and a Labline 9140 ATP Photometer. On the assay date the firefly lantern extract was rehydrated in 10 ml of TRIS buffer per vial. The vials were centrifuged for two minutes at 4500xG and the contents combined. Standard curves were read and calculated on each assay date using known concentrations of ATP salts (Sigma FF-ATP).

3.1.4 Chlorophylls

To determine chlorophylls, individual glass slides from each station were placed into plastic slide mailers containing 15 ml of 90% (V/V) acetone, then placed on ice for transport. At the laboratory the samples were frozen at -5 C for a 24-hour dark extraction period. Following this extraction the acetone/chlorophyll solution was transferred into test tubes and centrifuged for 2 minutes at 3500xG. Quantities of chlorophyll a were calculated by measuring the absorbance of the sample at wavelengths of 645, 630, 663, and 750 nm using a Perkin-Elmer 55E spectrophotometer. A correction for phaeophytin was included by acidifying the

sample with 2 drops of hydrochloric acid (10%) and rereading the absorbance of the sample at 663 nm. The calculated quantity of chlorophyll present in the 15-ml samples was converted to an areal basis.

3.1.5 Primary Productivity

To measure primary production rates, four microscope slides from each station were transferred into Plexiglas primary productivity chambers designed specifically for evaluating lotic communities (Rodgers, 1977; Rodgers et al, 1978a). These chambers consisted of a 1.9-l polystyrene cyindrical container equipped with a removable lid and a battery powered submersible pump to provide water circulation.

Rodgers (1977) has shown that the pump moved a volume of up to 360 ml per minute, corresponding to a turnover rate of 5.36 minutes. Chambers used to measure dark carbon fixation were completely covered with black tape. To measure carbon uptake four colonized slides were transferred to the lotic productivity chambers and the chamber incubated in situ for four hours during maximum solar insolation. Both light (clear) and dark chambers received 1.0 ml (5.0 uCi) of 14-C as $NaCO_3$ (Specific activity = 1.1 X 10 7 dpm per ml) (New England Nuclear NEC 0865). Control chambers consisted of identical lotic primary production chambers that received 50

ml of 5 % formalin. Following incubation each slide was immediately scraped into a 3-dram stopper vial and placed into a tightly capped 20-ml scintillation vial (Fisher Polyseal). In later artificial stream studies each stopper vial received 0.5 ml of 5% formalin to halt photosynthesis. Samples were placed on ice and transported to the Virginia Tech laboratory. A wet oxidation method (Shimshi,1969) was used to prepare samples for liquid scintillation counting. Two ml of 0.025 N NaOH was pipetted into the bottom of each scintillation vial, and 1 ml of saturated chromic acid (saturated solution of CrO_3 in concentrated H_2SO_4) was pipetted into each dram vial. The vials were tightly capped and placed into a boiling water bath for 1 hour (Figure 2). Following cooling to room temperature and overnight refrigeration at 4 C, the stopper vials were carefully removed with forceps and 12 ml of TX100 scintillation cocktail (667 ml scintanalyzed tolulene (Fisher T-313) 333 ml Triton X-100 + 5.5 g PPO + 0.1 g POPOP) added to the NaOH. Samples were held overnight to allow for the decline of minor chemiluminescence resulting from the addition of the scintillation cocktail to sodium hydroxide (Patterson and Green, 1965) and then counted (Beckman LS=3150T). Counting efficiencies were determined externally by the channel-ratio method and were around 75%. Primary production as mg C m-2 hr-1 was calculated as follows:

$$P = \frac{14\text{-}Ct \times Ci \times V \times 1.064}{14\text{-}Ci \times A \times T}$$

where:

14-Ct = 14-carbon content of aufwuchs in dpm

Ci = initial dissolved inorganic carbon (ug l-1)

V = volume of incubation chamber

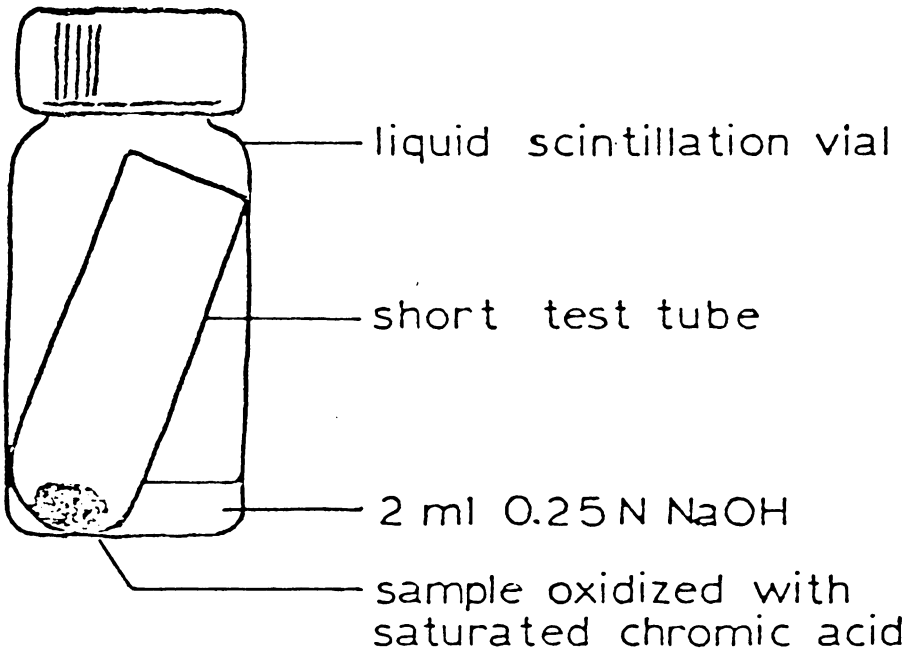
1.064 = isotopic correction factor for 14-C (APHA, 1975)

14-Ci = initial 14-C (dpm) injected into the incubation chamber

A = area of microscope slide

T = incubation time (hours).

Figure 2. Schematic of the oxidation apparatus for primary productivity samples.


liquid scintillation vial
short test tube
2 ml 0.25 N NaOH
sample oxidized with
saturated chromic acid

3.1.6 Sulfate Assimilation

During some of the initial field experiments sulfate assimilation rates by colonized glass slides and the corresponding attached aufwuchs community were determined. Glass slides were transferred to the lotic incubation chambers described above which then received 1 ml of carrier-free Na_2 35-SO_4 (NEN-041) diluted to approximately 100 uCi per ml with deionized distilled water (specific activity = 2.22 X 10 8 dpm per ml). $ Following a four-hour incubation period for both light and dark chambers, the tagged aufwuchs community was scraped into a 20-ml liquid scintillation vial with a single edge razor blade and the vial tightly capped. Vials were transported to the laboratory on ice where a liquid scintillation counting method described by Rodgers (1977) was used. This method was modified from the techniques of Mahin and Lofberg (1970) , and Jeffay et al (1960). To each vial 0.3 ml of cold perchloric acid was added and mixed with the sample by gentle swirling. Then 0.2 ml of hydrogen peroxide cold (4 C) was added to each vial and the contents quickly and tightly capped. The vials were placed in a 70 C water bath for one hour with occasional agitation. The samples were then cooled to room temperature and refrigerated overnight at 4 C. To prepare the samples for liquid scintillation counting, 8 ml of ethylene

glycol and 10 ml of a toluene based scintillation cocktail (6 g PPO in one liter of scintanalyzed toluene, Fisher T-313) were added to each vial and the contents vortexed until clear. Samples were analyzed for 35-sulfur by counting on a Beckman LS-3150T liquid scintillation counter. Both internal and external standardization techniques were used. Assimilatory sulfate reduction rates were calculated using the following equation:

$$S = \frac{35\text{-St} \times Si \times V}{35\text{-Si} \times A \times T \times Z}$$

where:

S = assimilatory sulfate reduction (mg S per square meter per hour)

35-St = 35-Sulfur content of aufwuchs after incubation (dpm)

Si = initial SO_4 concentration in mg/l

V = volume of incubation vessel

35-Si = initial 35-S (dpm) injected into vessel

A = area of substrate

T = incubation time in hours

Z = 32 mg S per mole SO4

3.2 ARTIFICIAL STREAM EXPERIMENTS

To determine specific environmental effects of fly ash, heavy metals , sulfates and thermal effluents upon the structure and function of aufwuchs communities a series of artificial streams were utilized. These previously constructed streams consisted of six 4 m X 35 cm X 39 cm aluminum fish hatchery troughs painted with an inert white epoxy, (Figure 3). Each stream was divided longitudinally by a white plexiglas divider and provided with a removable screen cover. Each stream consisted of an anterior mixing box which received the water source and selected chemical treatments for this series of experiments (Figure 4).

Figure 3. Diagram of the Glen Lyn artificial stream system.

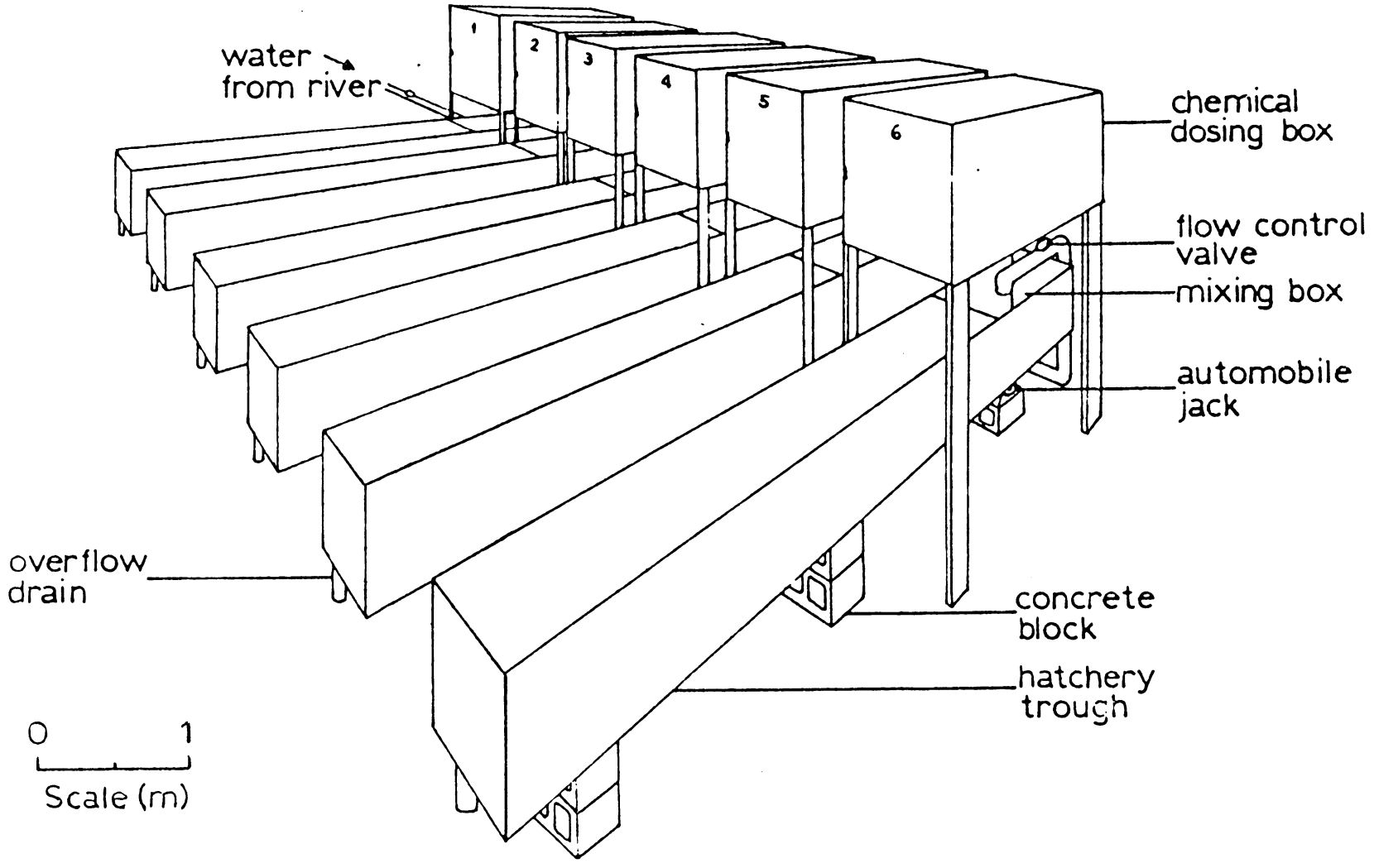
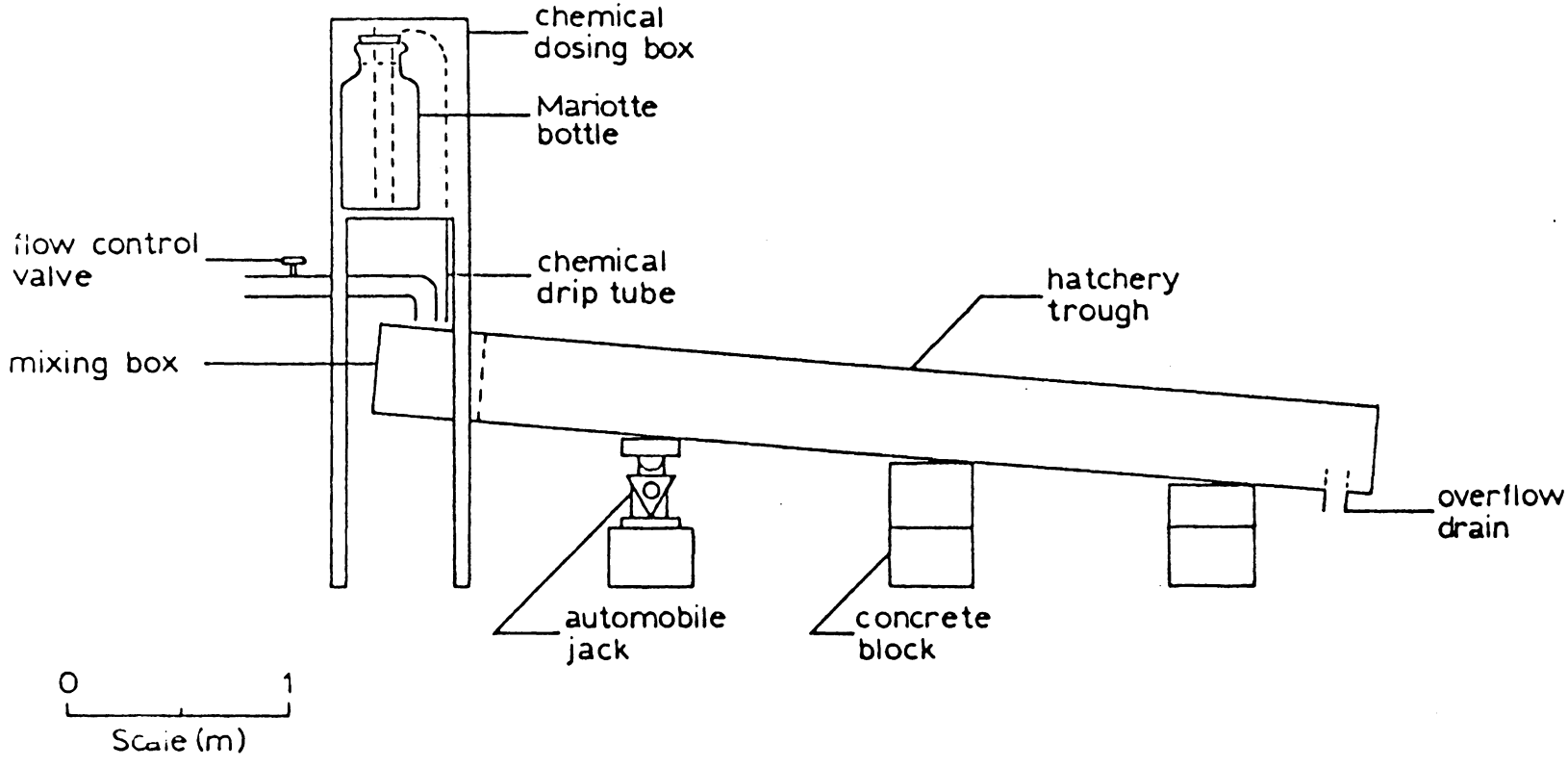

water
from river
1
2
3
4
5
6
chemical
dosing box
flow control
valve
mixing box
automobile
jack
overflow
drain
concrete
block
hatchery
trough
0
1
Scale (m)

Figure 4. Schematic cross section of the artificial stream system.


chemical
dosing box
Mariotte
bottle
flow control
valve
chemical
drip tube
hatchery
trough
mixing box
overflow
drain
automobile
jack
concrete
block
0
1
Scale (m)

Two 1-horsepower submersible well pumps (Gould 25EL10412) delivered New River water from a location within the pump house directly behind the intake screens of the power plant. This water was delivered through 2 PVC pipe and brass valves to the streams on a once-through basis. The flow rate to each stream was 15 l/min (9 cm/sec) and the water depth maintained at 4-5 cm with plastic standpipes . For experiments measuring structural and functional parameters of the aufwuchs community, plexiglas diatometers holding glass microscope slides were placed at one-meter intervals along the length of each stream. New River water served as a source of propagules for colonization, and in addition, several medium sized cobbles were placed in the anterior mixing box of each stream to simulate a more natural substrate. Prior to the initiation of each experiment the artificial streams were thoroughly scrubbed to remove potential grazing organisms.

Two schemes were utilized to introduce fly ash and heavy metal and sulfates respectively into the streams. In each case the dosing apparatus was contained within a wooden box located at the anterior end of the streams.

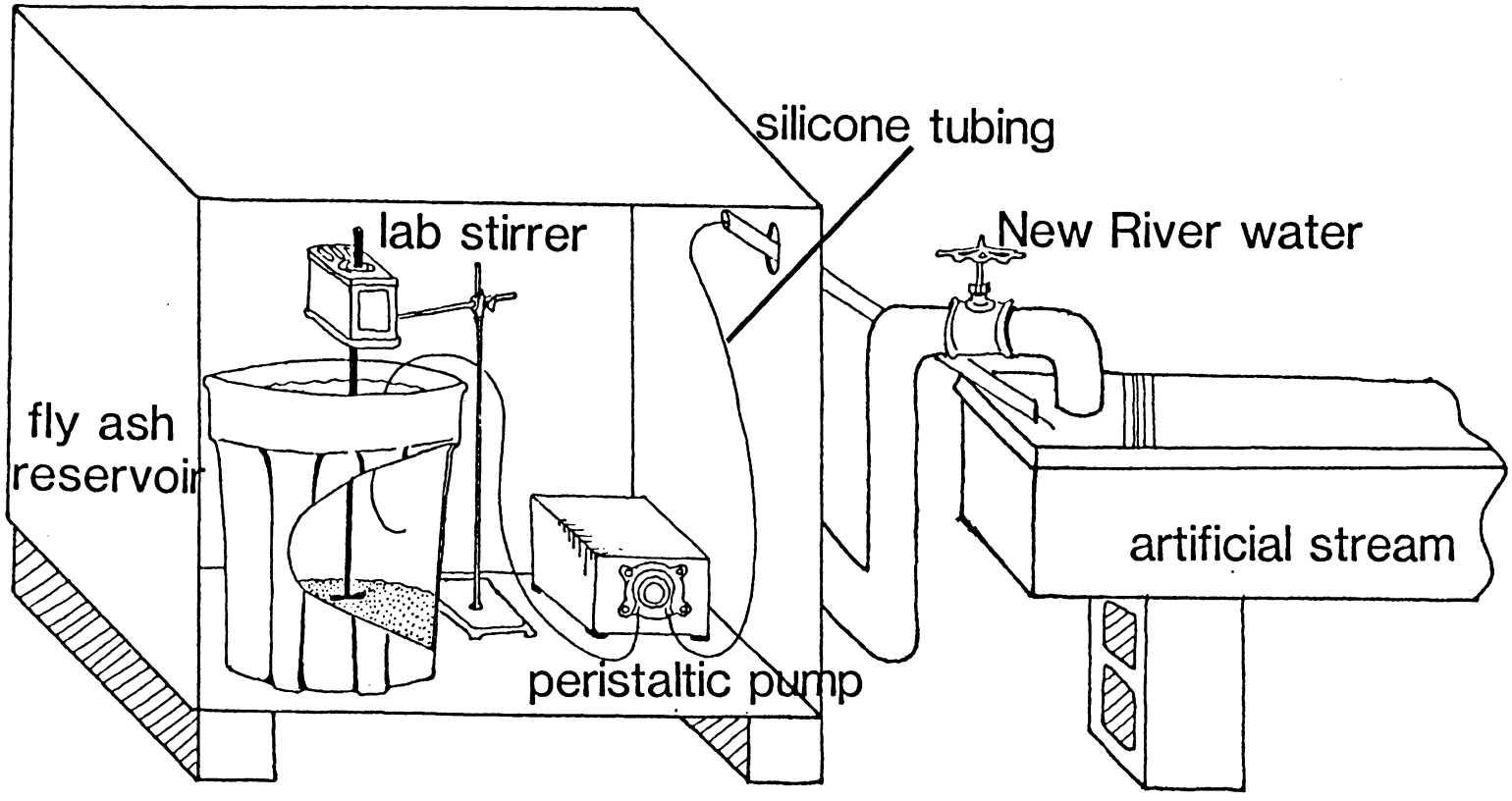
3.2.1 Fly Ash

Hot, dry fly ash was obtained in metal trash cans directly from the bottom of an electrostatic precipitator at the Glen Lyn plant. This ash was mixed in varying amounts with New River water and maintained in suspension in 50-l plastic reservoirs by single speed laboratory stirrers (Eberbach 7005) equipped with 18-inch stirring rods. This suspension was pumped into selected streams through silicone tubing with a variable speed peristaltic pump (Cole-Parmer Masterflex). Insulation and heat tape allowed for continual dosing despite subfreezing ambient temperatures. Dosing rates were calculated from the following equation:

$$\text{Dosing Rate} = \frac{\text{Stream flow rate x desired chemical concentration}}{\text{Stock solution concentration}}$$

Levels of total suspended solids (TSS) in dosed and reference streams were regularly monitored by filtering 150 ml of stream water through pre-tared glass fiber filters. The filters were dried at 55 C for 24 hours and weighed to determine TSS in ppm. Using this apparatus fly ash was successfully introduced into the artificial stream system at levels ranging from 25 to 100 ppm (Figure 5).

Figure 5. Diagram of apparatus used to introduce fly ash into artificial stream system.


silicone tubing
lab stirrer
New River water
fly ash
reservoir
artificial stream
peristaltic pump

Two different strategies were employed to determine the effects of coal ash upon structural and functional parameters of the aufwuchs community. Uncolonized diatometers were placed in streams receiving fly ash and allowed to become colonized for 14 - 21 days in the ash influenced stream prior to removal and analysis. Alternatively, diatometers were allowed to become completely colonized with native aufwuchs organisms in streams receiving only New River water. Fly ash was then introduced into the stream and the aufwuchs community sampled sequentially .

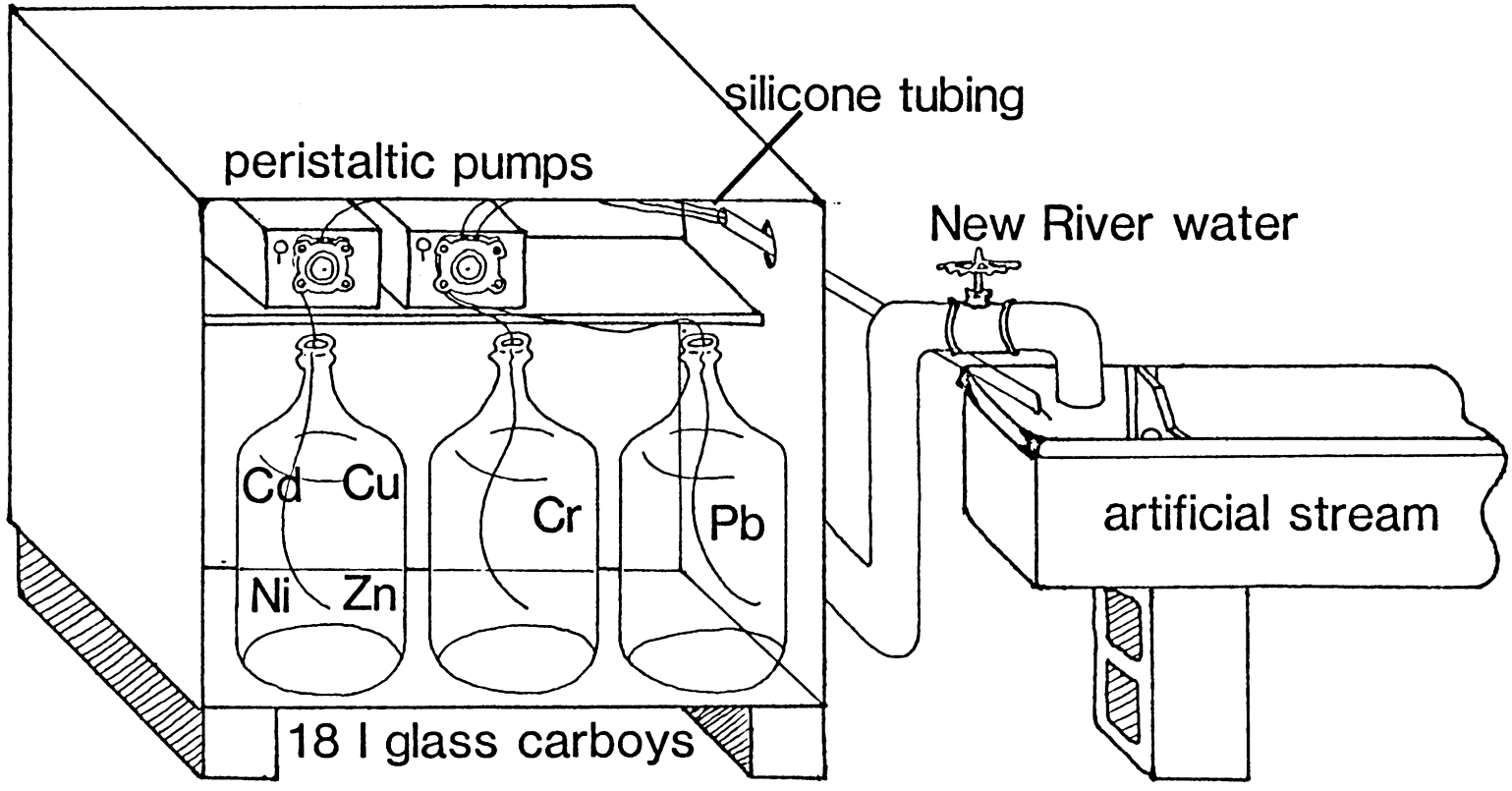
3.2.2 Heavy Metal Dosing

Selected heavy metals (Cr,Cd,Cu,Ni,Pb,and Zn) were added simultaneously to artificial streams at levels modeling the ambient concentrations found in the fly ash basin effluent. To prevent the formation of insoluble metal salts, 18-liter acid-washed glass carboys received a solution of either Pb, as $CH_3(COO)_2Pbx3H_2O$, Cr, as $K_2Cr_2O_7$, or a combination of the remaining four as $CuSO_4x5H_2O$, $ZnSO_4x7H_2O$, $CdCl_2x5H_2O$, and $NiCl_2x6H_2O$. All heavy metal solutions were formulated with distilled deionized water.

Variable speed peristaltic pumps (Cole-Parmer Masterflex) equipped with three cascaded pump heads delivered the metal salt solutions from a protected dosing box to the artificial

streams through silicone tubing (Figure 6). Dosing rates were calculated as in the fly ash experiments.

Figure 6. Schematic of the system used to introduce heavy metals into the artificial stream system.


silicone tubing
peristaltic pumps
New River water
Cd
Cu
Cr
Pb
Ni
Zn
artificial stream
18 l glass carboys

Ambient water samples were monitored regularly for metal concentration by acidifying stream samples to be analyzed with atomic absorption spectrophotometry. In addition, the aufwuchs community was analyzed for metal uptake by digesting samples in a solution of 1 : 2 : 1 HCl to H_2O to Nitric acid. These samples were also measured with atomic absorbtion spectrophometry.

3.2.3 Sulfate Dosing

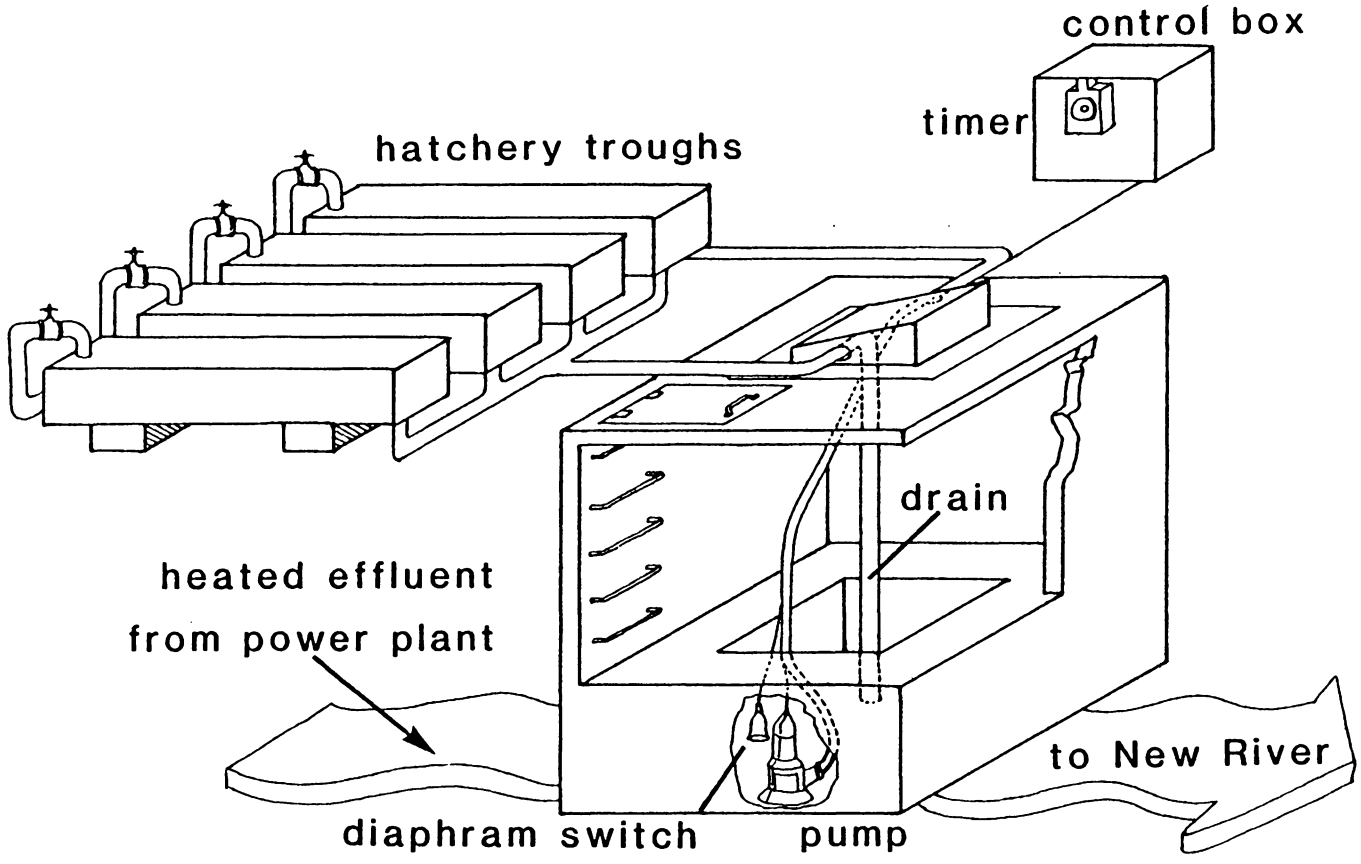
Sulfate as $NaSO_4$ was introduced into the artificial streams by a method identical to the heavy metal dosing scheme. Target in-stream sulfate levels were calculated to model the measured sulfate differential in the Adair Run system (delta SO_4 = 30 ppm) , and a quantity of twice this amount. Ambient, in stream concentrations of sulfate and sodium were monitored during the sulfate dosing experiments.

3.2.4 Thermal Loading

To determine the effect of an elevated temperature upon selected parameters of the aufwuchs community a series of four artificial streams was constructed near the Glen Lyn laboratory. A Gorman-Rupp submersible mine dewatering pump (S2A1) delivered heated effluent from the cooling water tunnel of the Glen Lyn power plant's number 5 unit to four

epoxy painted metal troughs. The design and alignment of these streams was identical to the other artificial streams which served as reference streams for the thermal loading experiments (Figure 7). Since the thermal effluent from the power plant is chlorinated automatically for 20 minutes three times per day to a level of 0.2 mg/l, an automatic timer (AMF Paragon 4004-20) was wired into the system to shut down the pump during the periods of chlorination. Standpipes at the end of each stream prevented the water level from receding during these periods. To prevent accidental damage to the pump when the power plant unexpectedly ceased operation, a diaphragm type pressure valve was included which turned off the pumping system when the heated effluent reached a designated depth.

Figure 7. Schematic of the thermal stream system.


control box
timer
hatchery troughs
drain
heated effluent
from power plant
to New River
diaphram switch
pump

3.3 STATISTICAL ANALYSIS

Analysis of data and computations were accomplished using the Statistical Analysis System (SAS) developed by Barr et al, (1979). Data were converted to a square meter basis by dividing the raw data by the area of the colonized slides (0.00375) (APHA, 1975). Data were tested for normality (Kolmogorov Procedure, Gold, 1979) and non-parametric statistical methods were applied to both normal and non-normal ranked data (Hollander and Wolfe, 1973). A Wilcoxon Sign Rank Test at the 0.05 level of significance revealed differences between Adair Run field stations or between artificial stream treatments (fly ash, heavy metals, SO_4, thermal enrichment), and reference streams. In addition data from heavy metal treatments was subjected to a one way analysis of variance (ANOVA) (Sokal and Rohlf, 1969). Differences between treatments were tested at the 5% level with Duncan's New Multiple Range Test.

Chapter IV

RESULTS

4.1 FIELD STUDIES

Two field stations, above and below the point of fly ash basin discharge into Adair Run, were evaluated for specific structural and functional parameters of aufwuchs communities from October 1979 to November 1980. During this period the fly ash basin reached maximum operating capacity, as determined by basin morphology and Total Suspended Solids discharge, and was operationally terminated by the power utility (American Electric Power Co.). Basin shutdown occurred in two phases, with the basin receiving one half of the original input beginning in mid July 1980, and no inputs after 6 August 1980. Sampling of aufwuchs colonizing artificial substrates occurred when the basin was in early operation, during the period when maximum capacity was reached, and subsequent to basin termination.

Fly ash introduced into Adair Run appeared to have no adverse environmental effects upon the aufwuchs community as determined by the structural and functional parameters measured. In general dry weights, ash free dry weights, chlorophyll a, and light carbon fixation rates were similar in both the up and downstream stations as the fly ash basin be-

gan to fill. The greatest differential between sampling stations was observed as the basin reached maximum capacity and was terminated, with higher values generally exhibited by the downstream, ash influenced station. Subsequent to basin termination in early August 1980, and following a period of recovery, parameter values of the downstream station dropped to approximate those of the upstream reference station. Specific data examples are given in the sections that follow.

4.1.1 Structural and Functional Parameters of Aufwuchs

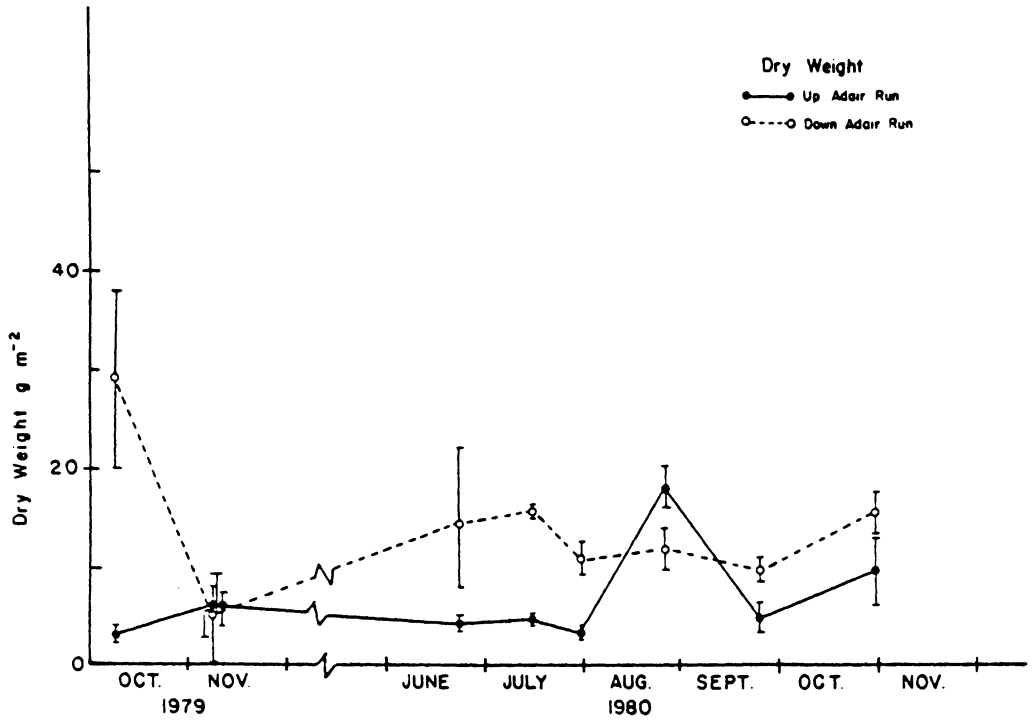
4.1.1.1 Dry Weights

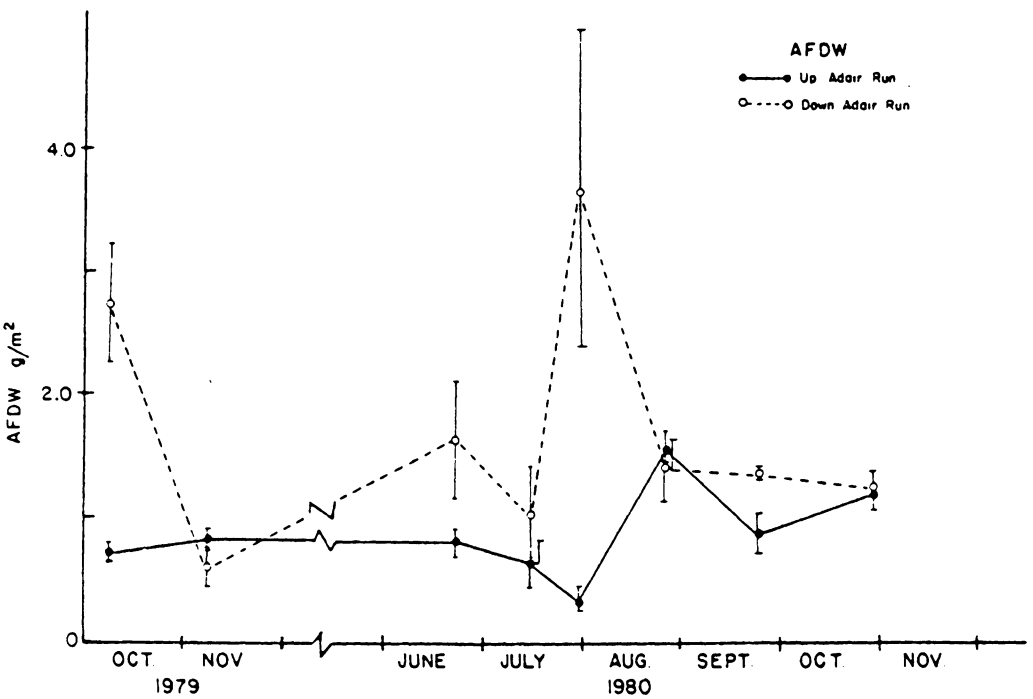
Averages of measured dry weights (n=4) ranged from 3.15 to 18.5 g m -2 at the upstream reference site, and from 6.35 to 29.2 g m -2 at the downstream effluent influenced station (Figure 8). Higher variability of dry weights was observed at the downstream station. Maximum differences between dry weight values for the two Adair Run stations occurred during June and July of 1980 (4.29 vs. 14.06 and 4.7 vs. 16.8 g m -2 respectively) (Figure 8). In October 1979, and June and July 1980, dry weights were significantly greater at the downstream station as analyzed with the Wilcoxon Sign Rank Test (Table 6).

4.1.1.2 Ash Free Dry Weights

Ash free dry weights exhibited a similar, although not identical, pattern. Values at the upstream site ranged from 0.72 g m -2 in October 1979, to 1.76 g m -2 in August 1980. At the downstream site, ranges were from 0.60 g m -2 in November 1979, to 3.7 g m -2 in July 1980. Again more variability was observed at the downstream station. The downstream station had significantly higher values in October 1979, and during June and July 1980. Following basin termination downstream values dropped to approximate those of the upstream reference station. The maximum observed differential between stations occurred on 31 July 1980, when upstream and downstream values were 0.26 and 3.7 g m -2 respectively (Figure 8).

Figure 8. Values for dry weights and ash free dry weights of aufwuchs in Adair Run from October 1979 to November 1980. Bars are one standard error of the mean.


Dry Weight
Up Adair Run
Down Adair Run
Dry Weight g m⁻²
40
20
0
OCT.
NOV.
1979
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1980


AFDW
Up Adair Run
Down Adair Run
AFDW g/m²
4.0
2.0
0
OCT.
NOV
1979
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1980

4.1.1.3 Chlorophyll a

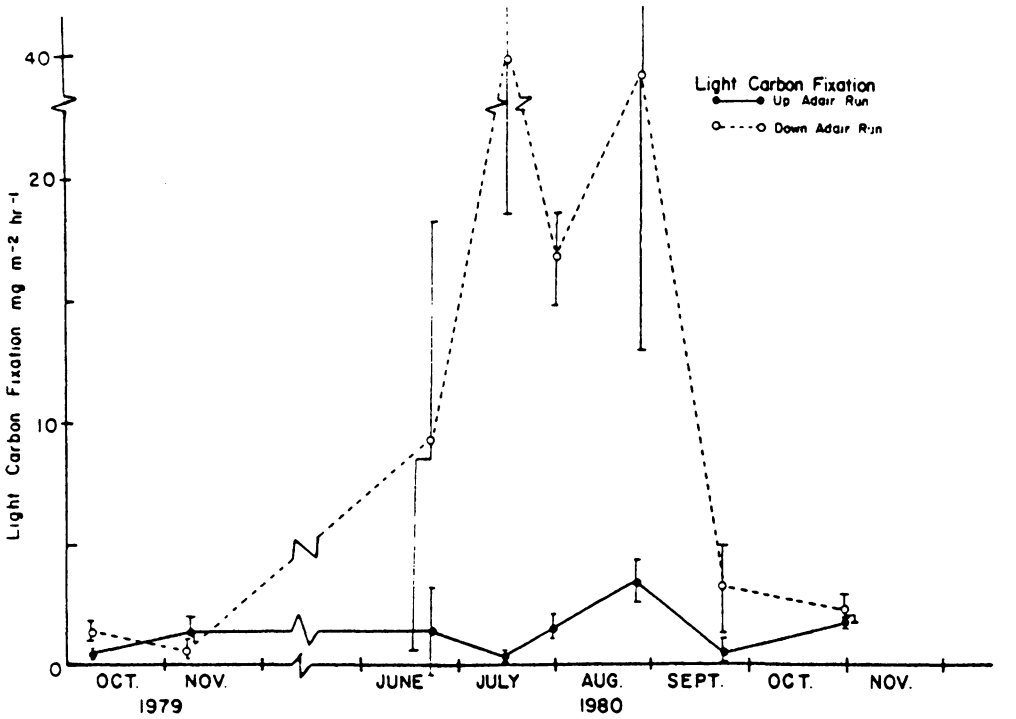
Chlorophyll a values ranged from 3.56 mg m -2 in October 1979, to 287 mg m -2 in June 1980 (Figure 9). Downstream values ranged from 34.7 mg m -2 in October 1979 to 282 mg m -2 in June 1980. Significant differences were not observed between stream stations until July 1980, but continued until November 1980. In general, except for measurements in November 1979, a higher variability was observed at the downstream site. Maximum separation of chlorophyll a values occurred in July 1980 when upstream and downstream sites measured 53.2 and 294 mg m -2, respectively. Following basin shutdown in August 1980, downstream values more closely approached those of the upstream reference station.

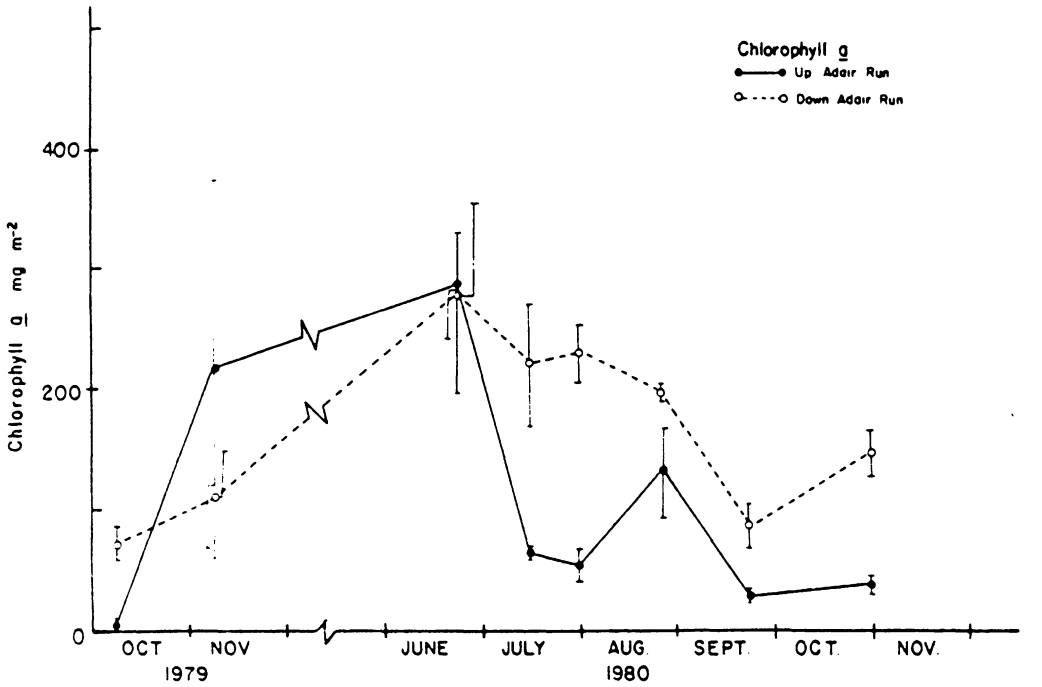
4.1.1.4 Light Carbon Fixation

In October and November of 1979 during early fly ash basin operation, values observed at both stations were between 1.0 and 2.0 mg C m -2 hr -1 (Figure 9). During maximum operating capacity (July - August 1980) the upstream values remained at 2.0, but the downstream station reached values between 18.0 and 40.0 mg C m -2 hr -1. In September and October of 1980, after basin shutdown, levels measured in the downstream station dropped to closely approximate those of the upstream community (1.81 vs 2.15 mg m -2 hr -1). Values

were significantly different from 14 July to 23 September 1980. Maximum differnces between stations occurred on these dates, with greater variability observed in downstream communities.

Figure 9. Values for light carbon fixation rates and chlorophyll a of aufwuchs in Adair Run from October 1979 to November 1980. Bars are one standard error of the mean.

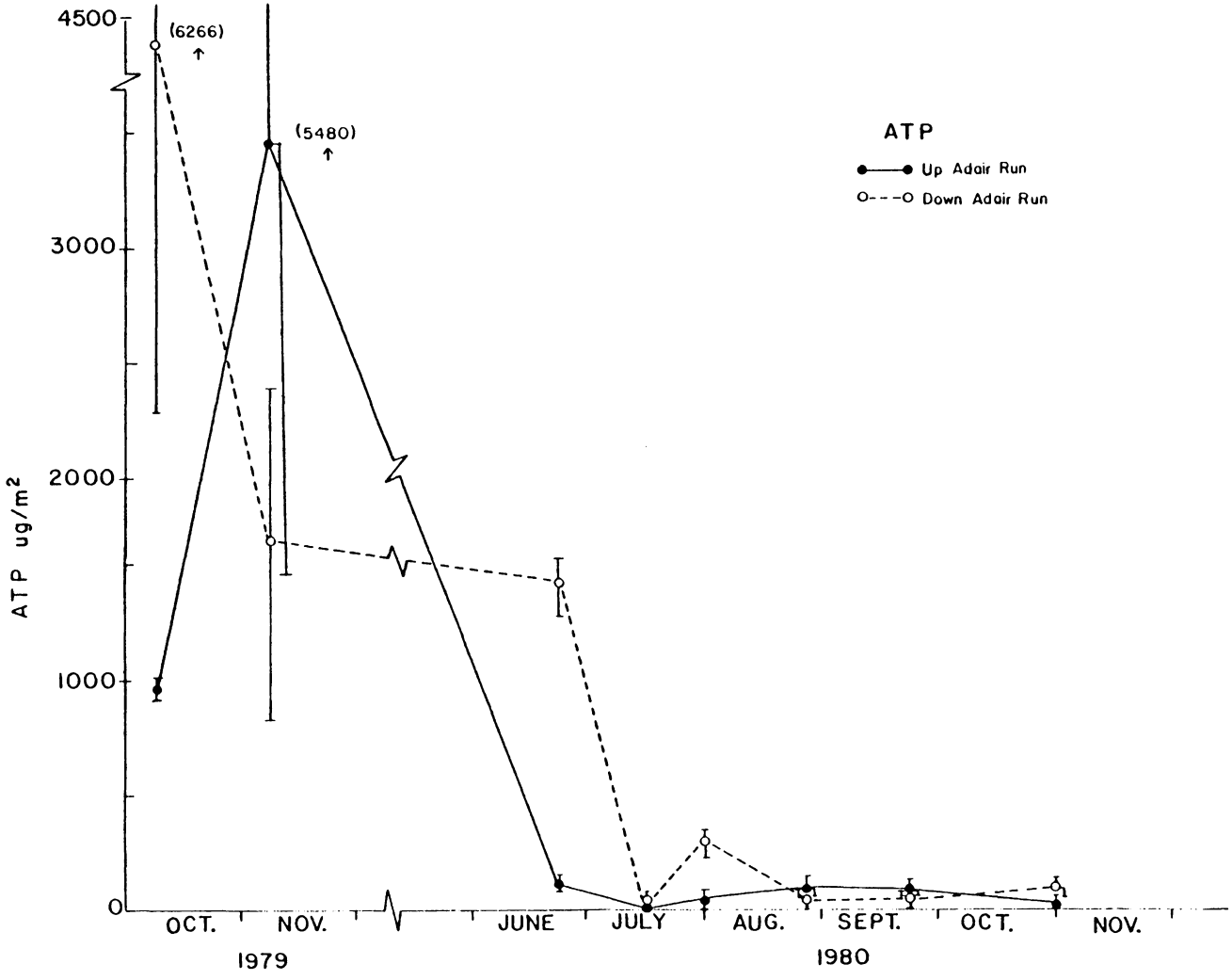

Light Carbon Fixation
Up Adair Run
Down Adair Run
Light Carbon Fixation mg m-2 hr-1
40
20
10
0
OCT.
NOV.
1979
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1980


Chlorophyll g
Up Adair Run
Down Adair Run
Chlorophyll g mg m-2
400
200
0
OCT
NOV
1979
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1980

4.1.1.5 ATP

Measured ATP values at both the upstream and downstream station were highly variable, and failed to exhibit the trends seen in dry weight, AFDW and chlorophyll a. Values were highest at the upstream station in November 1979 (3480 mg m -2) and lowest in July and October 1980 (6.27 and 9.67 mg m -2) (Figure 10). Downstream values ranged from 4266 mg m -2 In October 1979, to 37.7 mg m -2 in July 1980. Significant differences between stations were observed on only two sampling dates; 20 June and 31 July 1980. Following basin shutdown in August 1980, measured ATP values were similar at both Adair Run stations.

Figure 10. Values for ATP of aufwuchs in Adair Run from October 1979 to November 1980. Bars are one standard error of the mean.


4500
3000
2000
1000
0
ATP ug/m²
(6266)
(5480)
ATP
Up Adair Run
Down Adair Run
OCT.
NOV.
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1979
1980

4.1.1.6 Dark Carbon Fixation

In general dark carbon fixation rates were low, ranging from 0.014 to 0.78 mg C m -2 hr -1 at the upstream station, and from 0.03 to 0.68 mg C m -2 hr -1 at the downstream site (Table 7). Dark carbon fixation rate values on any given sampling date were not significantly different and no influence of basin fill-up or shutdown was observable via this parameter.

4.1.1.7 Light and Dark Sulfate Assimilation

Measured values for sulfate assimilation by aufwuchs were extremely low in the Adair Run system. Light assimilation ranged from 0.02 to 50 ug S m -2 hr -1 in Adair Run (Table 7). Dark values ranged from 0.02 to 57 ug S m -2 hr -1. Both parameters decreased in value from October 1979 to 14 July 1980, at which point this line of investigation was terminated.

Table 6. Results of Wilcoxon Sign Rank Test for Adair Run data at the 0.05 level of significance. D+ indicates that downstream values are significantly higher than upstream values. U+ indicates upstream values are significantly higher. NS indicates no significant differences between stations. Blanks are due to insufficient data.

Parameter	Date							
	10-04-79	11-04-79	6-20-80	7-14-80	7-31-80	8-28-80	9-23-80	10-31-80
Dry Weight	-	NS	-	D+	-	NS	D+	NS
Ash Free Dry Weight	-	NS	-	NS	-	NS	D+	NS
Chlorophyll a	-	NS	NS	D+	D+	D+	D+	D+
ATP	-	NS	D+	NS	D+	NS	NS	NS
Light Carbon Fixation	-	-	D+	D+	D+	D+	NS	NS
Dark Carbon Fixation	-	-	NS	NS	NS	NS	NS	NS
Light Sulfur Assimilation	-	NS	NS	D+	-	-	-	-
Dark Sulfur Assimilation		U+	NS	D+	-	-	-	-

Table 7. Additional structure and functional parameters of periphyton communities in Adair Run and water chemistry parameters.

	Sampling Date and Station															
	10-04-79		11-04-79		6-20-80		7-14-80		7-31-80		8-28-80		9/23-80		10-31-81	
Measured	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
Dark Carbon Fixation $mg/m^2/hr$	0.018	0.03	0.014	0.036	0.08	0.10	0.079	0.058	0.349	0.215	0.78	0.68	0.067	0.071	0.105	0.105
Light Sulfur Assimilation $\mu g/m^2/hr$	0.5	13.0	4.4	9.0	5.0	8.8	0.002	0.1	-	-	-	-	-	-	-	-
Dark Sulfur Assimilation $\mu g/m^2/hr$	0.3	2.0	0.9	9.0	3.0	4.4	0.02	0.03	-	-	-	-	-	-	-	-
TSS ppm	17	36	21	40	20	94	12.5	71.5	11.3	51.7	10.9	39.7	15.0	27.2	18.0	22.0
SO_4 ppm	18.3	69.6	15.8	41.2	30.7	56.5	19.0	32.0	18.0	30.3	60	90	25.5	32.0	-	-
Temperature ^oC	14.0	18.5	6.5	8.0	16.0	18.5	20.0	22.0	19.5	21.0	18.0	18.5	20.0	20.0	6	6
pH	7.3	7.2	6.6	8.1	8.1	8.5	7.8	9.2	7.6	8.0	7.2	7.1	7.3	7.7	7.4	7.4

4.1.2 Physical-Chemical Parameters

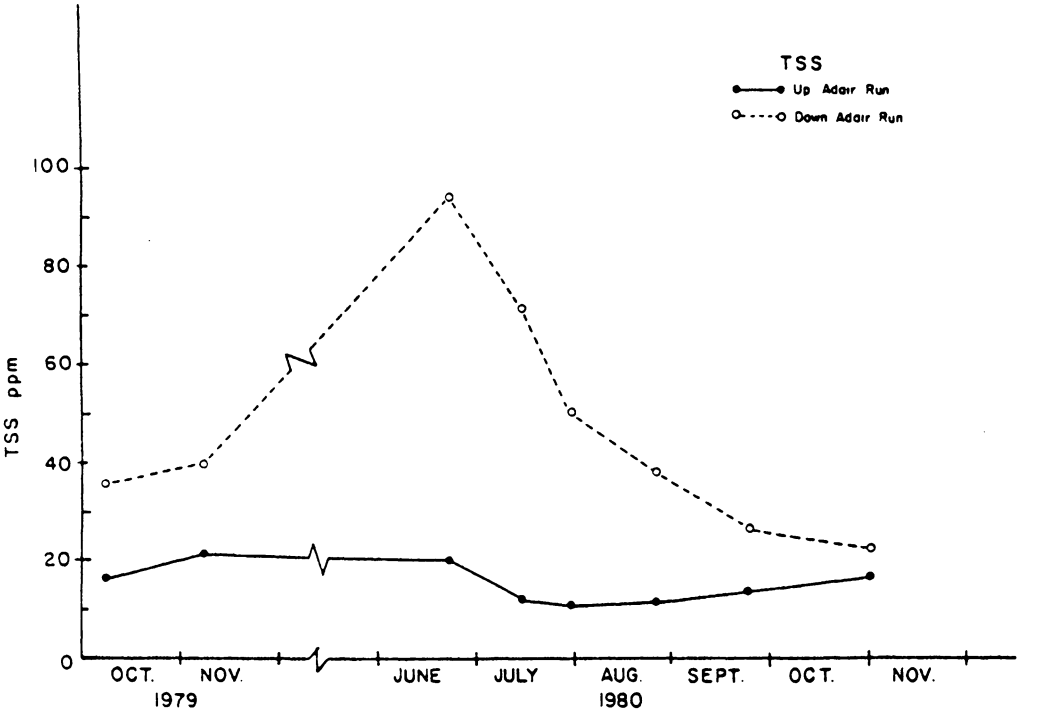
4.1.2.1 TSS

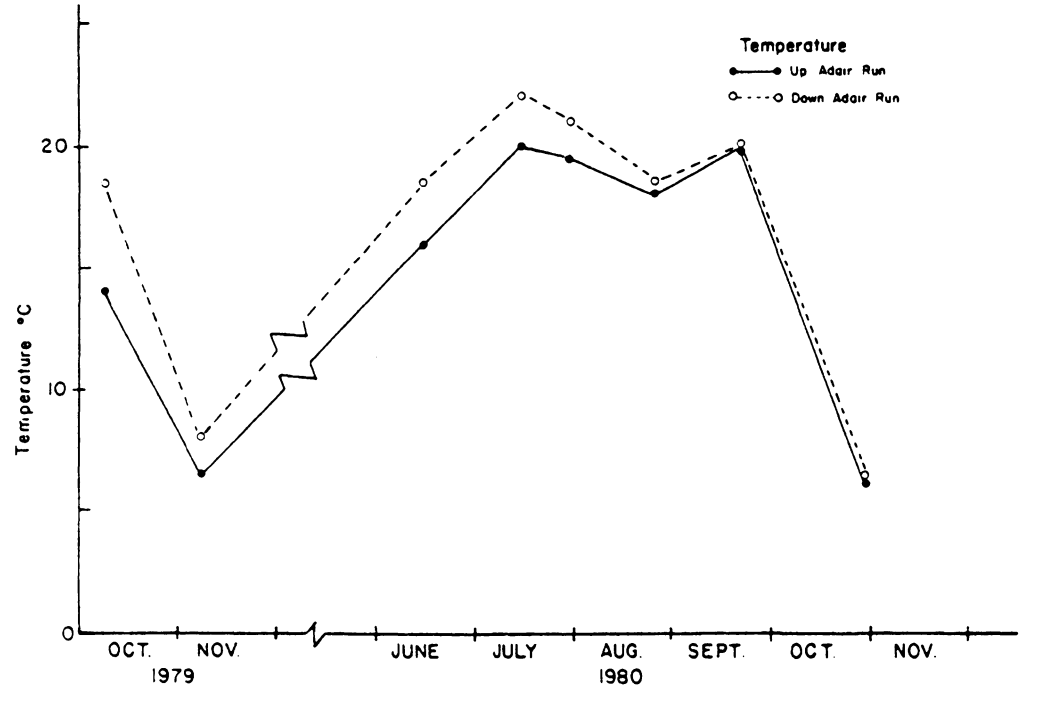
Measured TSS values at the upstream station were consistent throughout the study period, ranging from 10.9 to 21.0 ppm (Figure 11). Downstream values were always higher, ranging from 22.0 to 94.0 ppm. The maximum downstream value of 94.0 ppm occurred just prior to initial basin shutdown. TSS values dropped to approach those of the upstream station during the months of September and November 1980.

4.1.2.2 Temperature

Temperatures at the downstream station were consistently about 4 C greater than those of the upstream station (Figure 11). Following basin shutdown in August measured values at both stations were similar.

Figure 11. Values for total suspended solids and temperature in Adair Run from October 1979 to November 1980.


TSS
Up Adair Run
Down Adair Run
TSS ppm
100
80
60
40
20
0
OCT.
NOV.
1979
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1980


Temperature
Up Adair Run
Down Adair Run
Temperature °C
20
10
0
OCT.
NOV.
1979
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1980

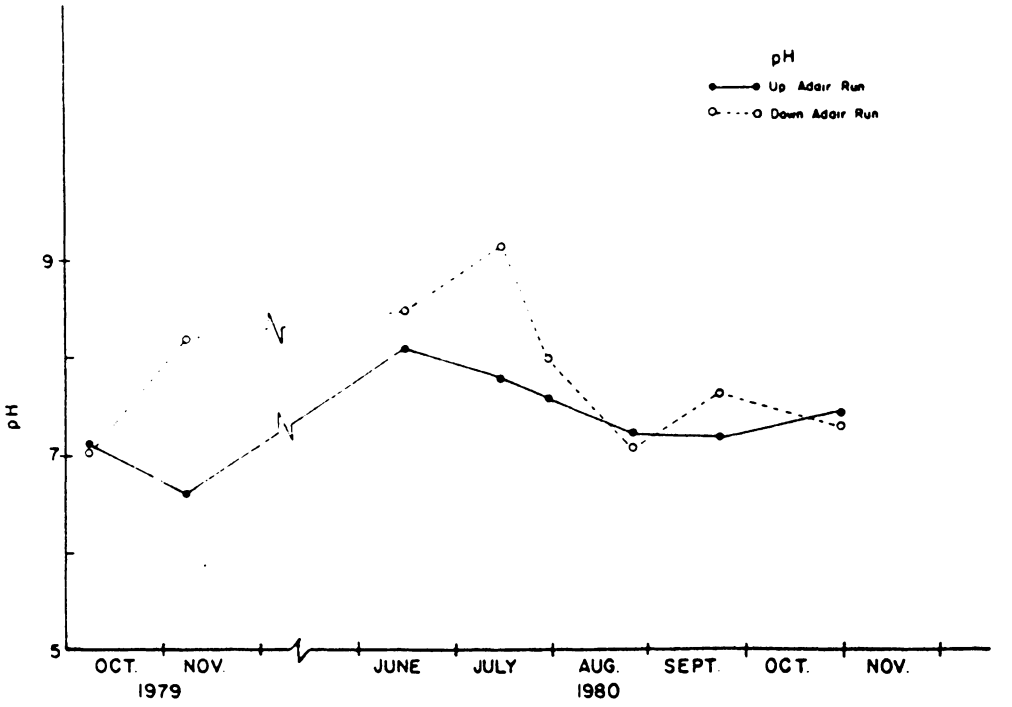
4.1.2.3 pH

pH ranges at the upstream site were from 6.6 to 8.1 (Figure 12). Downstream values climbed to 9.2 in July 1980, then dropped to approximate upstream values (7.1 - 8.0). Maximum differences between the two stations occurred on 14 July when downstream values were 1.2 units greater (9.2 vs 7.8) than the upstream reference station.

4.1.2.4 Nutrients

Of the nutrients measured (nitrate, phosphate, and sulfate), only sulfate exhibited any trends that could be attributed to an influence from the fly ash basin (Figure 12). Nitrates ranged from 0.2 to 0.7 mg l -1 at both stations, phosphates from 0.05 to 0.7 mg l -1 (Figure 13). Sulfates exhibited a maximum separation of 40 mg l -1 between stations during the period of maximum basin capacity. Values of 17.0 to 32.0 mg l -1 at the upstream station were approached by downstream values following basin termination.

Figure 12. Values for pH and sulfates in Adair Run from October 1979 to November 1980.


pH
Up Adair Run
Down Adair Run
9
7
5
pH
OCT.
NOV.
1979
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1980

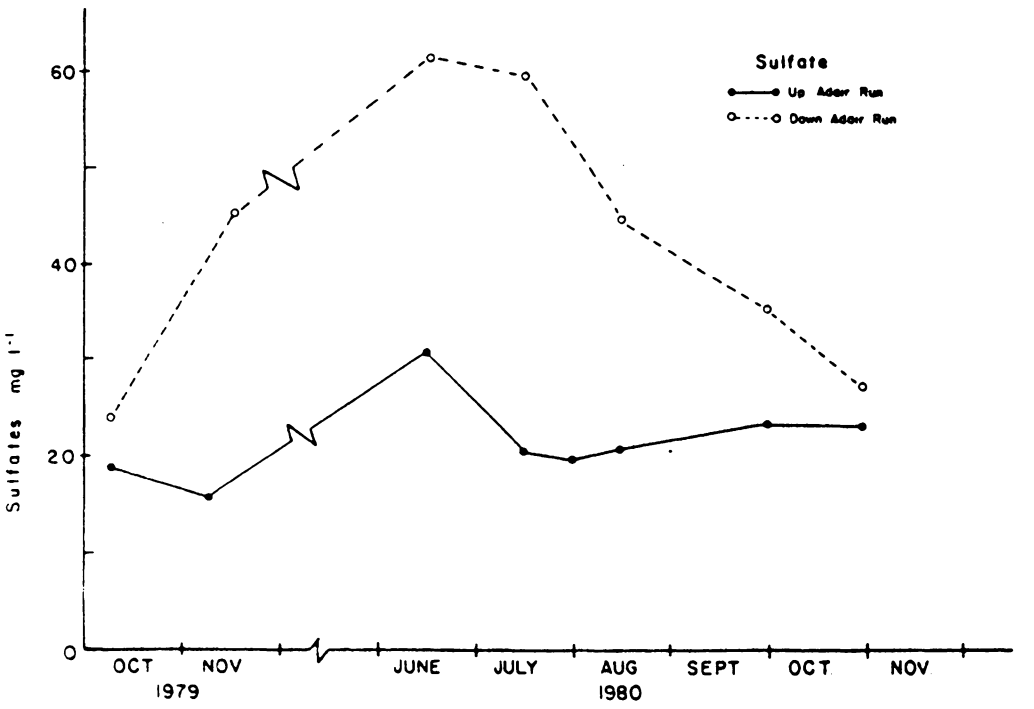
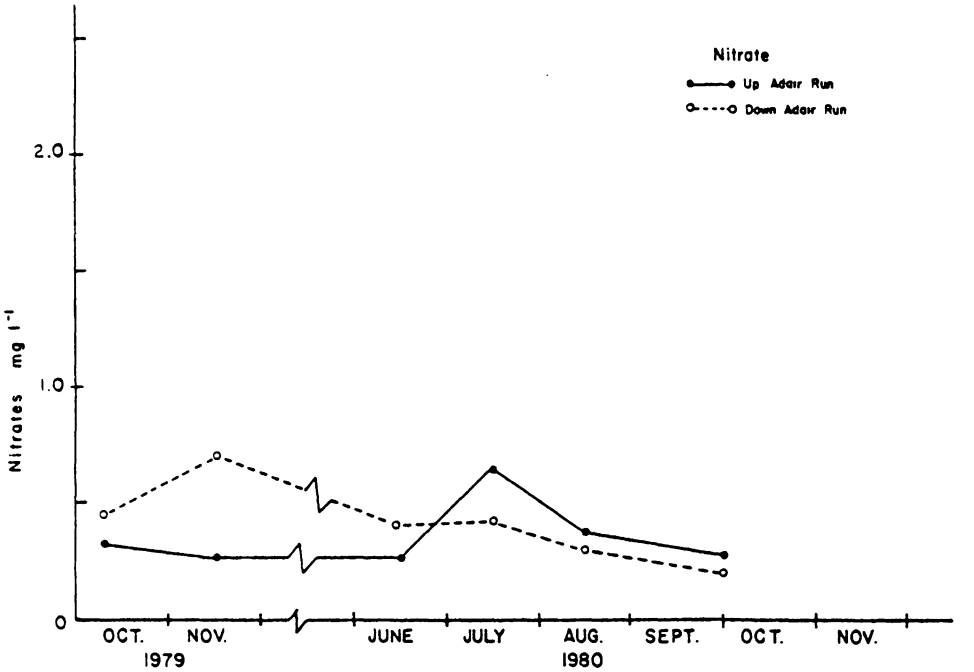
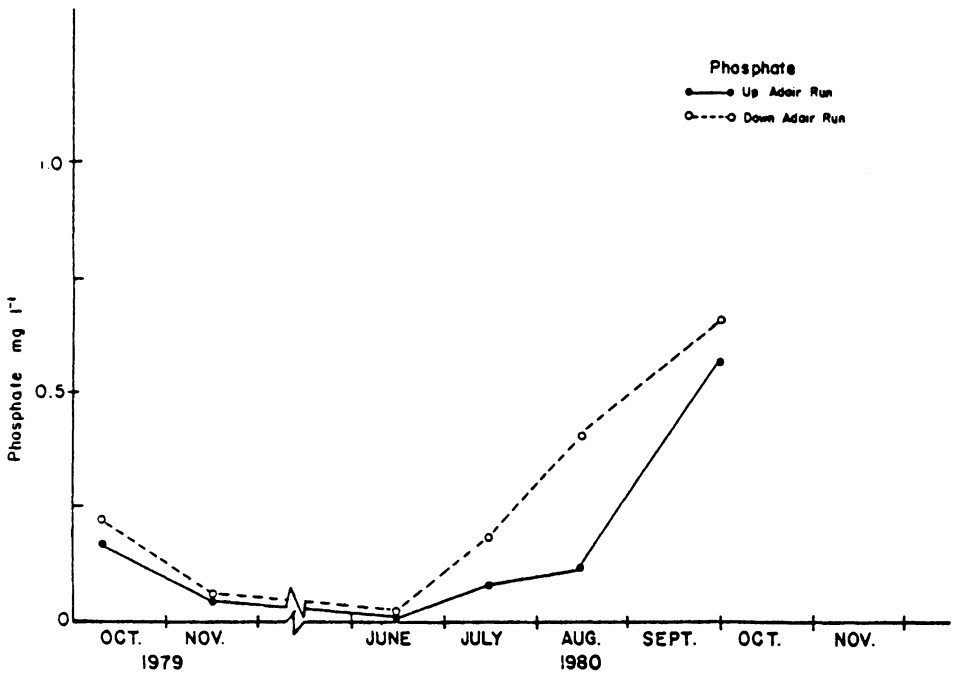

Sulfate
Up Adair Run
Down Adair Run
60
40
20
0
Sulfates mg l⁻¹
OCT
NOV
1979
JUNE
JULY
AUG
SEPT
OCT
NOV
1980

Figure 13. Values for nitrates and phosphates in Adair Run from October 1979 to November 1980.


Nitrate
Up Adair Run
Down Adair Run
Nitrates mg l⁻¹
2.0
1.0
0
OCT.
NOV.
1979
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1980


Phosphate
Up Adair Run
Down Adair Run
Phosphate mg l⁻¹
1.0
0.5
0
OCT.
NOV.
1979
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.
1980

4.2 ARTIFICIAL STREAM STUDIES

4.2.1 Ash Dosing with Uncolonized Diatometers

During the first phase of investigations designed to assess the impact of fly ash upon structural and functional parameters of aufwuchs communities, ash was introduced into artificial streams containing uncolonized diatometers at various TSS levels. Following a colonization period of 14 to 21 days the communities were assessed. The results were be examined in terms of high (80-100 ppm), medium (25-40 ppm), and low (8.0-10) TSS values.

4.2.1.1 High TSS (80-100 ppm)

Three experiments were conducted with diatometers exposed to high suspended ash concentrations on 20 November 1979, 31 July 1980, and 1 October 1980 (Table 8).

Dry weights and AFDW were not significantly greater in the ash dosed stream, but were once in the reference stream (Table 9). Chlorophyll a was significantly higher in the reference stream on two sampling dates, and higher in the ash stream one time. ATP values were not significantly different between streams on two occasions, and greater in the reference stream on one date. Light carbon fixation rates were significantly greater in the reference streams on two occasions, not significantly different once. Dark car-

bon fixation rate, and light and dark sulfur assimilation rates were significantly greater one time in the reference stream, and not significantly different on other sampling dates.

4.2.1.2 Medium TSS (25-40 ppm)

During the two sampling dates when medium quantities of fly ash were introduced into the artificial streams (7 May 1980, 14 July 1980), only light carbon fixation rates were consistently significantly greater in the ash dosed stream. Dry weights, AFDW, and dark carbon fixation rates were not significantly different at any time, and values for chlorophyll a and ATP were higher on one date, and lower on the next.

4.2.1.3 Low TSS (8.0-10.0 ppm)

Two experiments were conducted on 20 June 1980 and 1 October 1980 to determine the effects of fly ash at low suspended concentrations. In June 1980 no measured parameters of the ash stream had a significant departure from reference stream values. In October 1980 dry weights and ATP values were significantly higher in the ash dosed stream; chlorophyll a and light and dark carbon fixation rates were higher in the reference stream.

Table 8. Structural and functional parameters of periphyton communities in artificial streams exposed to fly ash.

Parameter Measured	Sampling Date and Station							
	11-20-79		5-07-80		6-20-80		7-14-80	
	Ash Stream	Reference Stream	Ash Stream	Reference Stream	Ash Stream	Reference Stream	Ash Stream	Reference Stream
Dry Weight g/m^2	15.6	19.4	41.8	9.8	11.4	4.40	23.1	22.9
AFDW g/m^2	2.12	1.48	3.44	1.09	1.09	0.96	3.29	3.70
Chlorophyll a mg/m^2	27.3	248	432	946	152	233	593	293
ATP ug/m^2	1160	1733	9.80	37.3	198	1303	17.2	14.4
Light Carbon Fixation $mg/m^2/hr$	0.921	1.485	20.0	6.25	2.97	1.70	27.4	12.2
Dark Carbon Fixation $mg/m^2/hr$	0.034	0.077	0.053	0.099	0.06	0.024	0.33	0.22
Light Sulfur Assimilation $\mu g/m^2/hr$	0.7	3.5			0.5	0.4	0.1	0.09
Dark Sulfur Assimilation $\mu g/m^2/hr$	1.0	2,6			0.4	0.4	0.03	0.03
TSS ppm	80-100		40		10		25	
SO_4 ppm	9.90		7.55		12.0		10.7	
Temperature ^oC	10.0	10.0	16.0	16.0	21.0	21.0	24.0	25.0
pH	7.6	7.6	7.5	7.7	7.8	7.7	7.6	7.6

Table 8. Structural and Functional Parameters of Periphyton Communities in Artificial Streams exposed to fly ash. (continued)

Parameter Measured	Sampling Date and Station							
	7-31-80		10-01-80			10-31-80		
	Ash Stream	Reference Stream	Ash[1] Stream	Reference Stream	Ash[2] Stream	Ash[1] Stream	Reference Stream	Ash[2] Stream
Dry Weight g/m^2	21.8	27.3	3.83	5.22	6.98	19.9	5.1	18.5
AFDW g/m^2	1.34	4.38	0.66	2.11	0.98	1.94	1.66	1.95
Chlorophyll a mg/m^2	236	121	124	443	57.2	67.1	272	139
ATP ug/m^2	169	158	67.2	641	358	661	513	941
Light Carbon Fixation $mg/m^2/hr$	16.1	8.16	5.67	15.3	6.83	1.85	4.92	2.82
Dark Carbon Fixation $mg/m^2/hr$	0.23		0.09	0.11	0.054	0.005	0.06	0.03
Light Sulfur Assimilation $mg/m^2/hr$								
Dark Sulfur Assimilation $mg/m^2/hr$								
TSS ppm	80		8.0		80	36		21
SO_4 ppm	12.2		21.2		21.2	24.0	24.0	24.0
Temperature ^oC	26.0	26.0	19.5	19.5	19.5	12.0	12.0	12.0
pH	7.4	7.5	7.3	7.2	7.2	7.5	7.5	7.5

Table 9. Results of Wilcoxon Sign Rank Test for Artificial Stream Fly Ash Dosing Experiment data at the 0.05 level of significance. A+ indicates that ash dosed stream values are significantly higher than reference stream values. R+ indicates higher reference stream values. NS indicates no significant differences between treatments. Blanks are due to insufficient data.

Parameter	Date						
	11-20-79	5-07-80	6-20-80	7-14-80	7-31-80	(Ash 1) 10-01-80	(Ash 2) 10-01-80
Dry Weight	NS	_	_	NS	_	A+	R+
Ash Free Dry Weight	NS	_	_	NS	_	NS	R+
Chlorophyll a	R+	R+	NS	A+	A+	R+	R+
ATP	NS	R+	_	NS	NS	A+	R+
Light Carbon Fixation	NS	A+	NS	A+	A+	R+	R+
Dark Carbon Fixation	NS	NS	NS	NS	_	R+	R+
Light Sulfur Assimilation	R+	_	NS	NS	_	_	_
Dark Sulfur Assimilation	R+	_	NS	NS	_	_	_

4.2.2 Ash Dosing with Colonized Diatometers

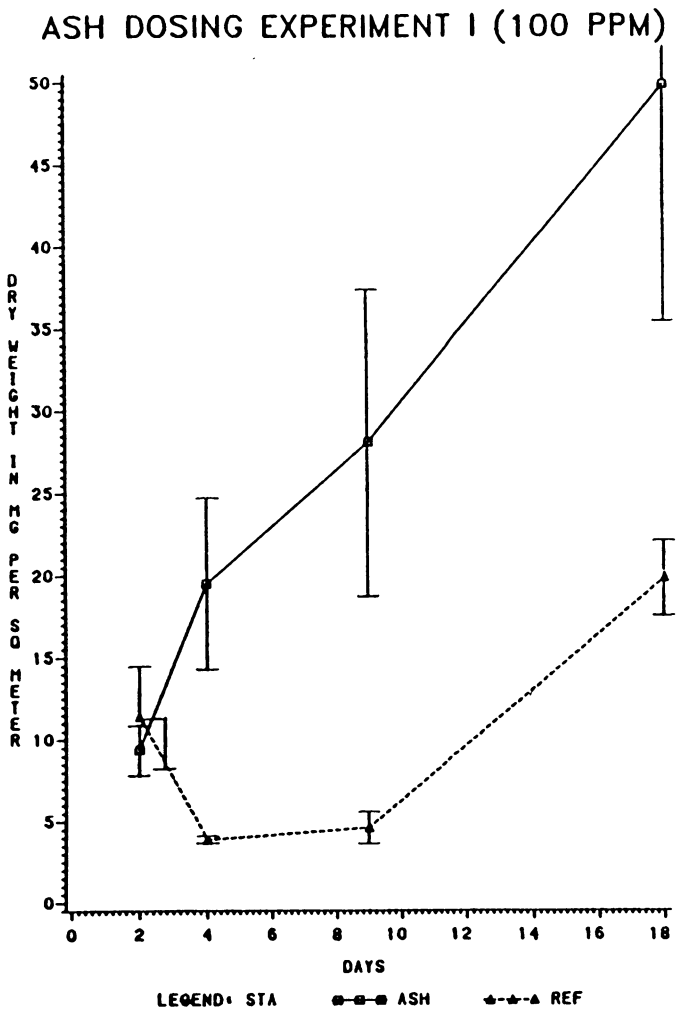
Artificial streams containing mature, colonized diatometers were dosed with fly ash for 18, 32, and 24 days at concentrations of approximately 100, 65, and 35 TSS (ppm). Since the introduced ash tended to settle out near the dosing apparatus outlet, and a gradual build-up of ash sometimes smothered the diatometers closest to the dosing apparatus, it proved difficult to accurately and consistently regulate the quantity of ash (as TSS) dosed into the streams. Values presented reflect fluctuations in dosing above and below the target concentrations by as much as 10 ppm TSS.

4.2.2.1 Ash Dosing Experiment I (100 ppm)

During the experiment with a high dosing concentration (100 ppm TSS) dry weights were initially (day 2) not significantly different in the dosed stream compared to reference values. By day 4, and until day 18, dry weights increased in both streams, and the ash dosed values remained significantly greater (Figure 14). Ash free dry weights were significantly greater in the reference stream, but no significance was demonstrated on subsequent days, although ash free dry weights continued to increase in both streams (Figure 14). Chlorophyll a values in the reference stream were sig-

nificantly greater on both day 2 and day 18, and not significant on days 4 and 9 (Figure 15). ATP values decreased in both streams during the 18 day experiment. Significant differences were observed on day 4, when the reference stream was significantly higher, and on day 18, when the ash stream had significantly more ATP per sample (Figure 15). Light carbon fixation rates fluctuated erratically during the experiment. From low values on day 2, both the dosed and reference stream exhibited increases, with a corresponding increase in variability. Dosed stream values then decreased until day 18, when reference values were significantly greater (Figure 16).

Figure 14. Values for dry weights and ash free dry weights for Ash Dosing Experiment I . Bars are one standard error of the mean.


ASH DOSING EXPERIMENT I (100 PPM)
DRY WEIGHT IN MG PER SQ METER
0
5
10
15
20
25
30
35
40
45
50
0
2
4
6
8
10
12
14
16
18
DAYS
LEGEND: STA
ASH
REF

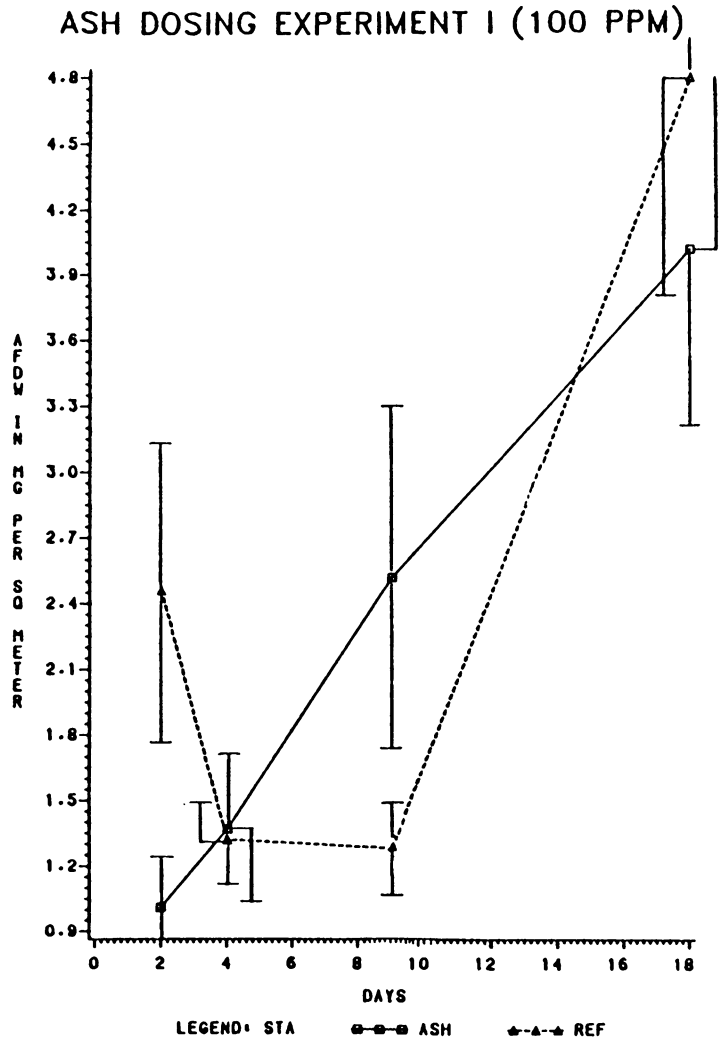
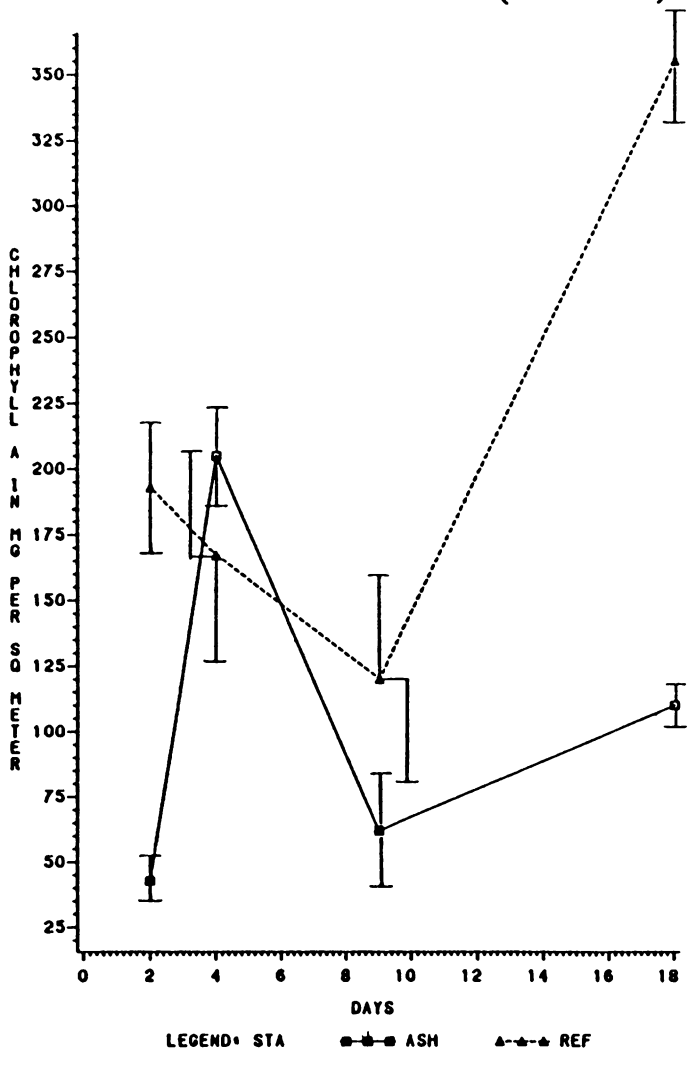

ASH DOSING EXPERIMENT I (100 PPM)
AFDW IN MG PER SQ METER
0.9
1.2
1.5
1.8
2.1
2.4
2.7
3.0
3.3
3.6
3.9
4.2
4.5
4.8
0
2
4
6
8
10
12
14
16
18
DAYS
LEGEND: STA
ASH
REF

Figure 15. Values for chlorophyll a and ATP from Artificial Stream Ash Dosing Experiment I. 19 November to 5 December 1980. Bars are one standard error of the mean.


ASH DOSING EXPERIMENT I (100 PPM)
CHLOROPHYLL A IN MG PER SQ METER
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
2
4
6
8
10
12
14
16
18
DAYS
LEGEND: STA
ASH
REF

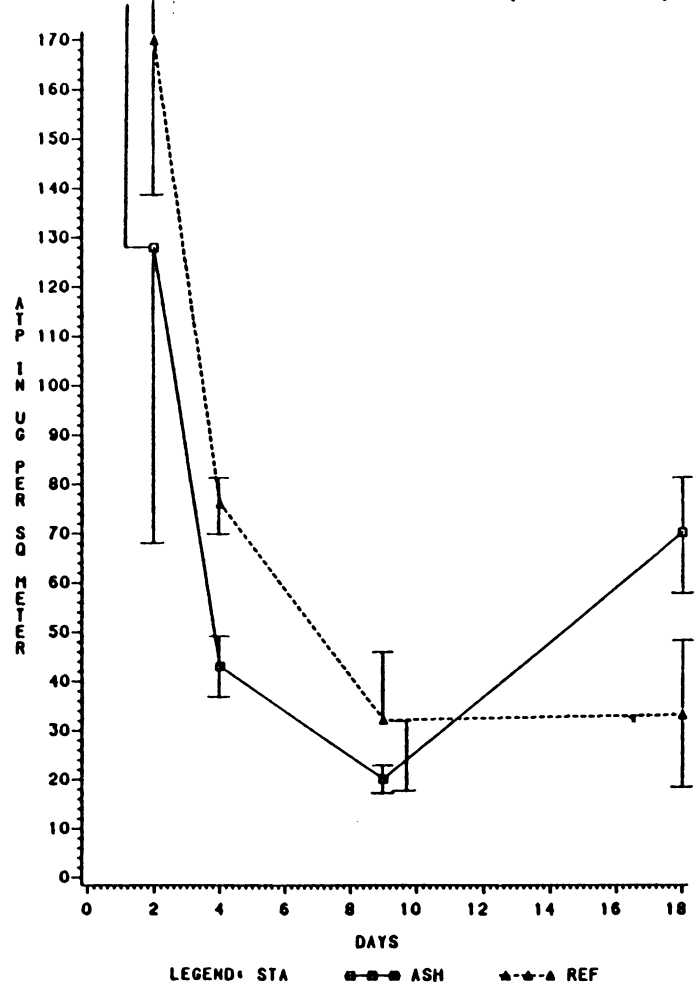

ASH DOSING EXPERIMENT I (100 PPM)
ATP IN UG PER SQ METER
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
16
18
DAYS
LEGEND: STA
ASH
REF

Figure 16. Values for light carbon fixation rates from Artificial Stream Ash Dosing Experiment I. 19 November to 5 December 1980. Bars are one standard error of the mean.

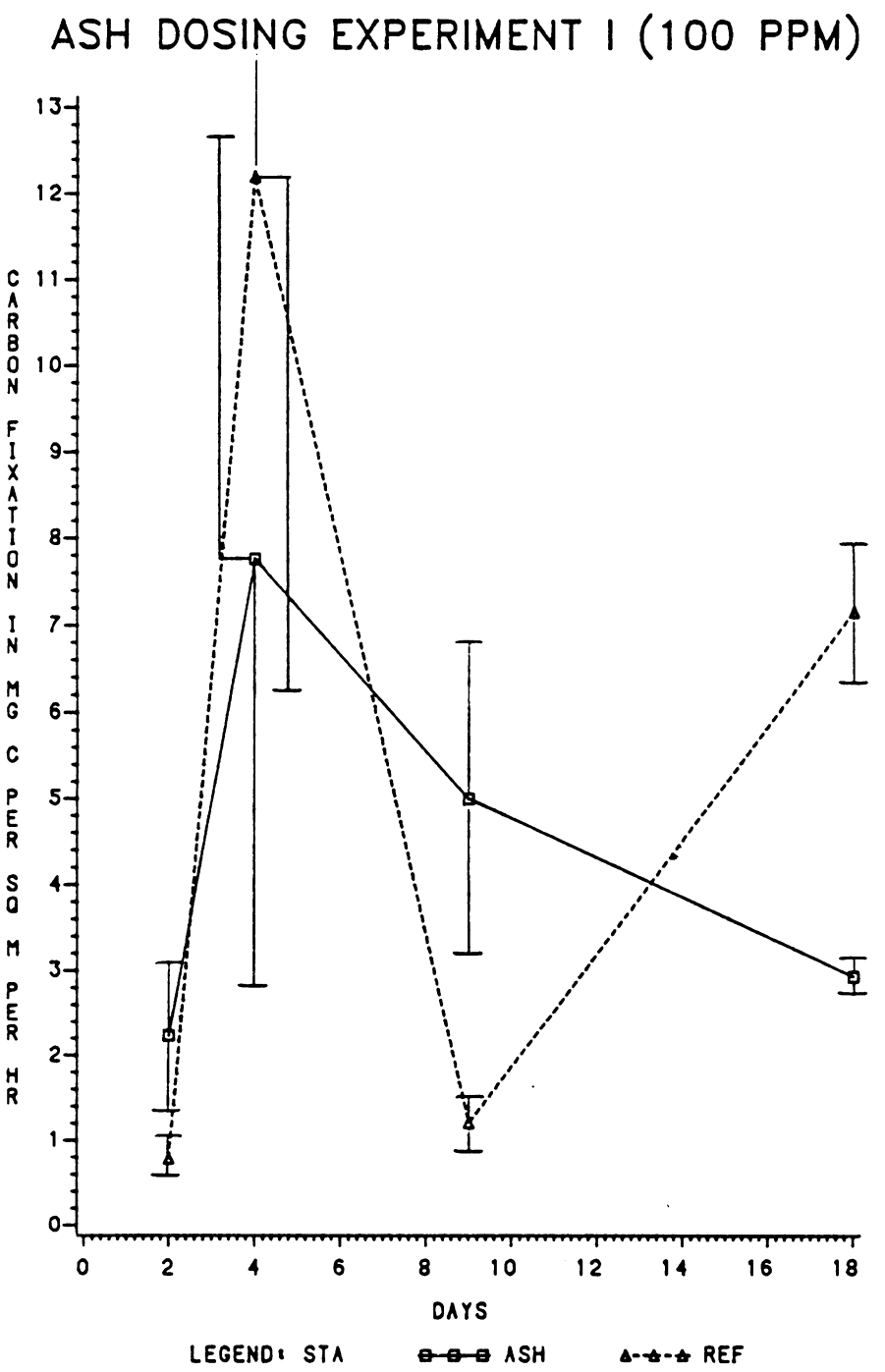

ASH DOSING EXPERIMENT I (100 PPM)
CARBON FIXATION IN MG C PER SQ M PER HR
0
1
2
3
4
5
6
7
8
9
10
11
12
13
0
2
4
6
8
10
12
14
16
18
DAYS
LEGEND: STA
ASH
REF

Table 10. Water chemistry data for ash dosing experiment (I) 100 ppm - 11/19/81-12/05/81.

	Day and Stream							
	2		4		9		18	
Parameter	Ash	Reference	Ash	Reference	Ash	Reference	Ash	Reference
Temperature ^{o}C	8.0	8.0	7.0	7.0	6.5	6.5	6.0	6.0
pH	7.5	7.5	7.3	7.3	7.2	7.2	7.4	7.4
Alkalinity mg l^{-1} $CaCO_3$	52	56	54	54	54	54	50	48
Turbidity JTU	1.5	1.7	2.0	1.7	3.0	2.5	1.7	1.5

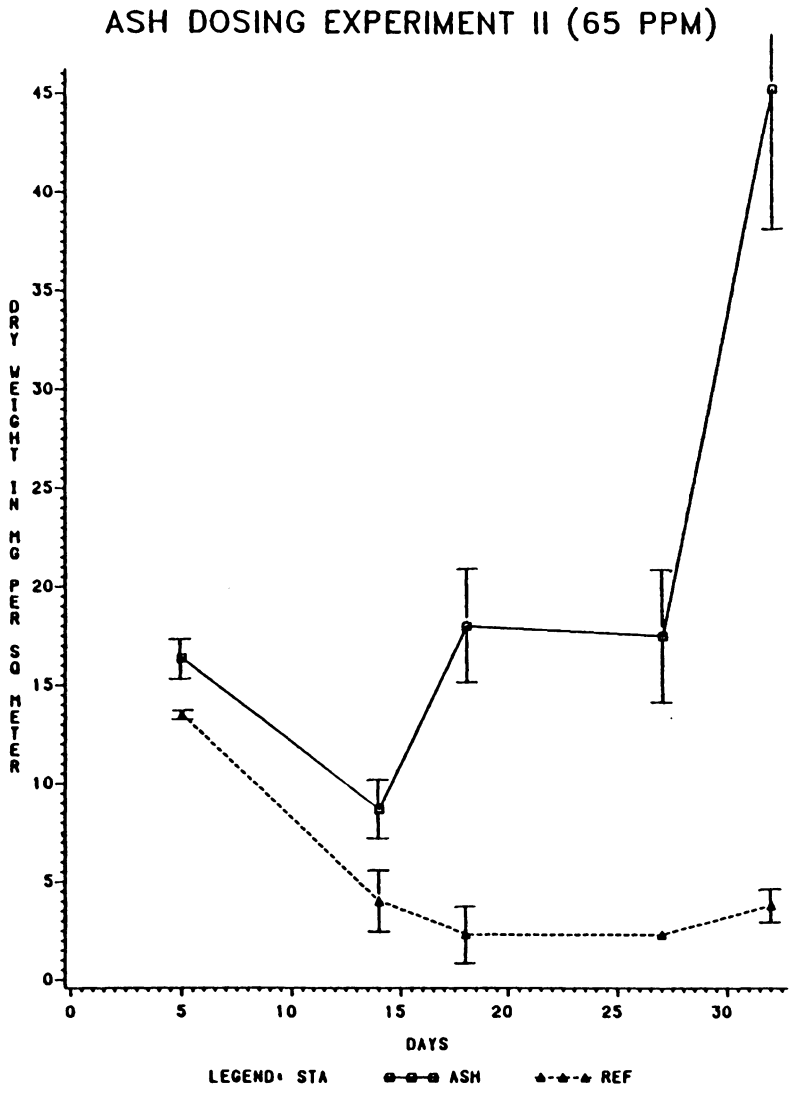
Table 11. Results of Wilcoxon Sign Rank Test for Artificial Stream Ash Dosing Experiment I (100 ppm) data at 0.05 level of significance. A+ indicates that ash dosed stream values are significantly higher than reference stream values. R+ indicates higher reference stream values. NS indicates no significant differences between treatments. Blanks are due to insufficient data.

Parameter	Day			
	2	4	9	18
Dry Weight	NS	A+	A+	A+
Ash Free Dry Weight	R+	NS	NS	NS
Chlorophyll a	R+	NS	NS	R+
ATP	NS	R+	NS	A+
Light Carbon Fixation	NS	NS	A+	R+

4.2.2.2 Ash Dosing Experiment II (65 ppm)

Dry weight and ash free dry weights exhibited similar trends during the 32-day dosing experiment with TSS concentrations of approximately 65 ppm. Both decreased in the reference streams from day 5 to day 27, with a slight increase on day 32. Following day 15, values in the ash dosed stream increased until day 32, exhibiting significantly higher values than the reference stream (Figure 17). Chlorophyll a values also decreased in the reference stream from day 5 to 27, with an increase by day 32, but the dosed stream values were significantly lower on days 5, 14, and 17, and significantly higher, although more variable, on day 32. ATP values were highly variable in both streams, reference value being greater, or not significantly different than the dosed stream values (Figure 18). Light carbon fixation rates were significantly lower in the ash dosed stream on day 5-17, then demonstrated no significant difference from the reference values, although both ash and reference streams greatly increased carbon fixation rates and variability on day 32 (Figure 19).

Figure 17. Values for dry weights and ash free dry weights from Artificial Stream Ash Dosing Experiment II. 1 Sept. to 28 Sept. 1981. Bars are one standard error of the mean.


ASH DOSING EXPERIMENT II (65 PPM)
DRY WEIGHT IN MG PER SQ METER
0
5
10
15
20
25
30
35
40
45
0
5
10
15
20
25
30
DAYS
LEGEND: STA
ASH
REF

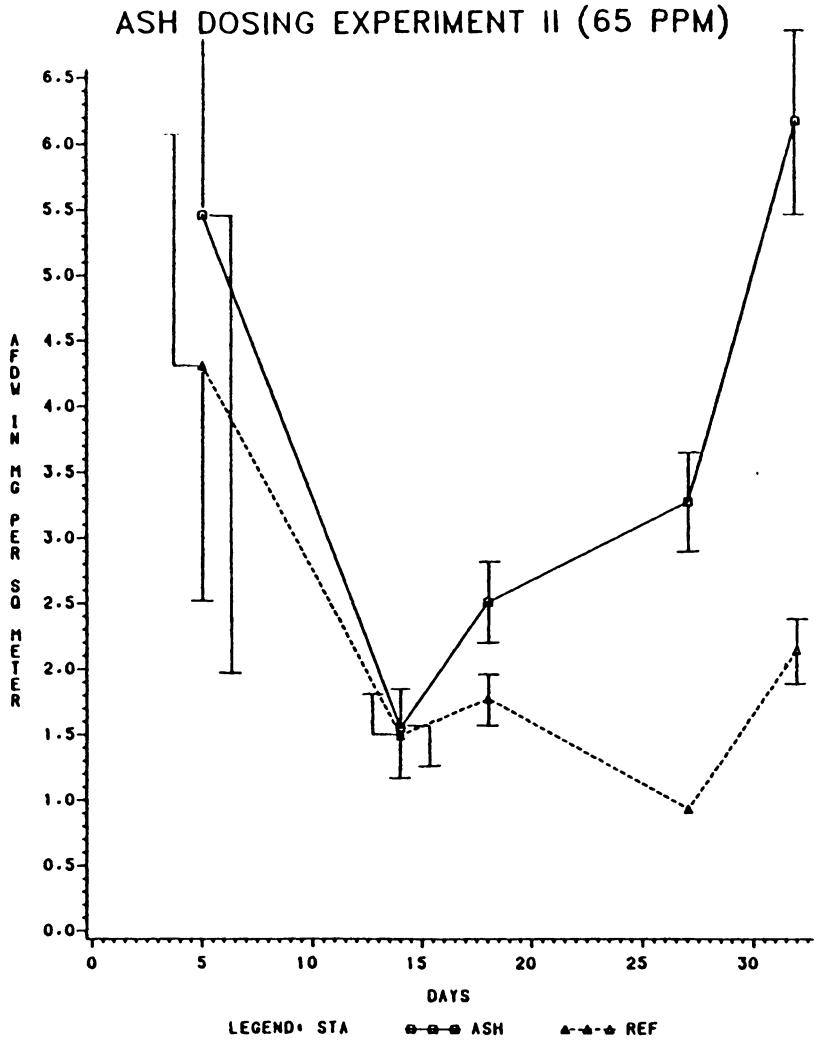
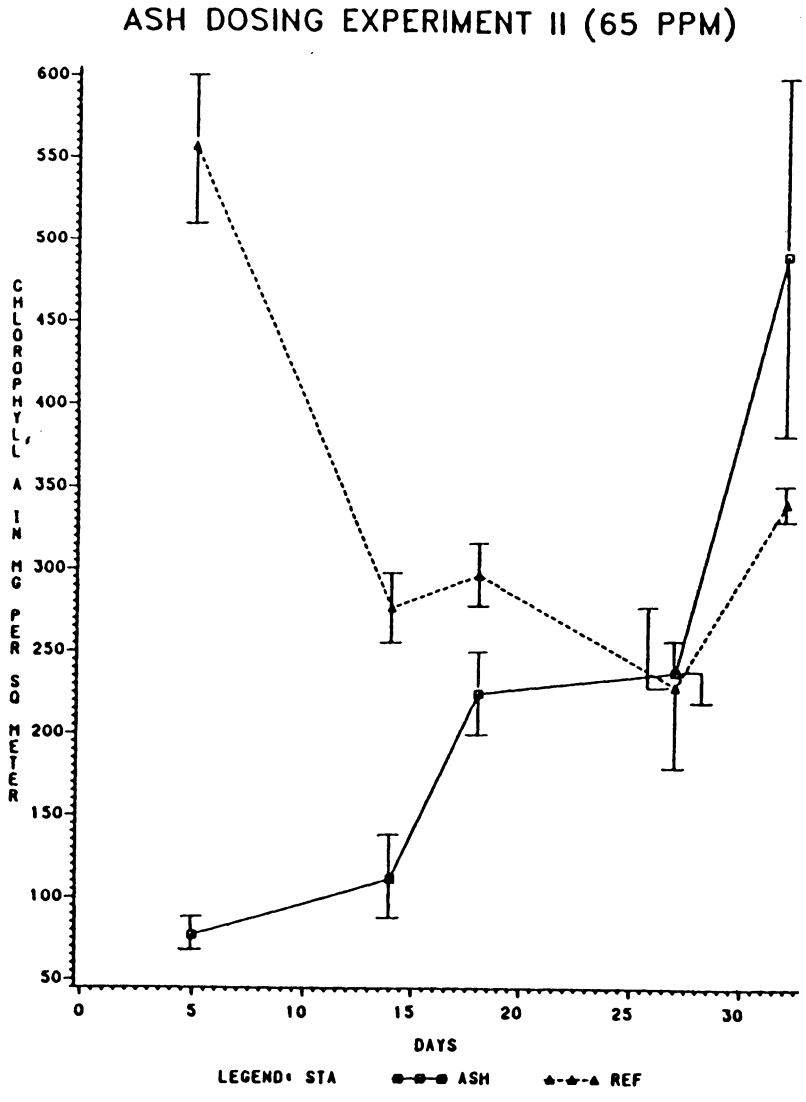

ASH DOSING EXPERIMENT II (65 PPM)
AFDW IN MG PER SQ METER
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
0
5
10
15
20
25
30
DAYS
LEGEND: STA
ASH
REF

Figure 18. Values for chlorophyll a and ATP from Artificial Stream Ash Dosing Experiment II. 1 Sept. to 28 Sept. 1981. Bars are one standard error of the mean.


ASH DOSING EXPERIMENT II (65 PPM)
CHLOROPHYLL A IN MG PER SQ METER
600
550
500
450
400
350
300
250
200
150
100
50
0
5
10
15
20
25
30
DAYS
LEGEND: STA
ASH
REF

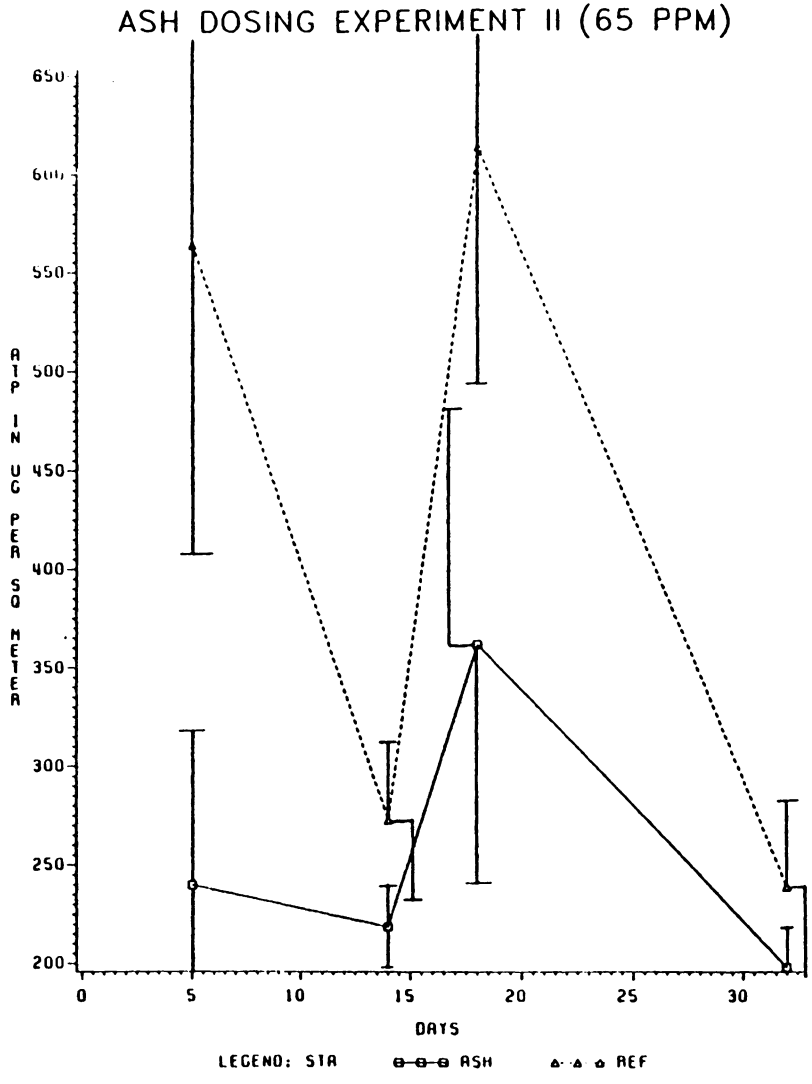

ASH DOSING EXPERIMENT II (65 PPM)
ATP IN UG PER SQ METER
650
600
550
500
450
400
350
300
250
200
0
5
10
15
20
25
30
DAYS
LEGEND: STA
ASH
REF

Figure 19. Values for light carbon fixation rates from Artificial Stream Ash Dosing Experiment II. 1 Sept. to 28 Sept. 1981.
Bars are one standard error of the mean.

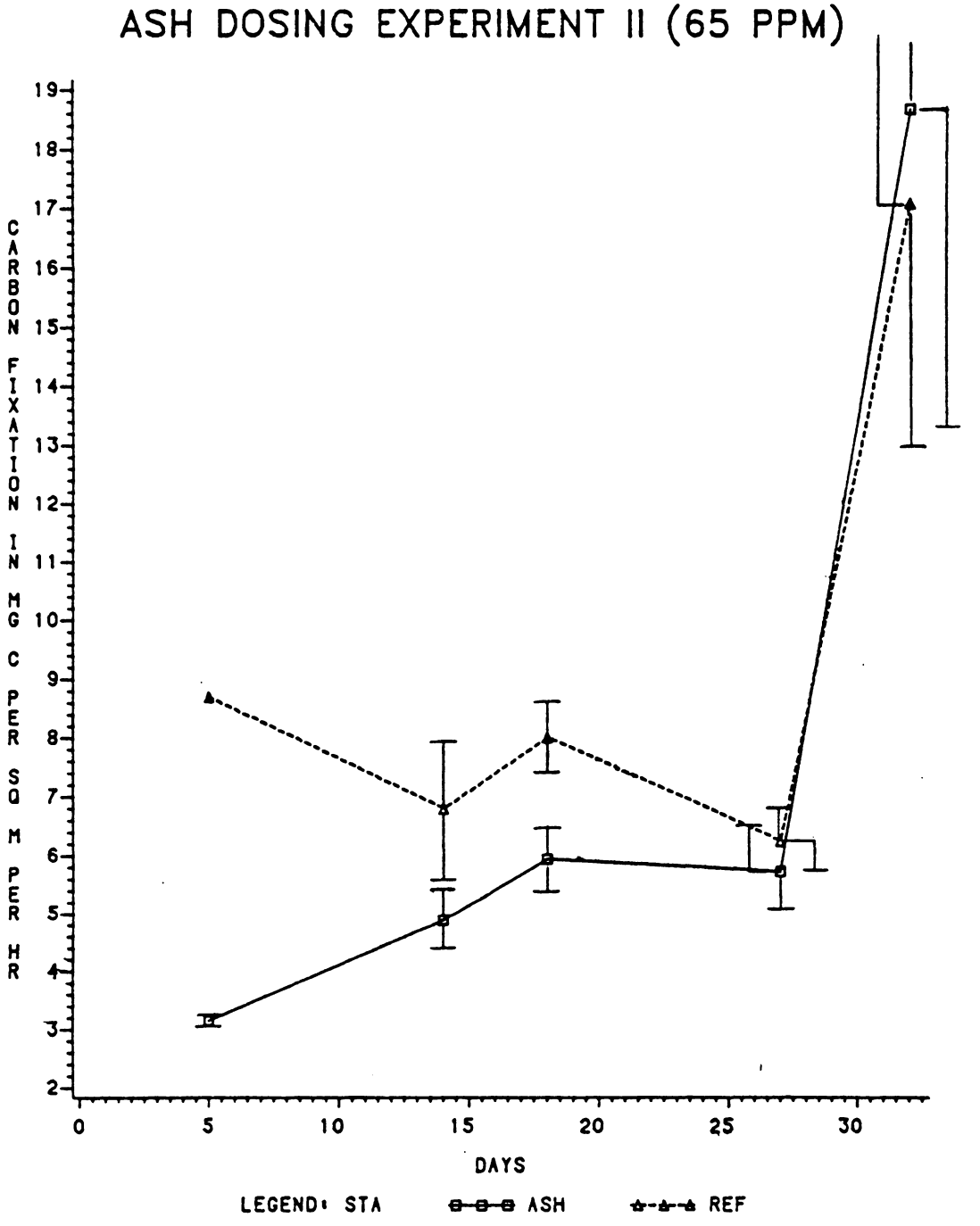

ASH DOSING EXPERIMENT II (65 PPM)
CARBON FIXATION IN MG C PER SQ M PER HR
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
0
5
10
15
20
25
30
DAYS
LEGEND: STA
ASH
REF

Table 12. Water chemistry data for ash dosing experiment (II) 65 ppm - 9/01/81 - 9/28/81.

Parameter	Day and Stream 5 Ash	5 Reference	14 Ash	14 Reference	18 Ash	18 Reference	27 Ash	27 Reference	32 Ash	32 Reference
Temperature °C	25.0	25.0	20.0	20.0	24.0	24.0	19.0	19.0	20.0	20.0
pH	8.4	8.6	7.2	7.6	7.7	7.8	7.8	7.8	8.2	8.3
Alkalinity mg l^{-1} $CaCO_3$	66	60	54	56	62	60	54	58	62	64
Turbidity JTU	1.5	1.6	2.5	2.0	1.7	1.7	1.0	1.0	1.5	1.3
TSS ppm	70	1.8			64.9	1.3	64.9		64.9	

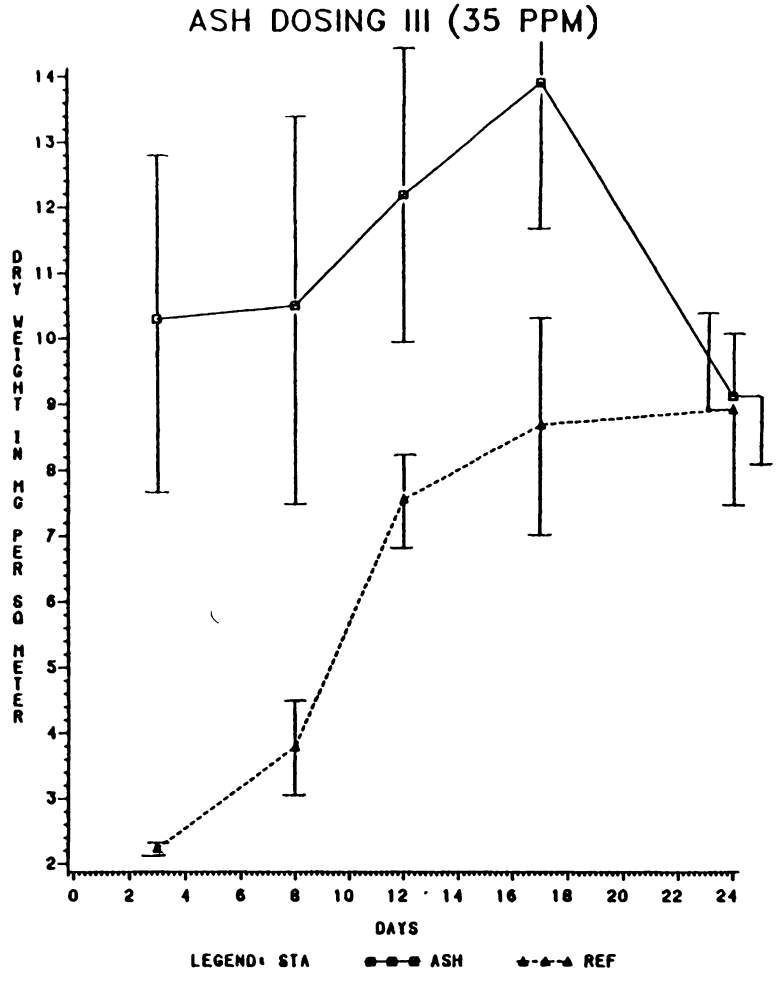
Table 13. Results of Wilcoxon Sign Rank Test for Artificial Stream Ash Dosing Experiment II (65 ppm) at the 0.05 level of significance. A+ indicates that ash dosed stream values are significantly higher than reference stream values. R+ indicates higher reference stream values. NS indicates no significant differences between treatments. Blanks are due to insufficient data.

Parameter	Day				
	5	14	18	27	32
Dry Weight	A+	A+	A+	A+	A+
Ash Free Dry Weight	NS	NS	A+	A+	A+
Chloropyll a	R+	R+	R+	NS	A+
ATP	R+	NS	R+	_	NS
Light Carbon Fixation	R+	R+	R+	NS	NS

4.2.2.3 Ash Dosing Experiment III (35 ppm)

At the lowest ash dosing experiment (35 ppm TSS) there was no consistent trend of the measured parameters. Dry weights were more variable and significantly greater in the dosed stream from day 3 to day 18 of the 24-day experiment. Ash free dry weights were significantly greater than the reference value on day 3, but reference values were significantly greater for the remaining days (Figure 20). Chlorophyll a values were not significantly different on day 3, and were significantly greater in the reference stream on days 8, 12, 17, and 24. Light carbon fixation rates were significantly greater in the reference stream on day 2. No significance between streams on days 8, 12, and 17 was observed, but the ash dosed stream demonstrated significantly higher carbon fixation rates on day 24 (Figure 21). For all of the parameters measured, a greater fluctuation in value over time, and a somewhat higher variability was noticed in the reference stream, when compared to values for the ash dosed stream.

Figure 20. Values for dry weights and ash free dry weights from Artificial Stream Ash Dosing Experiment III. 23 Sept. to 14 Oct. 1981. Bars are one standard error of the mean.


ASH DOSING III (35 PPM)
DRY WEIGHT IN MG PER SQ METER
DAYS
LEGEND: STA ASH REF

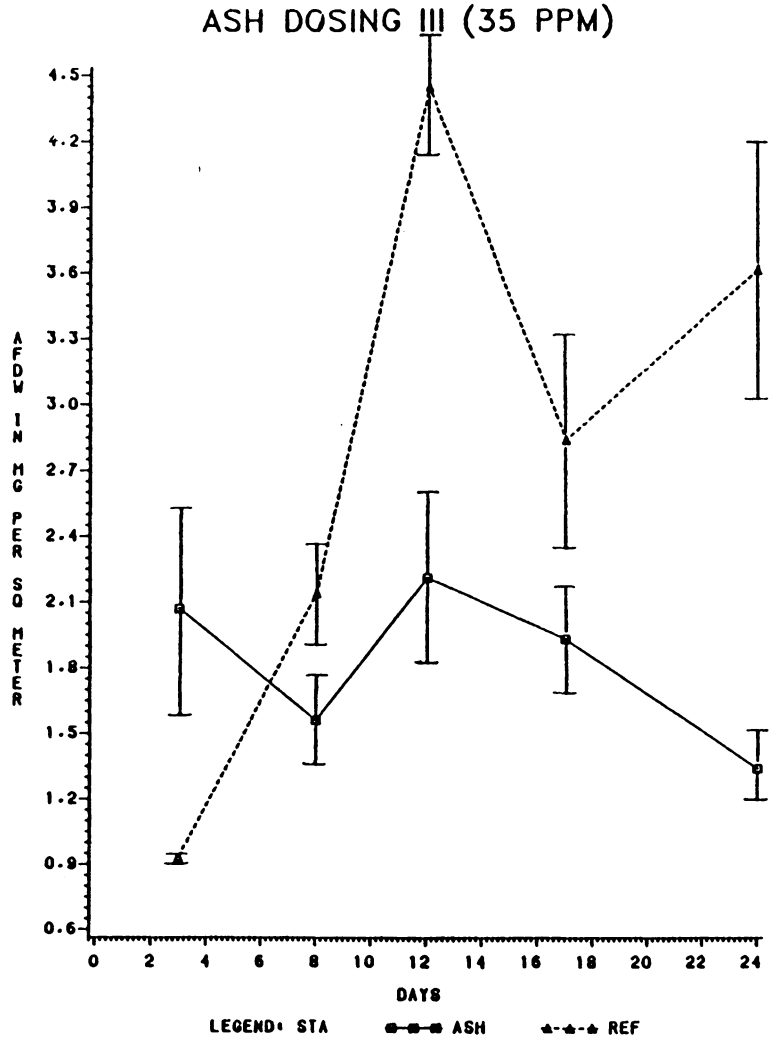
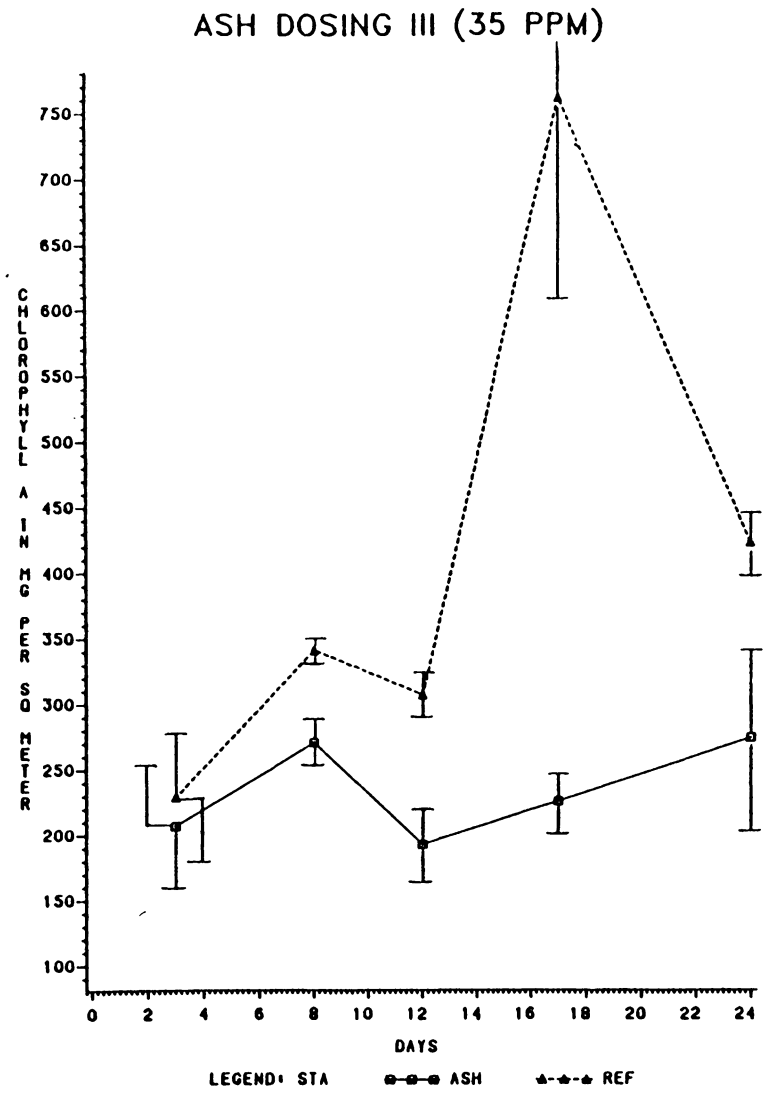

ASH DOSING III (35 PPM)
AFDW IN MG PER SQ METER
DAYS
LEGEND: STA ASH REF

Figure 21. Values for chlorophyll a and light carbon fixation rates for Artificial Stream Ash Dosing Experiment III. 23 Sept. to 14 Oct. 1980. Bars are one standard error of the mean.


ASH DOSING III (35 PPM)
CHLOROPHYLL A IN MG PER SQ METER
100
150
200
250
300
350
400
450
500
550
600
650
700
750
0 2 4 6 8 10 12 14 16 18 20 22 24
DAYS
LEGEND: STA ASH REF

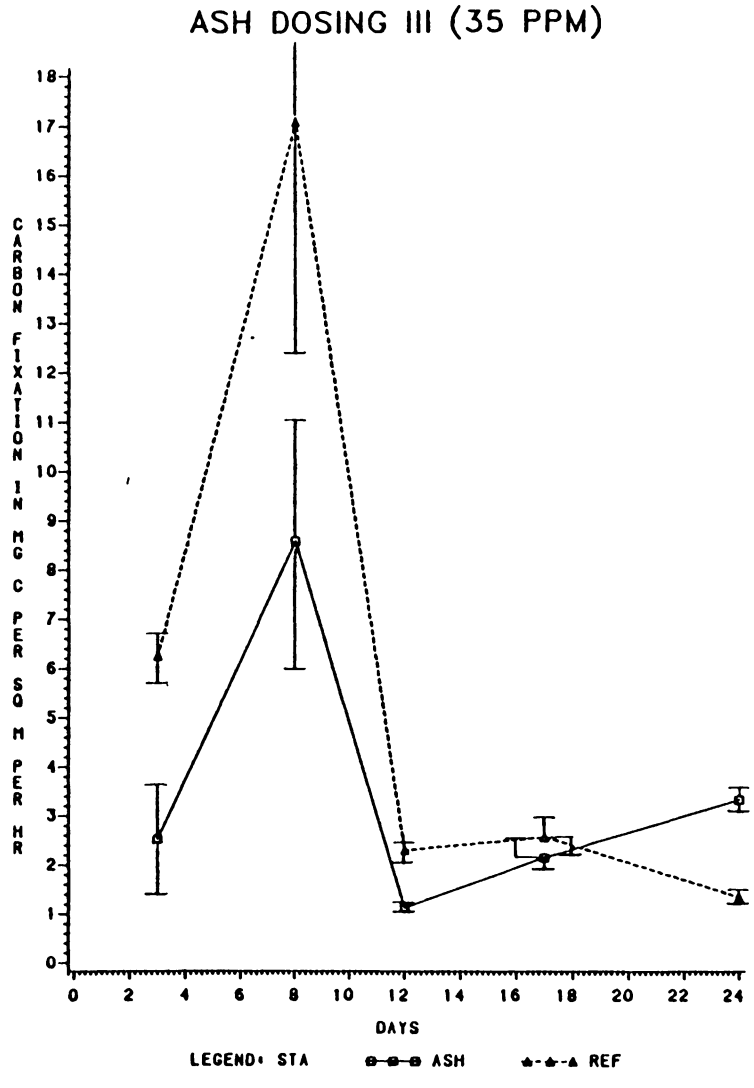

ASH DOSING III (35 PPM)
CARBON FIXATION IN MG C PER SQ M PER HR
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
0 2 4 6 8 10 12 14 16 18 20 22 24
DAYS
LEGEND: STA ASH REF

Table 14. Water chemistry data for ash dosing experiment (III) 35 ppm - 9/23/81 - 10/14/81.

	Day and Stream									
	3		8		12		17		24	
Parameter	Ash	Reference	Ash	Reference	Ash	Reference	Ash	Reference	Ash	Reference
Temperature ^{o}C	19.0	19.0	20.0	20.0	18.0	18.0	16.0	16.0	14.0	14.0
pH	7.8	7.8	8.2	8.3	8.3	8.3	8.3	8.0	8.4	8.1
Alkalinity mg l^{-1} $CaCO_3$	54	58	62	64	72	66	64	66	68	68
Turbidity JTU	1.0	1.0	1.5	1.3	2.2	1.0	1.0	1.0	1.1	1.2
TSS ppm	35.1		35.1							

Table 15. Results of Wilcoxon Sign Rank Test for Artificial Stream Ash Dosing Experiment III (35 ppm) data at the 0.05 level of significance. A+ indicates that ash dosed stream values are significantly higher than reference stream values. R+ indicates higher reference stream values. NS indicates no significant differences between treatments. Blanks are due to insufficient data.

Parameter	Day				
	3	8	12	17	24
Dry Weight	A+	A+	A+	A+	NS
Ash Free Dry Weight	A+	R+	R+	R+	R+
Chlorophyll a	NS	R+	R+	R+	R+
ATP	—	—	—	—	—
Light Carbon Fixation	R+	NS	NS	NS	A+

4.2.3 Heavy Metal Dosing

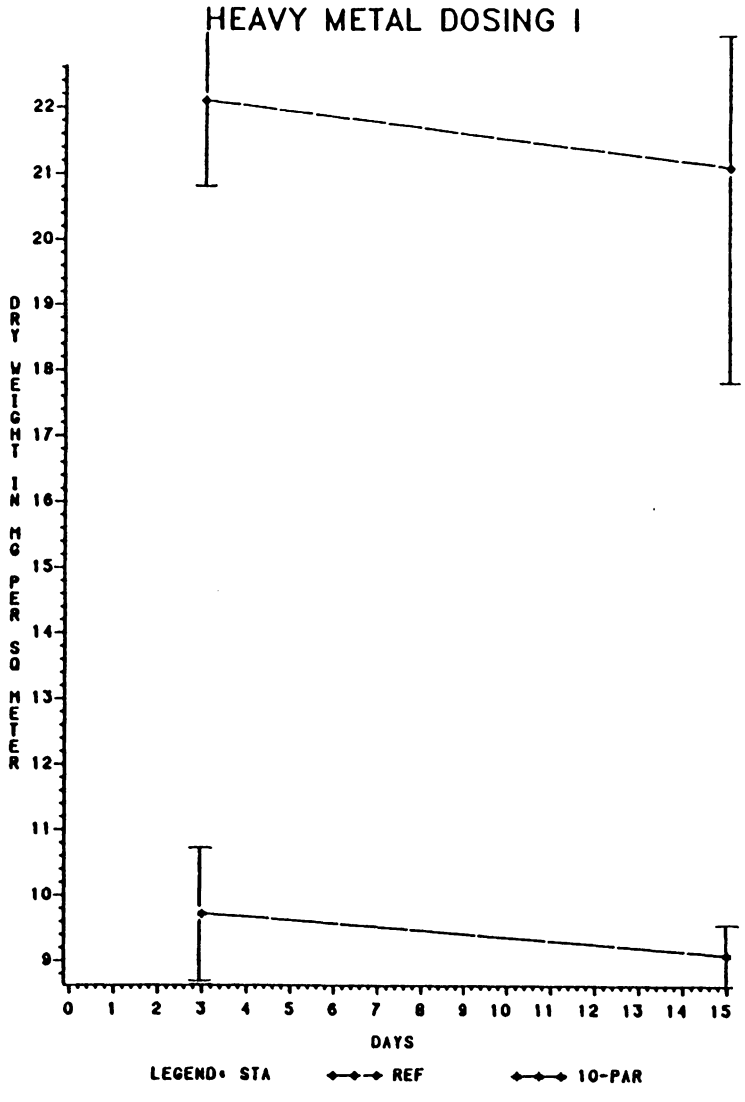
Three experiments were conducted to assess the environmental effects of six heavy metals on aufwuchs communities when introduced simultaneously. Heavy metal concentrations were calculated to model measured metal concentration in the Glen Lyn fly ash basin effluent (par), one-half this amount (0.5 par), and ten times this value (10 par) (Table 16). In general, target in-stream metal concentrations were achieved.

4.2.3.1 Heavy Metal Dosing I (10 Par)

At heavy metal concentrations approximately ten times greater than those measured in the fly ash basin effluent, severe depression of aufwuchs was observed. On days 3 and 15, following initiation of metal dosing, all parameters measured were significantly greater in the reference stream. (Table 18). Dry weights were approximately 12 mg m -2 less in the dosed stream. Ash free dry weights in the dosed streams were depressed to about one half of the reference value (Figure 22). Chlorophylls and ATP values were depressed by approximately 525 mg m -2, and 600 mg m -2, respectively in the metal dosed streams (Figure 23). Light carbon fixation was also extremely low in the dosed streams, ranging from 0.05 to 2.0 mg C m -2 hr -1. Reference values

ranged from 11.0 to 31.5 mg C m -2 hr -1 (Figure 24). In addition, all parameters (dry weight, ash free dry weight, chlorophyll a, ATP and light carbon fixation) exhibited a lower measured value on day 15 when compared to day 3. Reference values decreased for dry weights, ATP and light carbon fixation during this period. Ash free dry weights and chlorophyll a increased in the reference stream by day 15.

Figure 22. Values for dry weights and ash free dry weights from Artificial Stream Heavy Metal Dosing I. 13 May to 25 May 1981. Bars are one standard error of the mean.


HEAVY METAL DOSING I
DRY WEIGHT IN MG PER SQ METER
22
21
20
19
18
17
16
15
14
13
12
11
10
9
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS
LEGEND: STA
REF
10-PAR

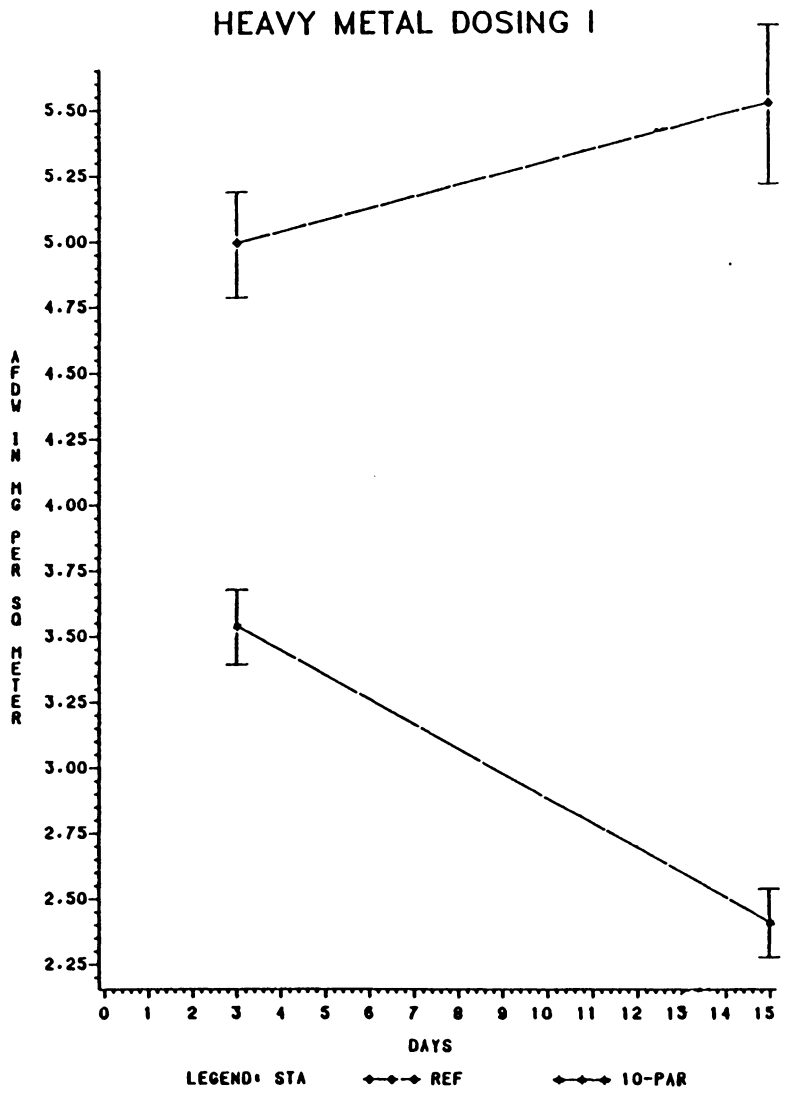
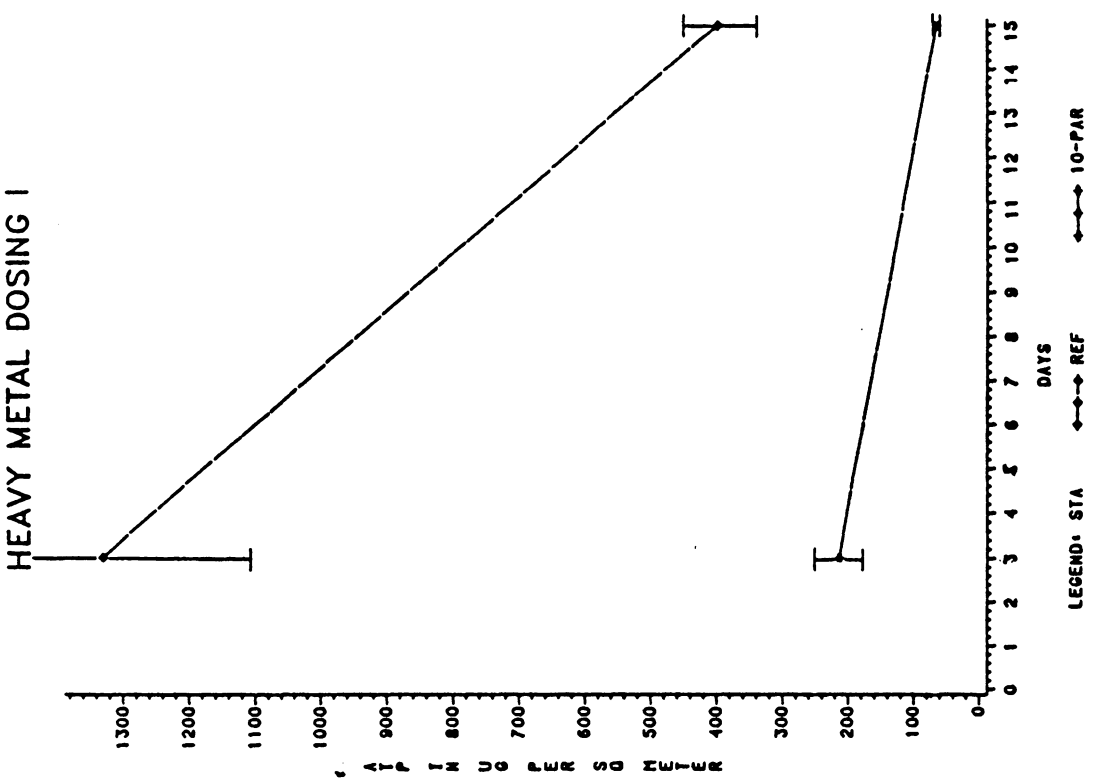

HEAVY METAL DOSING I
AFDW IN MG PER SQ METER
5.50
5.25
5.00
4.75
4.50
4.25
4.00
3.75
3.50
3.25
3.00
2.75
2.50
2.25
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS
LEGEND: STA
REF
10-PAR

Figure 23. Values for chlorophyll a and ATP from Artificial Stream Heavy Metal Dosing I. 13 May to 25 May 1981. Bars are one standard error of the mean.


HEAVY METAL DOSING I
ATP IN UG PER SQ METER
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS
LEGEND: STA
REF
10-PAR

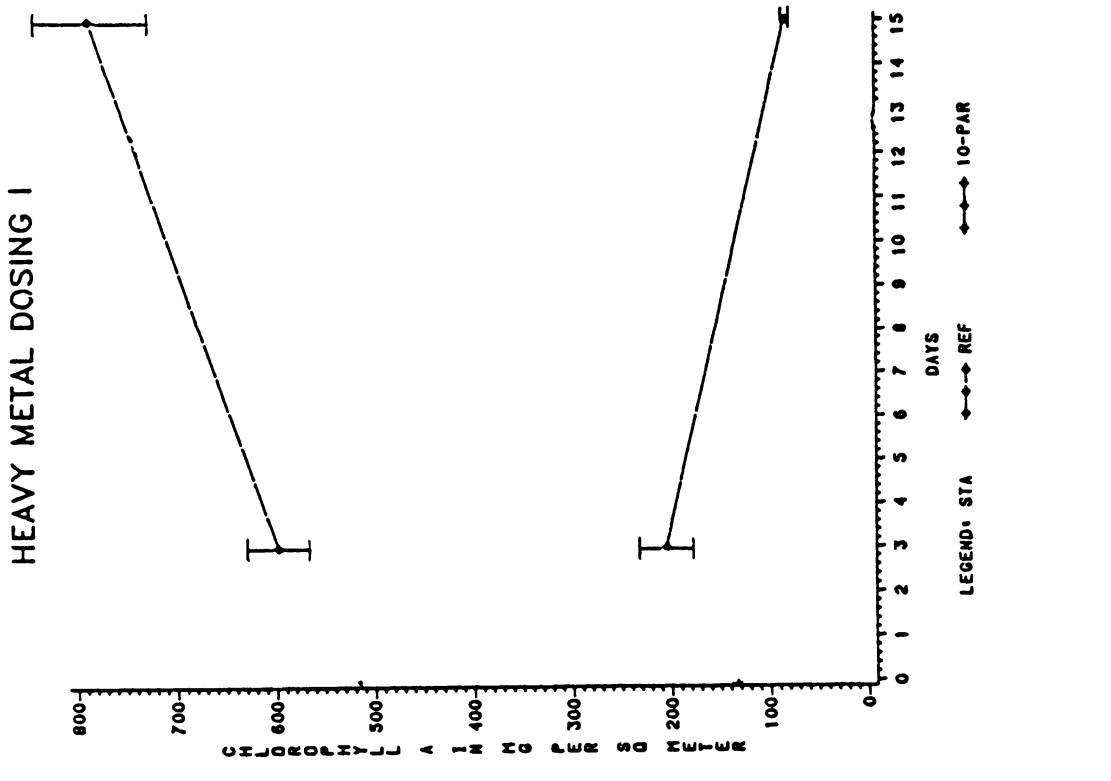

HEAVY METAL DOSING I
CHLOROPHYLL A IN MG PER SQ METER
800
700
600
500
400
300
200
100
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS
LEGEND: STA
REF
10-PAR

Figure 24. Values for light carbon fixation rates from Artificial Stream Heavy Metal Dosing I. 13 May to 25 May 1981. Bars are one standard error of the mean.

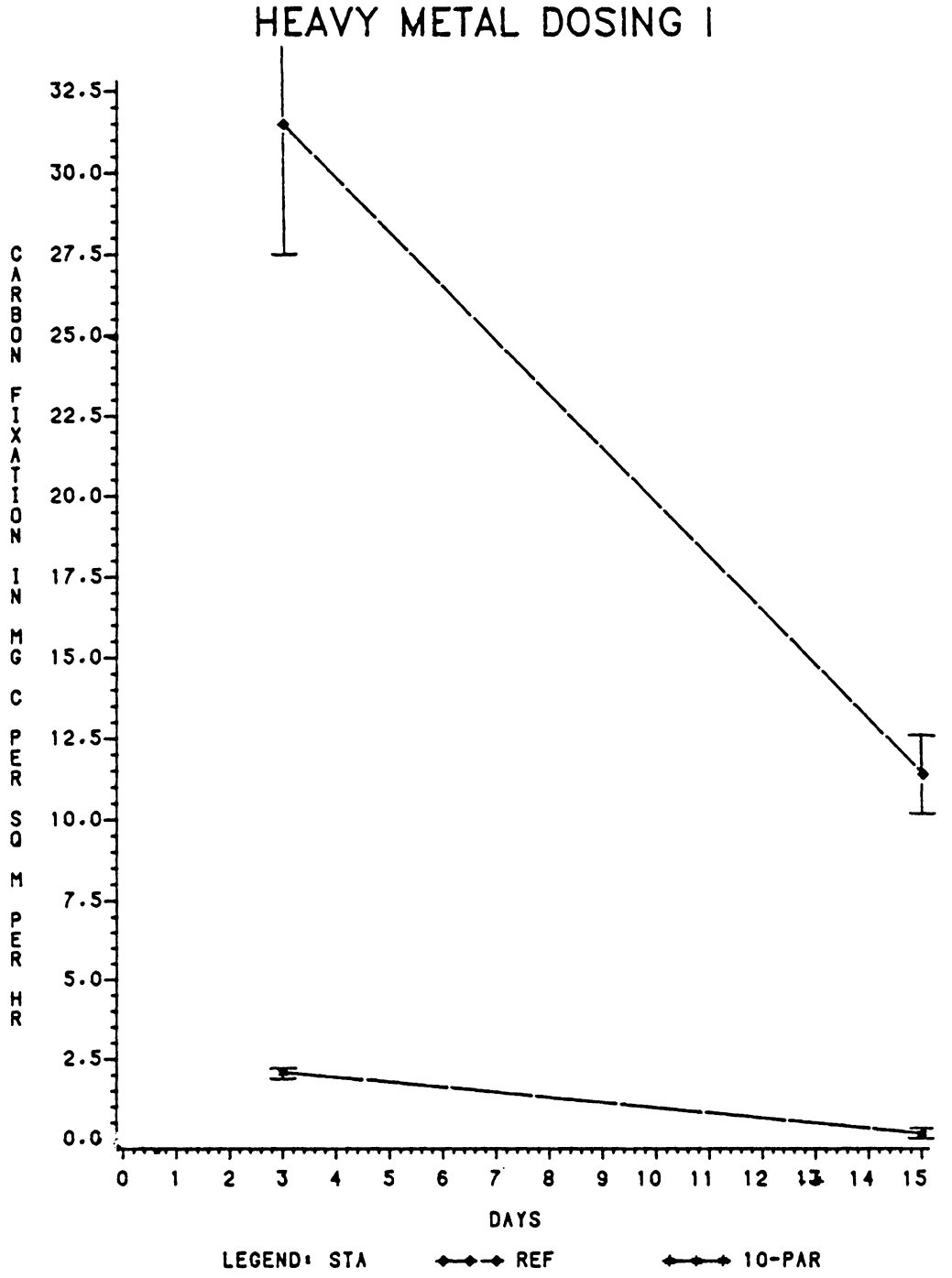

HEAVY METAL DOSING I
CARBON FIXATION IN MG C PER SQ M PER HR
32.5
30.0
27.5
25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
DAYS
LEGEND: STA REF 10-PAR

Table 16. Water chemistry data for heavy metal dosing experiment (I) - 5/13/81 - 5/25/81.

Parameter	Day and Stream 3 10-Par	3 Reference	15 10-par	15 Reference
Temperature ^{o}C	14.5	14.5	19.5	19.5
pH	6.7	6.6	7.2	7.2
Alkalinity mg l^{-1} $CaCO_3$	36	36	40	40
Turbidity JTU	8.2	8.0	3.2	3.1

Table 17. Target heavy metal dosing concentrations and measured in stream concentrations for heavy metal dosing experiments.

Metal	Exposure	Target conc. (mg/l)	Mean Measured conc. (mg/l)
Cadmium	Control	0.00	0.01
	Par	0.03	0.02
	10 Par	0.30	0.19
Chromium	Control	0.00	0.001
	Par	0.02	0.06
	10 Par	0.20	0.32
Copper	Control	0.00	0.001
	Par	0.03	0.04
	10 Par	0.30	0.36
Lead	Control	0.00	0.001
	Par	0.02	0.02
	10 Par	0.20	0.16
Nickel	Control	0.00	0.01
	Par	0.04	0.04
	10 Par	0.40	0.23
Zinc	Control	0.00	0.32
	Par	0.12	0.17
	10 Par	1.20	1.57

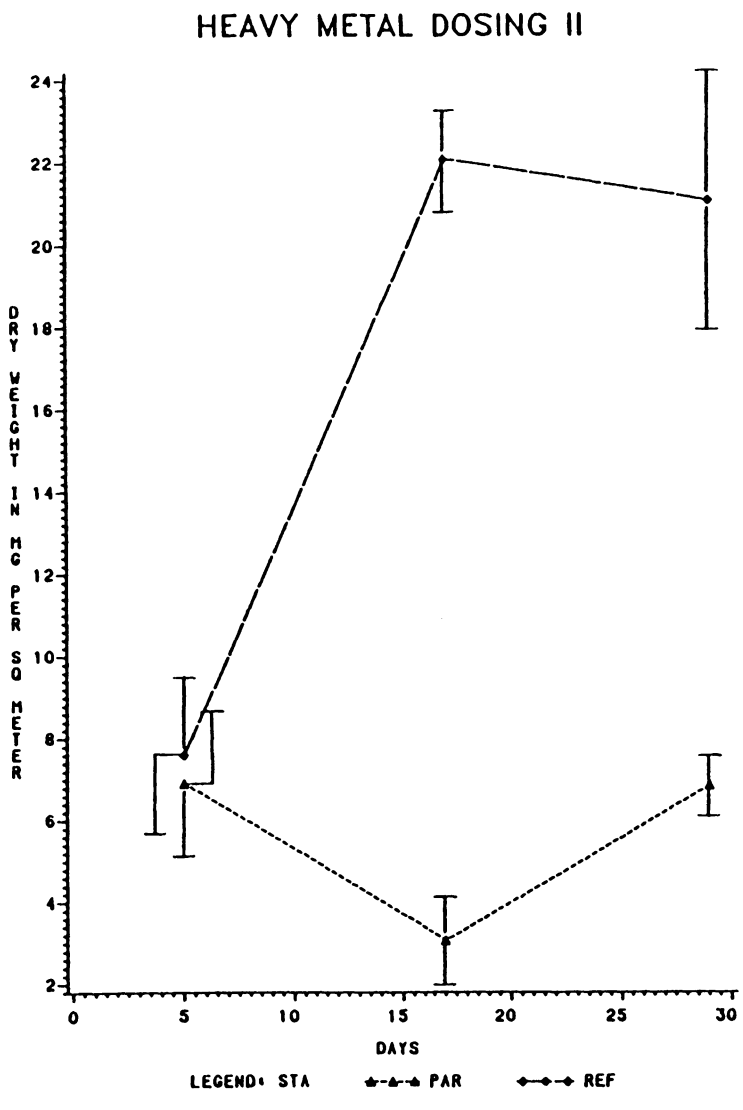
Table 18. Results of Wilcoxon Sign Rank Test for Artificial Stream Heavy Metal Dosing Experiment I data at the 0.05 level of significance. M+ indicates that metal dosed stream values are significantly higher than reference stream values. R+ indicates higher reference stream values. NS indicates no significant differences between treatments. Blanks are due to insufficient data.

Parameter	Day	
	3	15
Dry Weight	R+	R+
Ash Free Dry Weight	R+	R+
Chlorophyll a	R+	R+
ATP	R+	R+
Light Carbon Fixation	R+	R+

4.2.3.2 Heavy Metal Dosing II (Par)

Heavy metals dosed into the artificial stream system at concentrations approximately equal to those measured in the fly ash basin effluent (par) also appeared to depress the aufwuchs community, although not as severely as in the 10-par dosing experiment. On day 5 of the 29 day experiment values for dry weight, ash free dry weight and chlorophyll a in the dosed streams were not significantly different from the reference values (Table 20). By day 17, and on day 29 all were significantl lower than reference stream values (Figures 25 and 26). In each case, while reference parameters exhibited a rapid and substantial rise, dosed stream parameters demonstrated an initial depression on day 17, followed by an increase on day 29. ATP values were initially much greater in the dosed stream, then dropped as the reference values rose, and finally leveled out as the reference values dropped again (Figure 26). Light carbon fixation rates were significantly lower than reference rates on days 5 and 17, but continued to increase while the reference rate dropped so that on day 29 light carbon fixation rates were significantly greater in the metal dosed stream (Figure 27).

Figure 25. Values for dry weights and ash free dry weights for Artificial Stream Heavy Metal Dosing II. 2 May to 25 May 1981. Bars are one standard error of the mean.


HEAVY METAL DOSING II
DRY WEIGHT IN MG PER SQ METER
24
22
20
18
16
14
12
10
8
6
4
2
0
5
10
15
20
25
30
DAYS
LEGEND: STA
PAR
REF

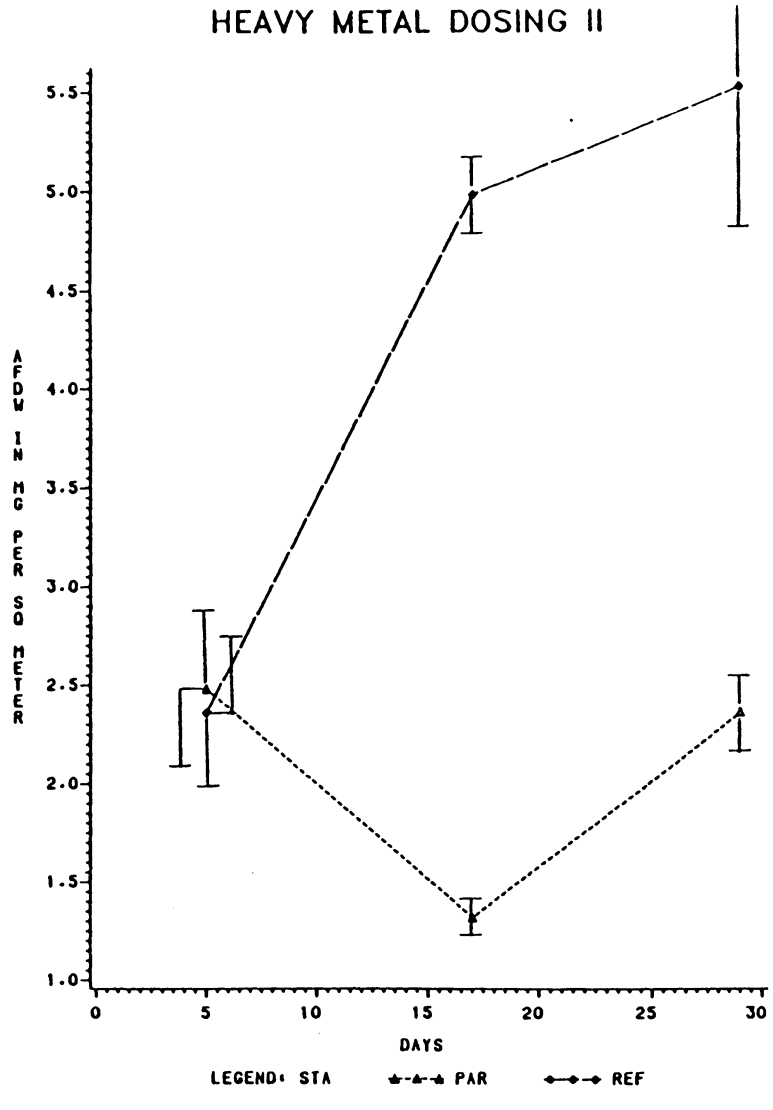
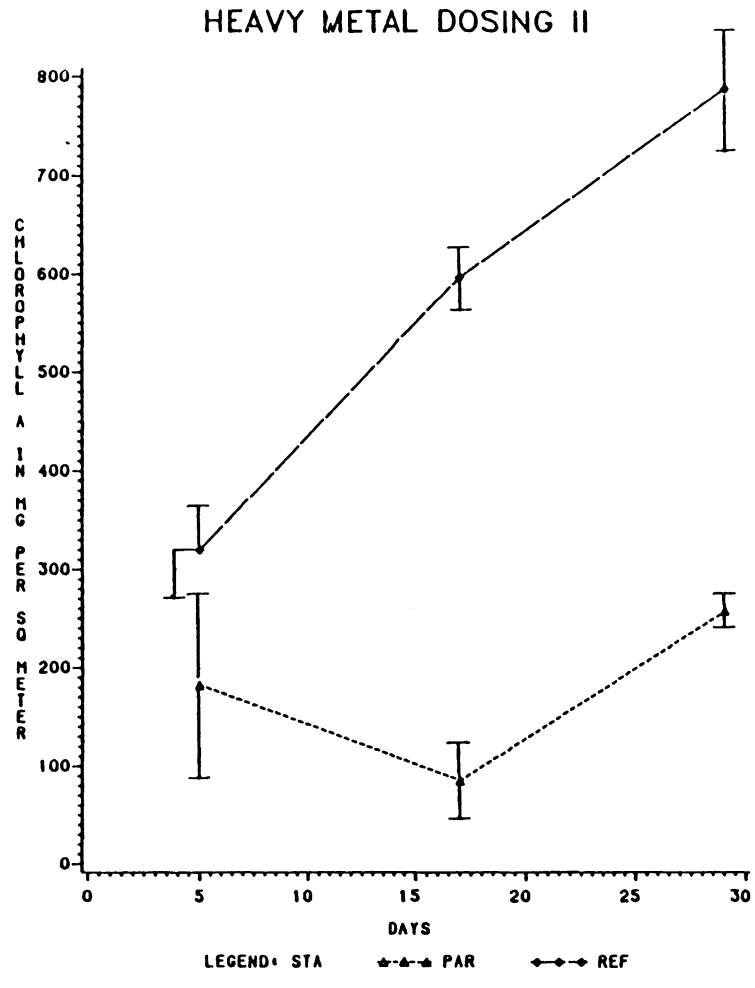

HEAVY METAL DOSING II
AFDW IN MG PER SQ METER
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0
5
10
15
20
25
30
DAYS
LEGEND: STA
PAR
REF

Figure 26. Values for chlorophyll a and ATP from Artificial Stream Heavy Metal Dosing II. 2 May to 25 May 1981. Bars are one standard error of the mean.


HEAVY METAL DOSING II
CHLOROPHYLL A IN MG PER SQ METER
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
DAYS
LEGEND: STA
PAR
REF

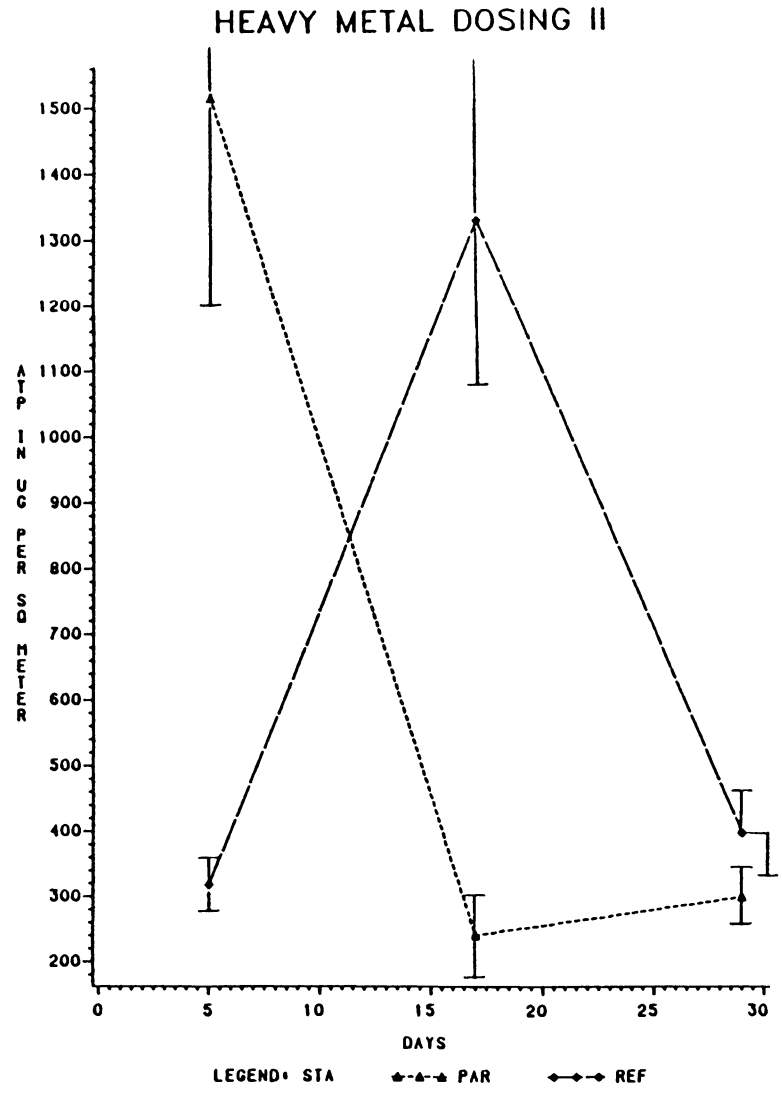

HEAVY METAL DOSING II
ATP IN UG PER SQ METER
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
0
5
10
15
20
25
30
DAYS
LEGEND: STA
PAR
REF

Figure 27. Values for light carbon fixation rates from Artificial Stream Heavy Metal Dosing II. 2 May to 25 May 1981. Bars are one standard error of the mean.

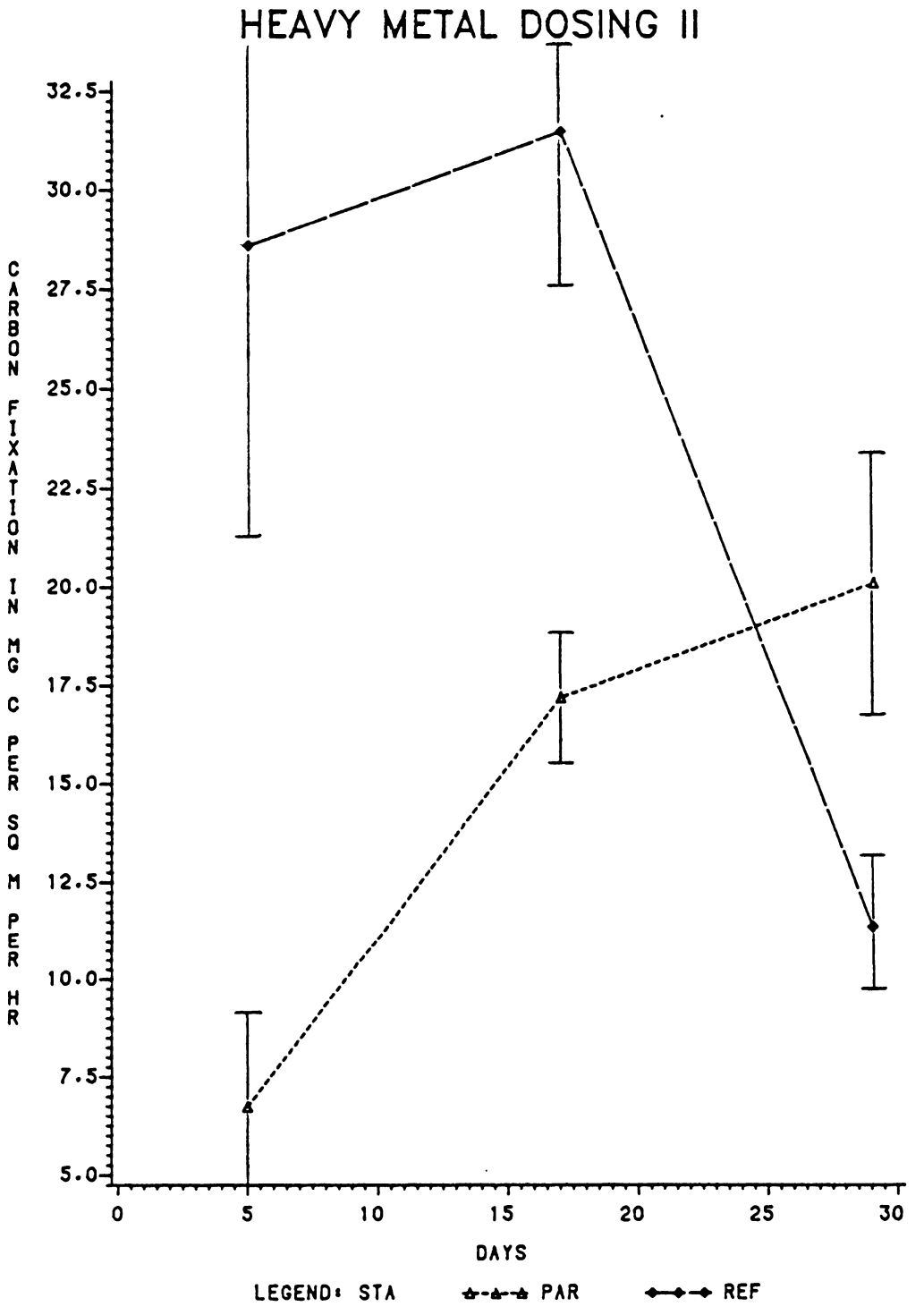

HEAVY METAL DOSING II
CARBON FIXATION IN MG C PER SQ M PER HR
32.5
30.0
27.5
25.0
22.5
20.0
17.5
15.0
12.5
10.0
7.5
5.0
0
5
10
15
20
25
30
DAYS
LEGEND: STA
PAR
REF

Table 19. Water chemistry data for heavy metal dosing experiment (II) - 5/02/81 - 5/25/81.

Parameter	Day and Stream 5 Par	5 Reference	17 Par	17 Reference	29 Par	29 Reference
Temperature ^{o}C	14.0	14.0	14.5	14.5	19.5	19.5
pH	7.1	7.0	6.5	6.6	7.2	7.2
Alkalinity mg l^{-1} $CaCO_3$	40	41	36	36	40	40
Turbidity JTU	3.1	2.8	8.5	8.0	3.1	3.1

Table 20. Results of Wilcoxon Sign Rank Test for Artificial Stream Heavy Metal Dosing Experiment II data at the 0.05 level of significance. M+ indicates that metal dosed stream values are significantly higher than reference stream values. R+ indicates higher reference stream values. NS indicates no significant differences between treatments. Blanks are due to insufficient data.

Parameter	Day		
	5	17	29
Dry Weight	NS	R+	R+
Ash Free Dry Weight	NS	R+	R+
Chlorophyll a	NS	R+	R+
ATP	M+	R+	R+
Light Carbon Fixation	R+	R+	M+

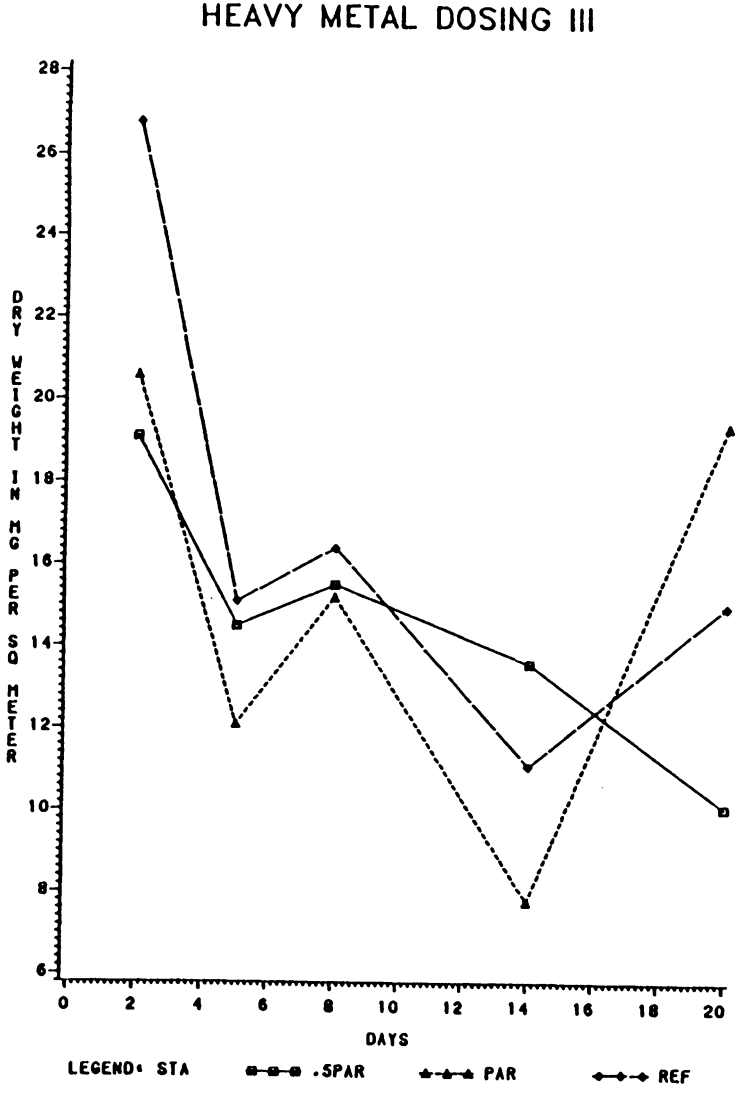
4.2.3.3 Heavy Metal Dosing III (0.5 Par, Par)

Streams dosed with heavy metal concentrations of one-half the concentrations measured in the fly ash basin effluent (0.5 par) were compared to par and reference streams for 20 days. Dry weights and ash free dry weights exhibited similar patterns during the study period. Both initially dropped in all streams during the first 14 days of the study, then increased by day 20. Par-dosed streams were lower than either the 0.5 par or reference stream until day 20, when values were significantly higher (Figure 28). For both dry weights and ash free dry weights par values were significantly higher on day 20, and reference values were significantly lower than 0.5 par values (Table 23). Chlorophyll a values in the reference and par dosed streams demonstrated a decrease until day 5, followed by a stable period through day 14, and an increase by day 20. The 0.5 par stream values increased from day 2 to day 5, then followed a pattern similar to the reference and par streams. On day 20 par values were significantly higher than observed values in the other streams. ATP values decreased in both metal dosed streams until day 8, and remained at low levels, while the reference stream ATP increased from day 5 to day 20 (Figure 29). Light carbon fixation rates in the 0.5 par and reference stream exhibited an intial decrease from day 2 to 8, and

then increased until day 20 when 0.5 par values were significantly greater than the reference stream. The par dosed stream showed initially significantly less carbon fixation activity, but this trend gradually increased by day 14 to be significantly greater than the reference value (Figure 30). On day 20 there was no significant difference between the reference or 0.5 par dosed stream (Table 23).

Heavy metals exhibited different accumulation patterns in the aufwuchs community depending upon the metal specie and the level of dosing concentration (Table 21). Cadmium and copper concentrations increased in both the par and 0.5 par streams until day 5, and remained fairly constant until a sharp decrease on day 20. Chromium appeared to be only slightly bioconcentrated although more was observed in the par stream. Nickel concentrations were higher than reference values in the par stream on day 2, and remained higher with little fluctuation. The 0.5 par values for nickel were consistently measured at about three times the reference concentration. Lead and zinc were also accumulated by the aufwuchs community under both dosing regimes, the par stream aufwuchs concentrating more metal than the 0.5 par stream community.

Figure 28. Values for dry weights and ash free dry weights from Artificial Stream Heavy Metal Heavy Metal Dosing III. 12 June to 30 June 1981.


HEAVY METAL DOSING III
DRY WEIGHT IN MG PER SQ METER
28
26
24
22
20
18
16
14
12
10
8
6
0
2
4
6
8
10
12
14
16
18
20
DAYS
LEGEND: STA
.SPAR
PAR
REF

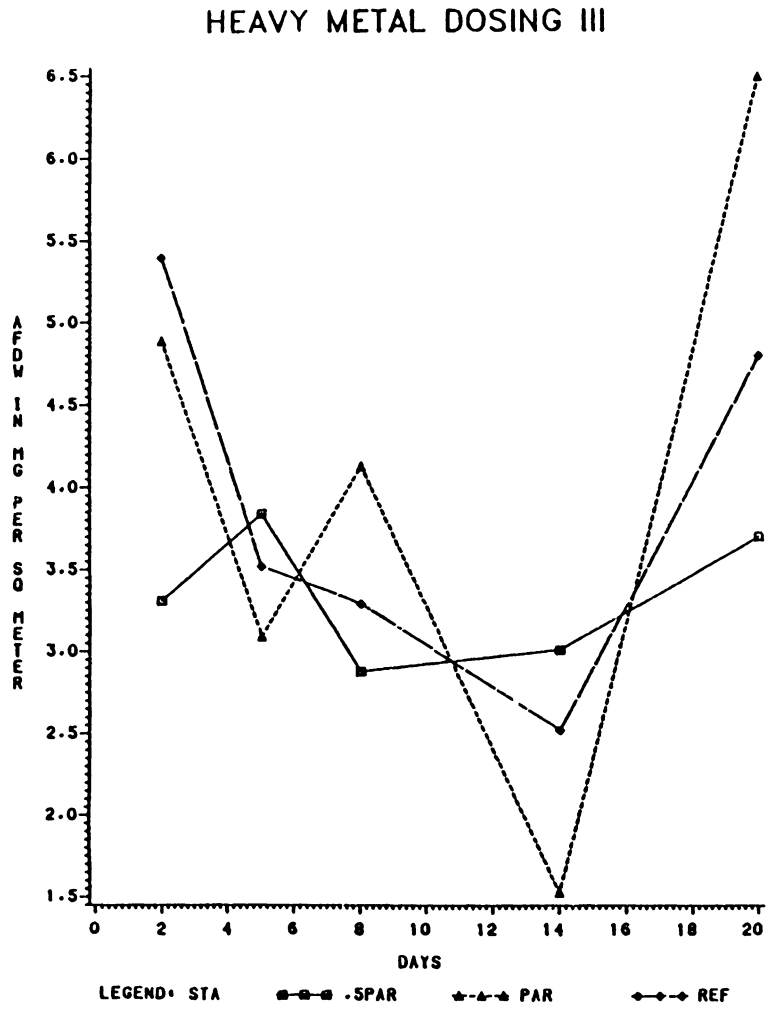
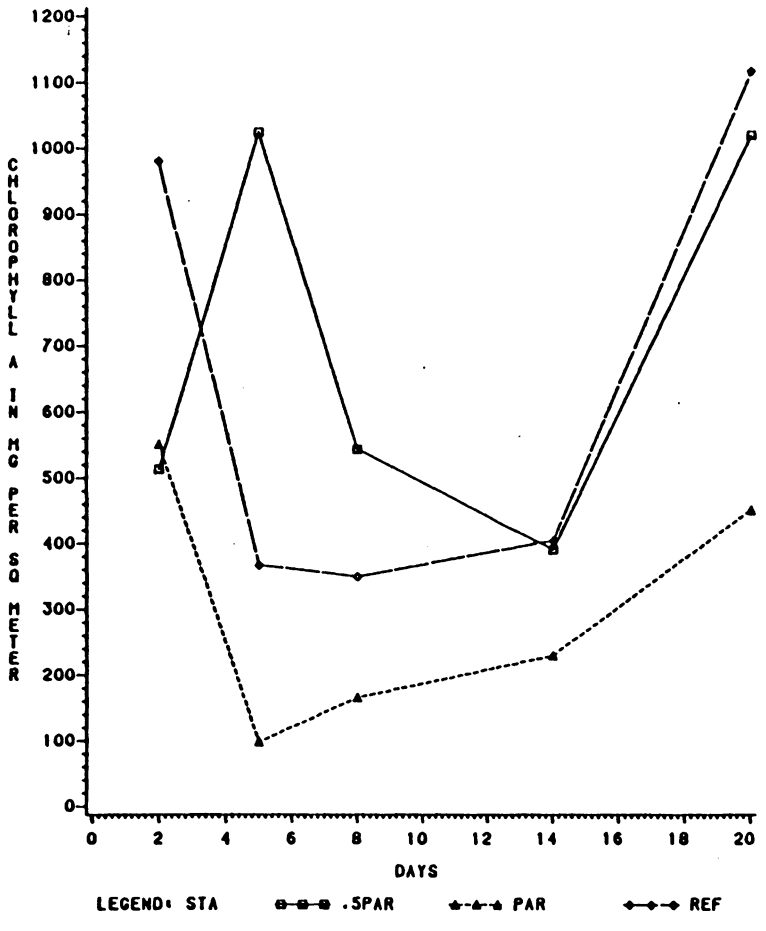

HEAVY METAL DOSING III
AFDW IN MG PER SQ METER
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0
2
4
6
8
10
12
14
16
18
20
DAYS
LEGEND: STA
.SPAR
PAR
REF

Figure 29. Values for chlorophyll a and ATP from Artificial Stream Heavy Metal Dosing III. 12 June to 30 June 1981.


HEAVY METAL DOSING III
CHLOROPHYLL A IN MG PER SQ METER
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0 2 4 6 8 10 12 14 16 18 20
DAYS
LEGEND: STA .5PAR PAR REF

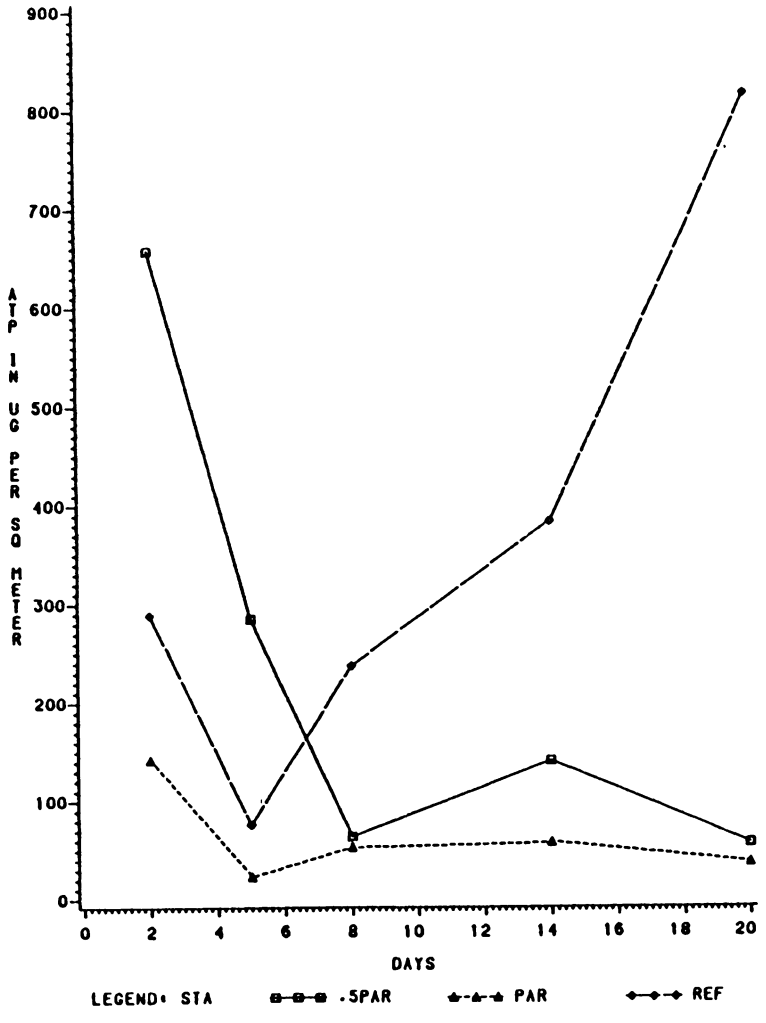

HEAVY METAL DOSING III
ATP IN UG PER SQ METER
900
800
700
600
500
400
300
200
100
0
0 2 4 6 8 10 12 14 16 18 20
DAYS
LEGEND: STA .5PAR PAR REF

Figure 30. Values for light carbon fixation rates from Artificial Stream Heavy Metal Dosing III. 12 June to 30 June 1981.

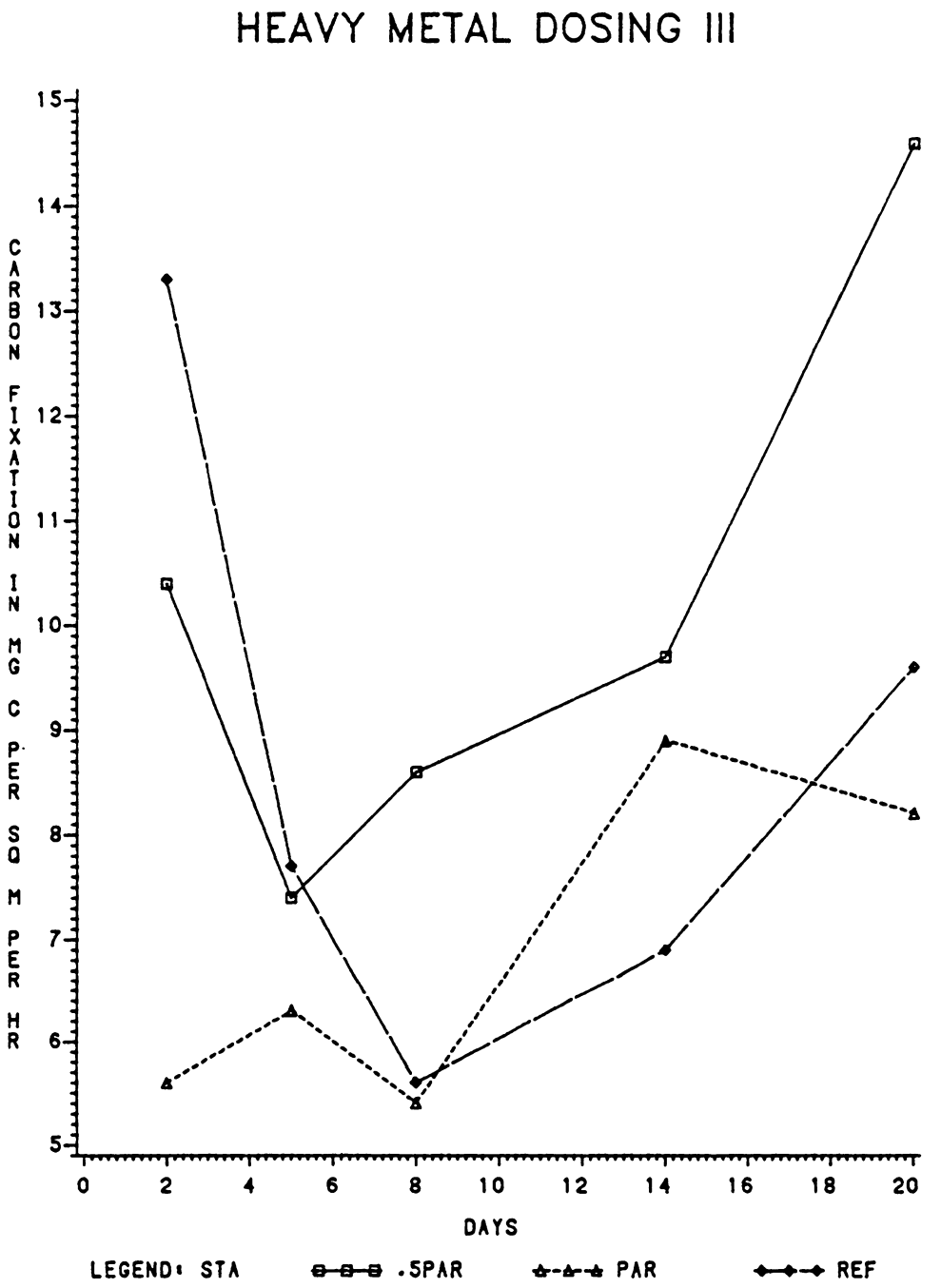

HEAVY METAL DOSING III
CARBON FIXATION IN MG C PER SQ M PER HR
15
14
13
12
11
10
9
8
7
6
5
0
2
4
6
8
10
12
14
16
18
20
DAYS
LEGEND: STA .SPAR PAR REF

Table 21. Metal content of aufwuchs from heavy metal dosing experiment (ug/g dry weight).

Metal	Treatment	Day 2	5	8	14	20
Cd	Reference	3.95	3.31	4.08	4.52	3.43
	0.5 Par	5.50	76.07	51.71	59.51	37.14
	Par	14.64	118.53	121.52	107.42	49.72
Cr	Reference	14.76	39.28	50.16	25.31	28.16
	0.5 Par	21.76	52.09	32.99	21.92	34.46
	Par	46.78	90.56	72.59	57.14	71.64
Cu	Reference	21.62	26.53	14.52	16.25	12.02
	0.5 Par	44.34	174.10	119.73	211.89	72.27
	Par	150.88	507.28	504.05	1130.09	398.64
Ni	Reference	118.08	67.21	66.75	32.30	83.77
	0.5 Par	165.72	248.60	168.40	189.54	134.54
	Par	424.83	510.65	527.77	602.02	740.60
Pb	Reference	83.11	152.90	94.02	89.98	33.84
	0.5 Par	124.44	298.11	204.57	229.90	238.04
	Par	104.62	524.52	264.61	701.80	602.64
Zn	Reference	10.99	318.53	453.55	225.48	108.85
	0.5 Par	15.70	940.18	540.29	966.66	903.80
	Par	46.59	2191.07	2118.31	2950.67	2344.28

Table 22. Artificial stream heavy metal uptake experiment: Water chemistry data (mg/l).

Parameter		Day 2	5	8	14	20
Cd	Reference		.025		.025	.025
	0.5 Par		.025		.025	.025
	Par		.025		.025	.025
Cr	Reference		.1		.1	.1
	0.5 Par				.1	.1
	Par		.285		.1	.1
Cu	Reference		.09		.09	.09
	0.5 Par				.09	.09
	Par		.09		.09	.09
Ni	Reference					
	0.5 Par					
	Par					
Pb	Reference		.001		.001	.001
	0.5 Par				.039	.001
	Par		.025		.034	.054
Zn	Reference		.037		.030	.018
	0.5 Par				.115	.063
	Par		.416		.160	.178
Temperature		23.0	24.5	22.0	24.5	24.5
pH		6.7	7.3	6.5	6.85	7.0
Turbidity (JTU)		8.6	2.7	7.5	4.8	4.5
Alkalinity		36	52	44	44	56

Table 23. Results of Duncan's New Multiple Range Test for artificial stream heavy metal dosing experiment III data at the 0.05 level of significance. Treatments with the same letter are not significantly different.

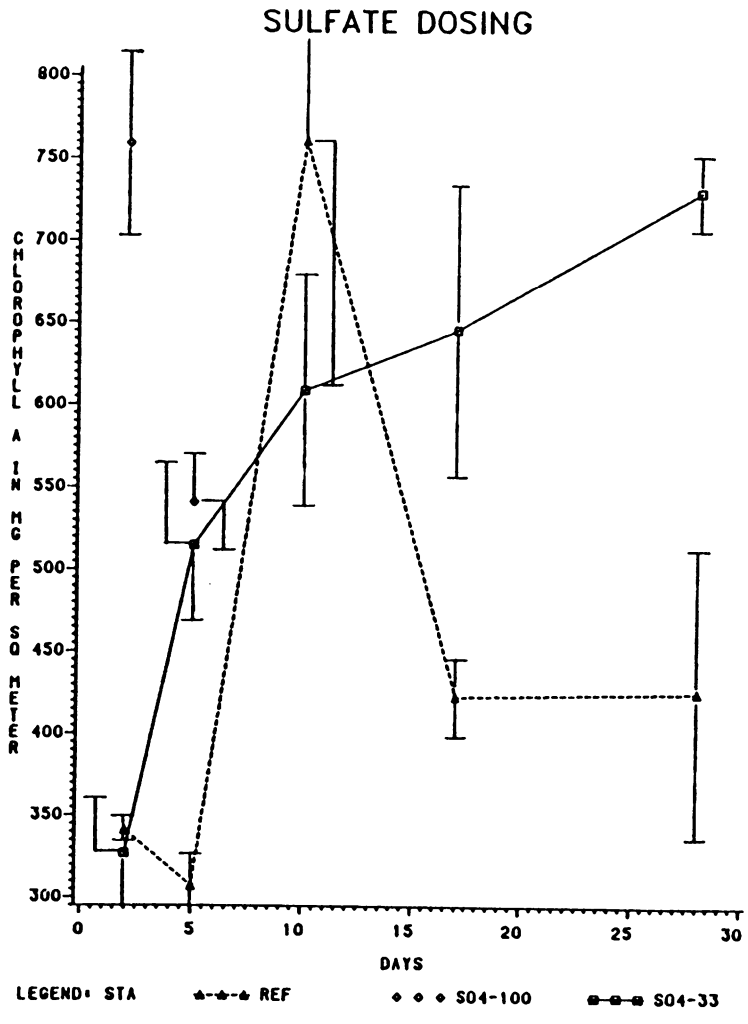
Parameter	Day 2		Day 5		Day 8		Day 14		Day 20	
Dry Weight	0.5 Par	A	0.5 Par	A	0.5 Par	A	0.5 Par	A	0.5 Par	A
	Par	A	Par	A	Par	A	Ref	A	Par	B
	Ref	B	Ref	A	Ref	A	Par	B	Ref	C
Ash Free Dry Weight	0.5 Par	A	0.5 Par	A	0.5 Par	A	0.5 Par	A	0.5 Par	A
	Par	B	Par	A	Par	A	Par	B	Par	B
	Ref	C	Ref	B	Ref	A	Ref	B	Ref	C
Chlorophyll a	0.5 Par	A	0.5 Par	A	0.5 Par	A	0.5 Par	A	Par	A
	Par	A	Par	B	Par	B	Ref	A	0.5 Par	B
	Ref	B	Ref	C	Ref	C	Par	B	Ref	B
ATP	0.5 Par	A	0.5 Par	A	0.5 Par	A	0.5 Par	A	0.5 Par	A
	Par	B	Par	B	Par	A	Par	B	Par	B
	Ref	C	Ref	B	Ref	B	Ref	B	Ref	B
Light Carbon Fixation	0.5 Par	A	0.5 Par	A	0.5 Par	A	0.5 Par	A	0.5 Par	A
	Par	B	Ref	A	Par	B	Par	B	Par	B
	Ref	C	Par	B	Ref	B	Ref	B	Ref	B

4.2.4 Sulfate Dosing

The effects of the potential sulfate enrichment, as Na2SO4, was investigated in the aufwuchs community utilizing the artificial stream system. During a 28-day experiment one stream received sulfate at 33 ppm, and another stream was dosed at 100 ppm (Table 24). This proved to be too high a concentration for the dosing apparatus, due to precipitation and subsequent blocking of the dosing tubes, so this concentration was terminated after five days. The actual in-stream measurements for dosed sulfate were 117 mg/l (Table 24). Dry weights were significantly higher in both the 33 and 100 ppm dosed streams compared to the reference stream, with no significant differences between sulfate dosed streams. On day 5 both dosed streams dropped to significantly lower levels than found in the reference stream. No significant differences between streams were observed although values in each continued to increase until day 17 (Tables 25 and 26). On day 28, dry weight values decreased in the reference stream so that the sulfate dosed stream values were significantly greater. Ash free dry weights demonstrated a similar trend, except the 100 ppm dosed stream was not significantly greater than the reference value on day 2. Ash free dry weights were significantly greater in the 33 ppm dosed stream on day 28 (Figure 31). Chlorophyll

a values were extremely high in the 100 ppm dosed stream on day 2, and remained significantly greater than reference values on day 5. The 33-ppm dosed stream values exhibited a consistent increase throughout the 28 day experiment, and were significantly higher in the dosed stream on days 5, 17, and 28. ATP values showed no consistent trend during the experiment. Neither of the dosed sulfate streams was significantly greater than the reference stream values on days 2, 5, and 17. On day 10 the 33-ppm stream ATP values were significantly greater than the reference stream values, but ATP quantities decreased in the dosed streams, so by day 28 the reference stream values were significantly greater (Figure 32). Light carbon fixation rates were significantly lower in the 100 ppm dosed stream on day 2. For the other streams, and days, no significant differences were observed until day 28, when a slight rise in carbon fixation rates in the 33 ppm dosed stream resulted in significantly higher values than those of the reference stream (Figure 33).

Figure 31. Dry weights and ash free dry weights from Artificial Stream Sulfate Dosing.
28 Sept. to 21 Oct. 1981.
Bars are one standard error of the mean.


SULFATE DOSING
CHLOROPHYLL A IN MG PER SQ METER
800
750
700
650
600
550
500
450
400
350
300
0
5
10
15
20
25
30
DAYS
LEGEND: STA
REF
S04-100
S04-33

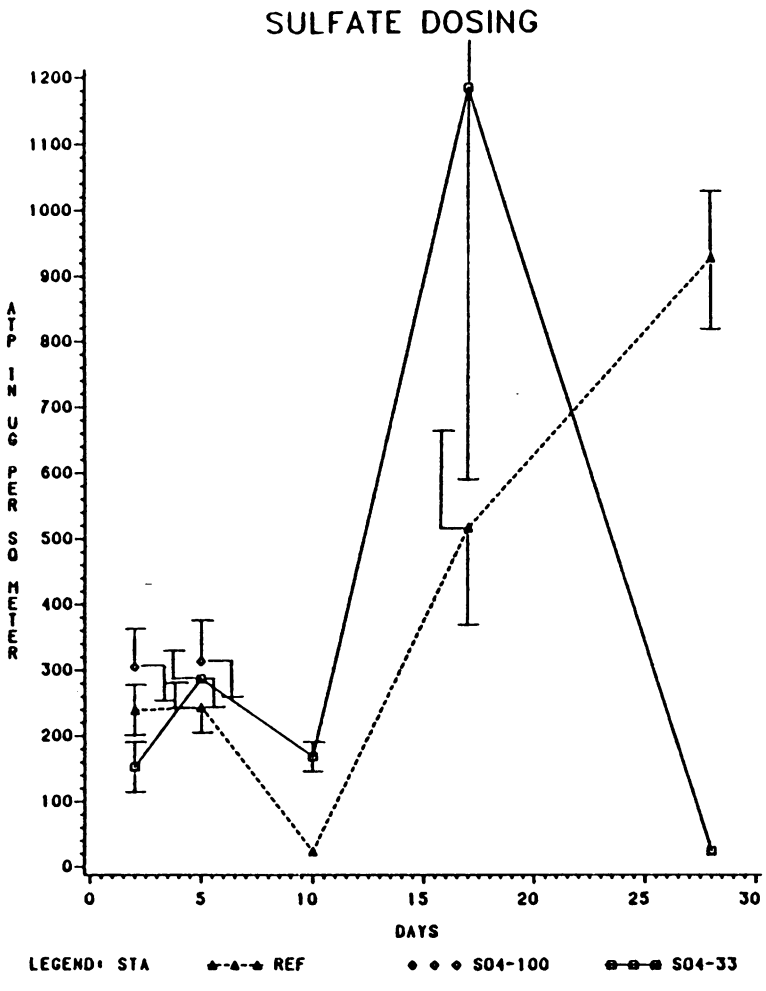
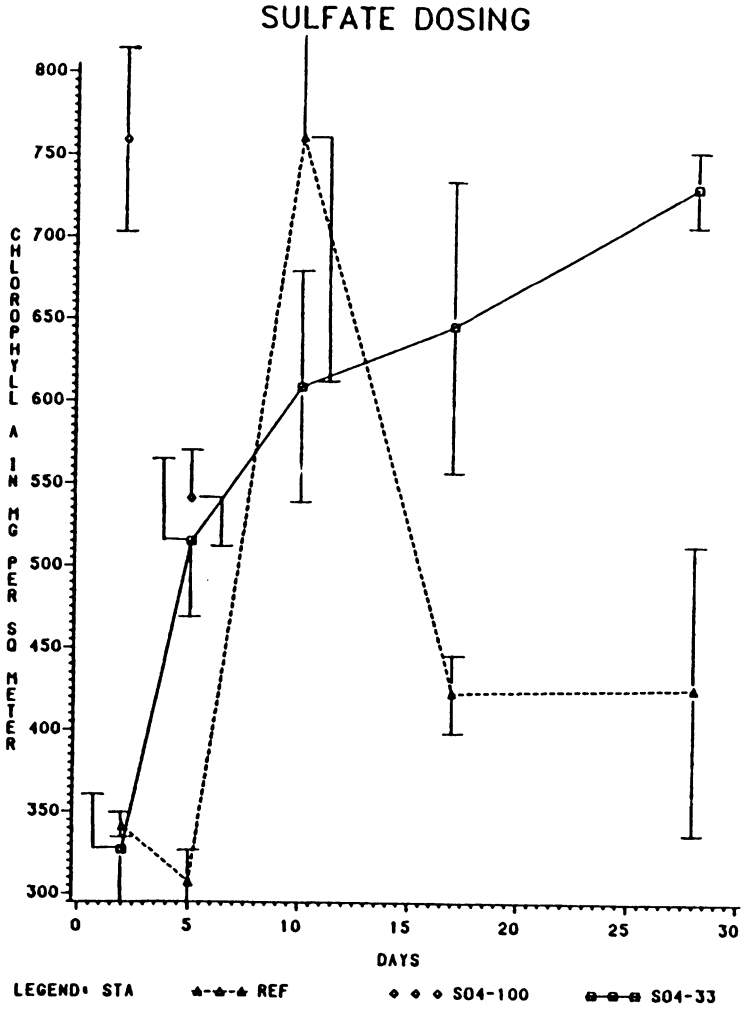

SULFATE DOSING
ATP IN UG PER SQ METER
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
DAYS
LEGEND: STA
REF
S04-100
S04-33

Figure 32. Values for chlorophyll a and ATP from Artificial Stream Sulfate Dosing.
28 Sept. to 21 Oct. 1981.
Bars are one standard error of the mean.


SULFATE DOSING
CHLOROPHYLL A IN MG PER SQ METER
800
750
700
650
600
550
500
450
400
350
300
0
5
10
15
20
25
30
DAYS
LEGEND: STA
REF
SO4-100
SO4-33

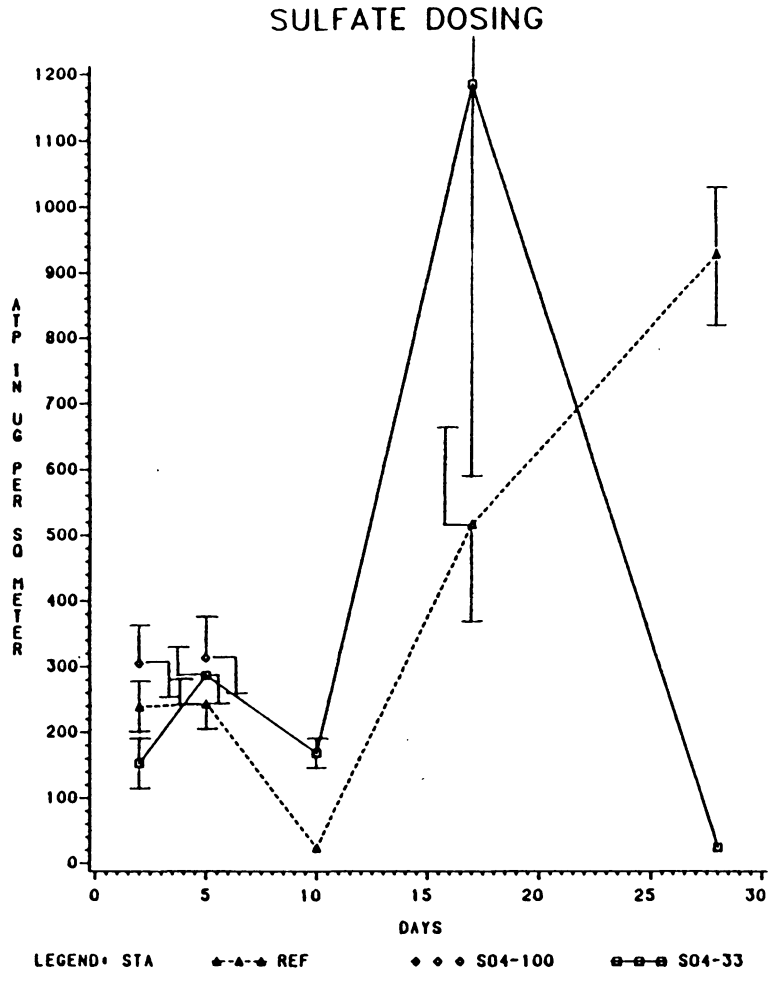

SULFATE DOSING
ATP IN UG PER SQ METER
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
DAYS
LEGEND: STA
REF
SO4-100
SO4-33

Figure 33. Value for light carbon fixation rates from Artificial Stream Sulfate Dosing.
28 Sept. to 21 Oct. 1981.
Bars are one standard error of the mean.

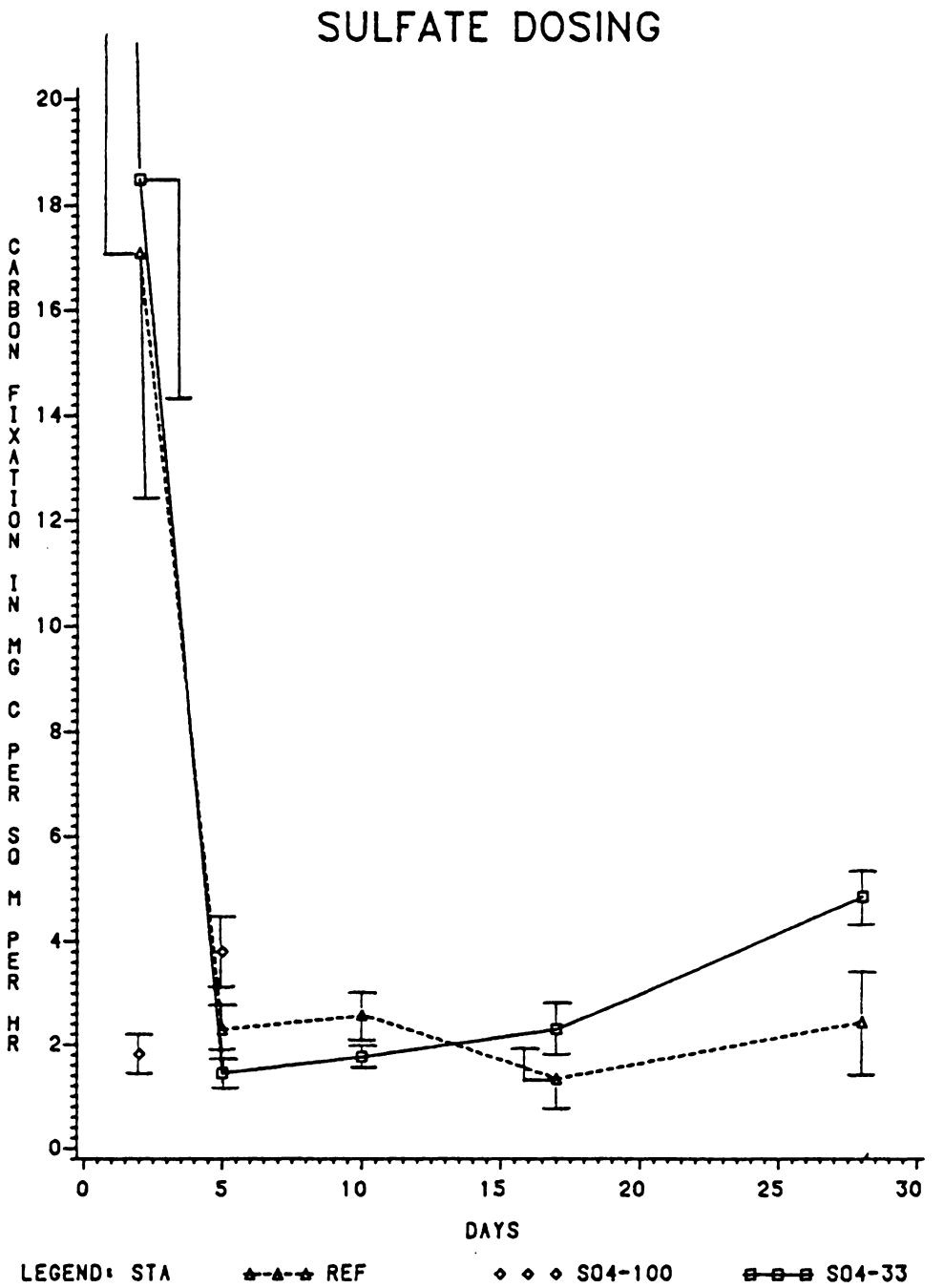

SULFATE DOSING
CARBON FIXATION IN MG C PER SQ M PER HR
0
2
4
6
8
10
12
14
16
18
20
0
5
10
15
20
25
30
DAYS
LEGEND: STA
REF
SO4-100
SO4-33

Table 24. Water chemistry data for sulfate dosing experiment - 9/28/80 - 10/21/81.

Parameter	Day and Stream 2 SO_4-33	2 Reference	2 SO_4-100	2 Reference	5 SO_4-33	5 Reference	5 SO_4-100	5 Reference	10 SO_4-33	10 Reference	17 SO_4-33	17 Reference	24 SO_4-33	24 Reference
Temperature ^oC	20.0	20.0	18.0	18.0	18.0	18.0	16.0	16.0	16.0	16.0	14.0	14.0	11.0	11.0
pH	8.4	8.3	8.6	8.3	8.6	8.3	8.1	8.0	8.1	8.0	8.3	8.1	8.1	8.2
Alkalinity mg l^{-1} $CaCO_3$	70	64	64	66	64	66	70	66	70	66	68	68	73	70
Turbidity JTU	1.3	1.5	1.8	1.3	1.8	1.3	0.9	1.0	0.9	1.0	1.6	1.2	1.2	1.0
Sulfate ppm			117		74									

Table 25. Results of Wilcoxon Sign Rank Test for Artificial Stream Sulfate Dosing Experiment (33 ppm) data at the 0.05 level of significance. S+ indicates that sulfate dosed stream values are significantly higher than reference stream values. R+ indicates higher reference stream values. NS indicates no significant differences between treatments. Blanks are due to insufficient data.

Parameter	Day 2	5	10	17	28
Dry Weight	S+	R+	NS	NS	S+
Ash Free Dry Weight	S+	R+	NS	S+	S+
Chlorophyll a	NS	S+	NS	S+	S+
ATP	NS	NS	S+	NS	R+
Light Carbon Fixation	NS	R+	R+	NS	S+

Table 26. Results of Wilcoxon Sign Rank Test for Artificial Stream Sulfate Dosing Experiment (100 ppm) data at the 0.05 level of significance. S+ indicates that sulfate dosed stream values are significantly higher than reference stream values. R+ indicates higher reference stream values. NS indicates no significant differences between treatments. Blanks are due to insufficient data.

Parameter	Day	
	2	5
Dry Weight	S+	R+
Ash Free Dry Weight	NS	R+
Chlorophyll a	S+	S+
ATP	NS	NS
Light Carbon Fixation	R+	S+

Table 27. Water chemistry data for thermal enrichment experiment - 10/21/81 - 11/09/81.

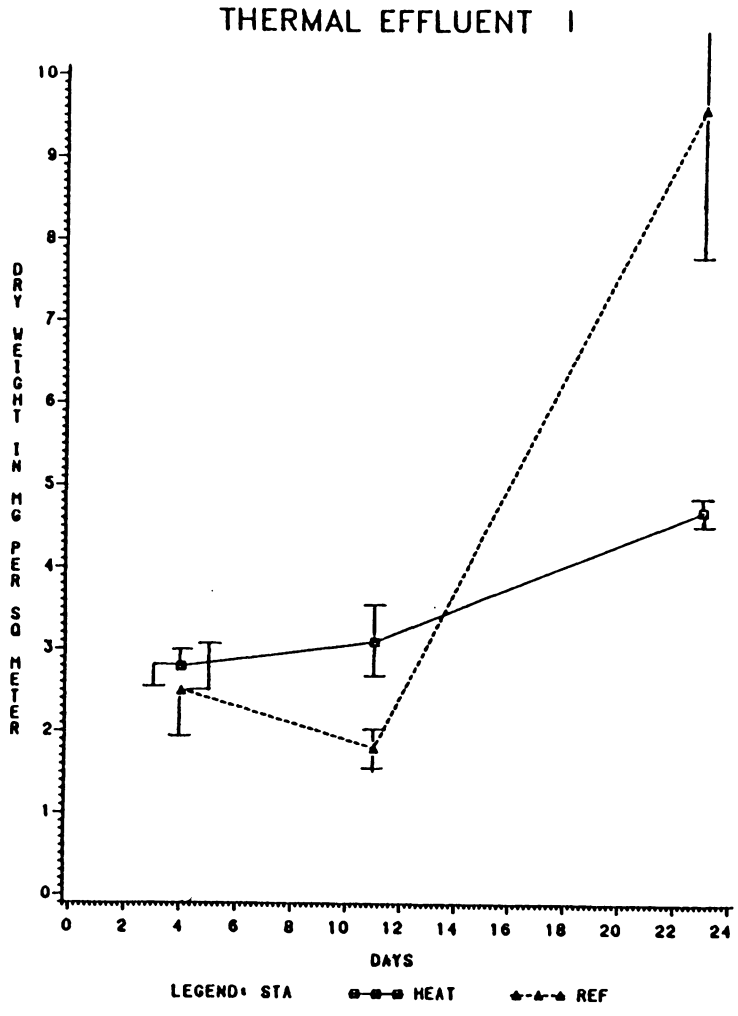
Parameter	Day and Stream 4 Thermal	4 Reference	11 Thermal	11 Reference	23 Thermal	23 Reference
Temperature ^{o}C	29.5	11.0	20.0	14.0	19.0	10.0
pH	8.0	8.2	7.7	7.7	7.8	7.6
Alkalinity mg l^{-1} $CaCO_3$	70	70	64	64	65	65
Turbidity JTU	1.2	1.0	2.3	1.9	1.0	1.2

4.2.5 Thermal Effluent I

Due to a decreased demand for electrical power by the American Electric Power network during the study period, the Glen Lyn unit supplying heated effluent to the artificial stream system was inoperative for several months. For this reason, only the results of one experiment conducted utilizing the heated artificial stream system were available for analysis. During the 23-day experiment to assess the environmental impacts of the thermal effluent upon the aufwuchs community, temperatures in the heated streams ranged from 19.0 to 29.0 C, those of the reference stream from 10.0 to 14.0 C, giving an average temperature differential of 11.0 C. Dry weights and ash free dry weights were not significantly different between reference and thermal streams on the first sampling day, day 4. On day 11, thermal stream dry weight values were significantly greater than reference values, and on day 23, reference values were significantly greater (Table 28). Ash free dry weights increased on days 11 and 23, as did the reference values, but the heated stream values remained significantly greater. Higher variability of both dry weights and ash free dry weights was observed in the reference streams (Figure 34). Chlorophyll a values were significantly greater in the reference stream on day 4, significantly greater in the heated stream on day 11, and not significantly different between streams on day 23.

ATP values were significantly greater in the reference stream on days 4 and 23 (Figure 35). There was no significant difference between treatments on day 11 for ATP values. Light carbon fixation rates were highly variable during this experiment, and were significantly greater in the thermal effluent stream only on day 23 (Figure 36).

Figure 34. Values for dry weights and ash free dry weights for Artificial Stream Thermal Effluent I. 21 Oct. to 9 Nov. 1981. Bars are one standard error of the mean.


THERMAL EFFLUENT I
DRY WEIGHT IN MG PER SQ METER
0
1
2
3
4
5
6
7
8
9
10
0 2 4 6 8 10 12 14 16 18 20 22 24
DAYS
LEGEND: STA HEAT REF

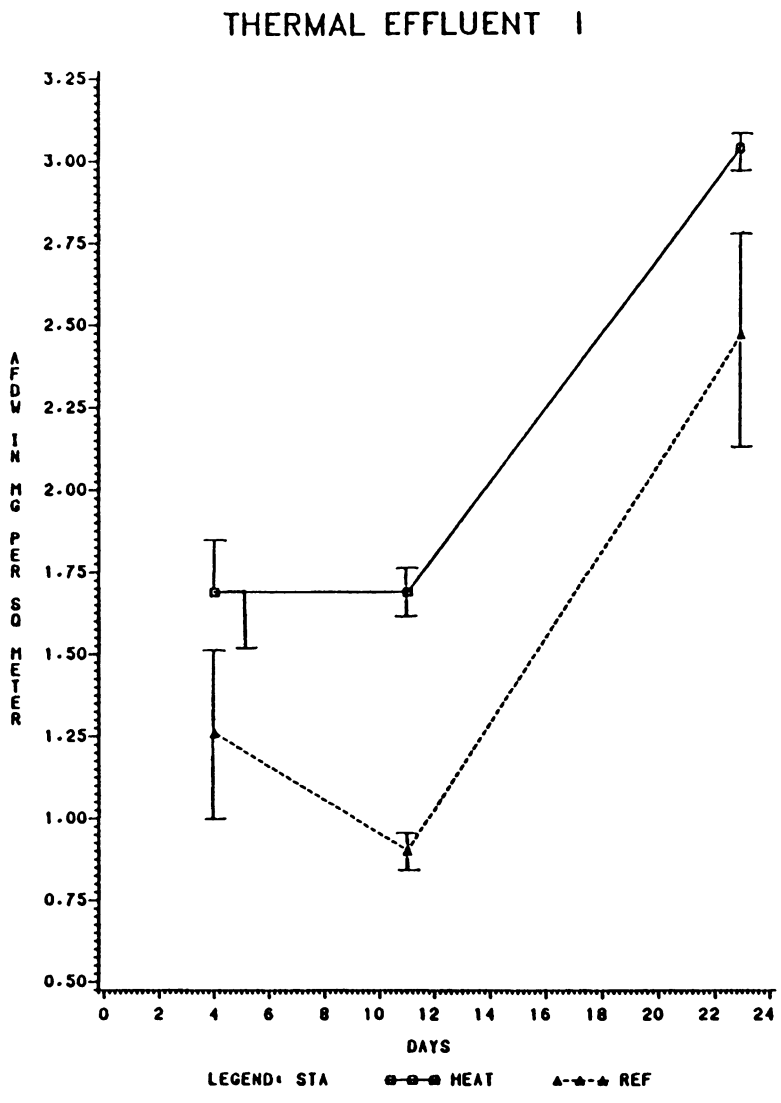
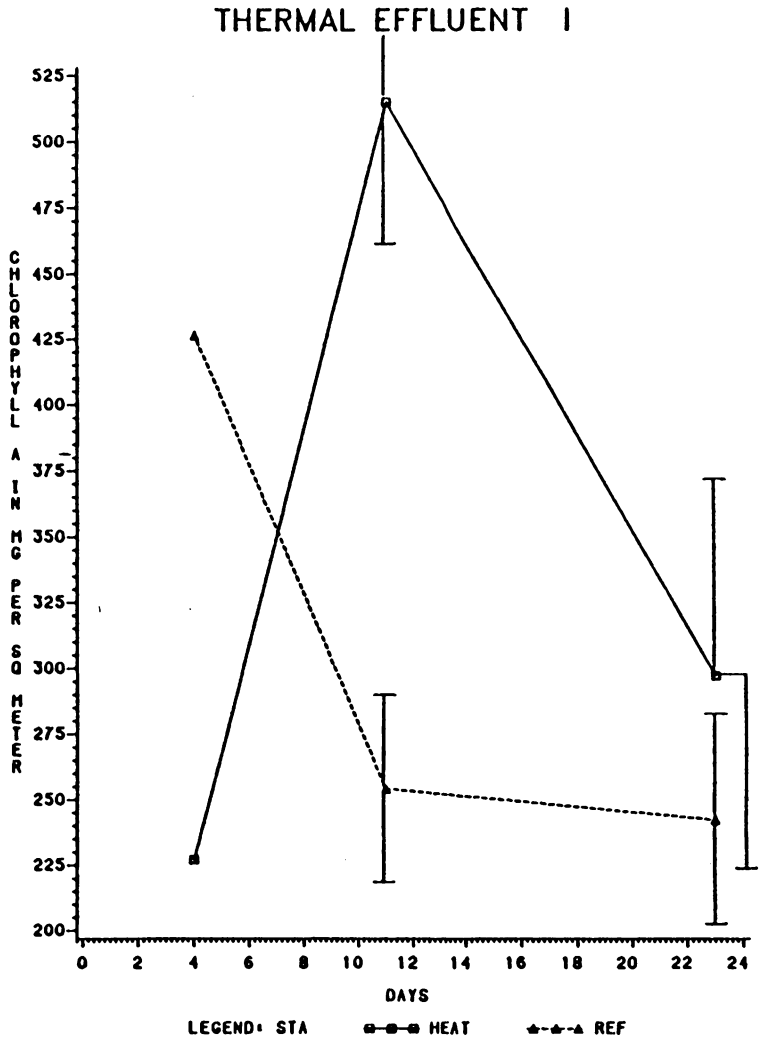

THERMAL EFFLUENT I
AFDW IN MG PER SQ METER
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
2.50
2.75
3.00
3.25
0 2 4 6 8 10 12 14 16 18 20 22 24
DAYS
LEGEND: STA HEAT REF

Figure 35. Values for chlorophyll a and ATP from Artificial Stream Thermal Effluent I. 21 Oct. to 9 Nov. 1981. Bars are one standard error of the mean.


THERMAL EFFLUENT I
CHLOROPHYLL A IN MG PER SQ METER
525
500
475
450
425
400
375
350
325
300
275
250
225
200
0 2 4 6 8 10 12 14 16 18 20 22 24
DAYS
LEGEND: STA HEAT REF

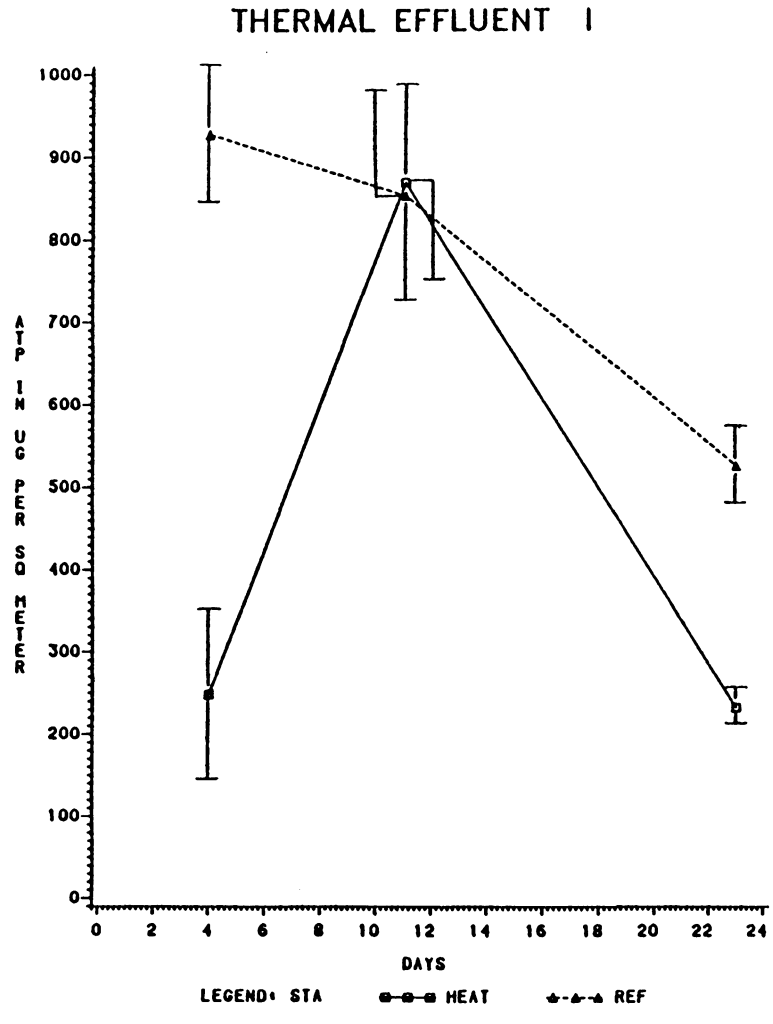

THERMAL EFFLUENT I
ATP IN UG PER SQ METER
1000
900
800
700
600
500
400
300
200
100
0
0 2 4 6 8 10 12 14 16 18 20 22 24
DAYS
LEGEND: STA HEAT REF

Figure 36. Values for light carbon fixation rates from Artificial Stream Thermal Effluent I.
21 Oct. to 9 Nov. 1981.
Bars are one standard error of the mean.

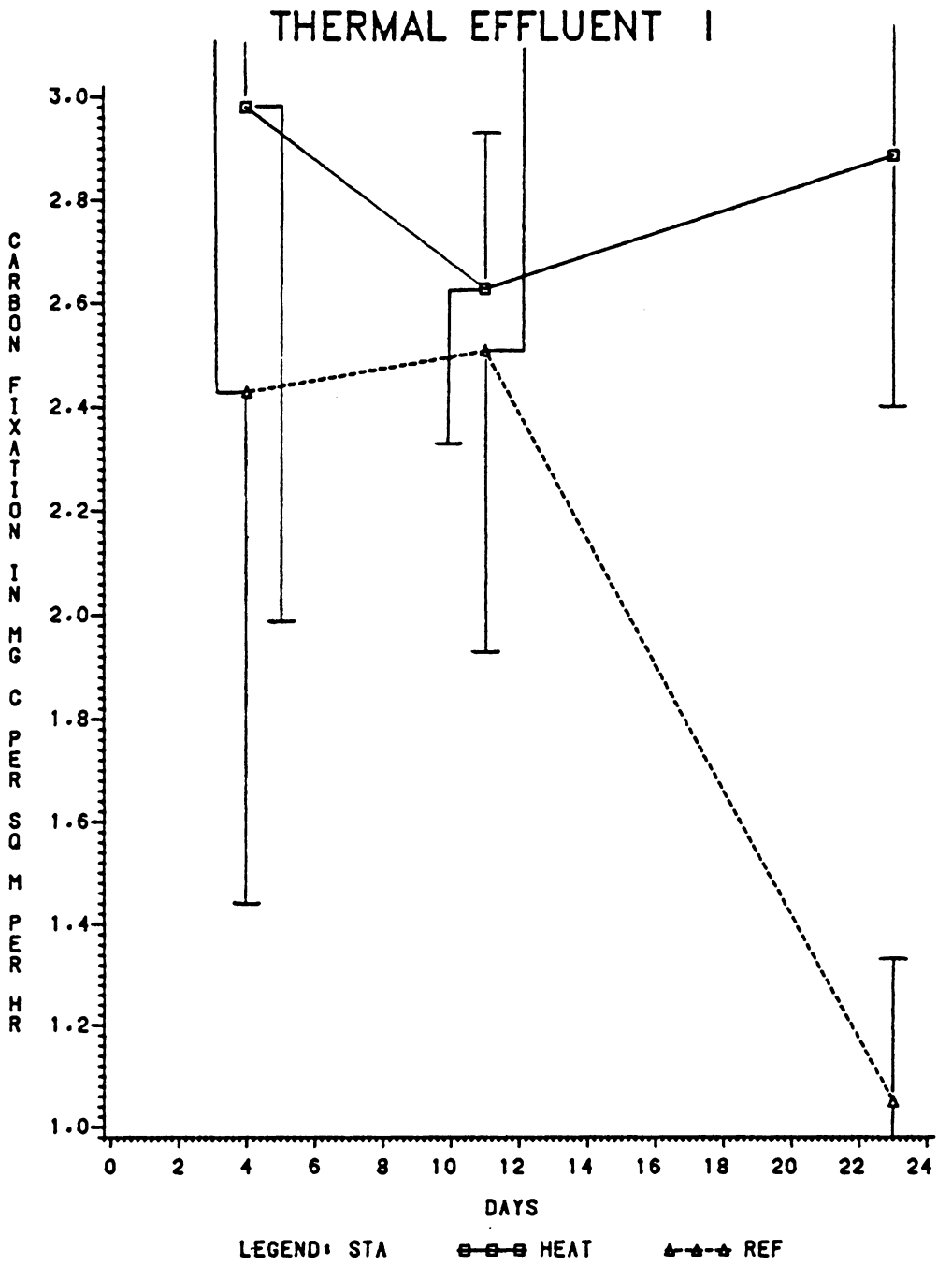

THERMAL EFFLUENT I
CARBON FIXATION IN MG C PER SQ M PER HR
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0
2
4
6
8
10
12
14
16
18
20
22
24
DAYS
LEGEND: STA
HEAT
REF

Table 28. Results of Wilcoxon Sign Rank Test for Artificial Stream Thermal Effluent Experiment I data at the 0.05 level of significance. H+ indicates that thermally enriched stream values are significantly higher than reference stream values. R+ indicates higher reference stream values. NS indicates no significant differences between treatments. Blanks are due to insufficient data.

Parameter	Day		
	4	11	23
Dry Weight	NS	H+	R+
Ash Free Dry Weight	NS	H+	H+
Chlorophyll a	R+	H+	NS
ATP	R+	NS	R+
Light Carbon Fixation	NS	NS	H+

Chapter V

DISCUSSION

5.1 FIELD STUDIES IN ADAIR RUN

The results of field studies conducted in Adair Run during operation of the fly ash basin indicated that the primary producing aufwuchs community is not adversely affected by the ash basin effluent, as measured by selected structural and functional parameters. Although total suspended solids (TSS), heavy metal concentrations, and pH values were consistently higher at the downstream ash-influenced station than at the upstream reference station, no inhibition of aufwuchs colonizing diatometers was measured. In fact, an increase in dry weight, ash free dry weight, chlorophyll a, and light carbon fixation rates at the downstream station indicated a potential stimulatory effect of the ash basin effluent to this community by as much as eight times the reference station value (light carbon fixation rates). Apparently increases in temperature (delta T=4.0 C) which resulted from solar energy gain during the long residence time of the sluice water within the settling basin, and a maximum increase in sulfate concentrations of 40 mg/l above the upstream reference station, were great enough to offset the inhibiting potential of increased suspended solids, pH ex-

cursions, and toxic heavy metals present at the downstream station. The return of downstream structural and functional parameters to values closely modeling the upstream reference values shortly following the termination of inputs to the fly ash basin, and a corresponding convergence of measured physical and chemical qualities indicates that observed differences between the two stations is directly attributable to the addition of the fly ash basin effluent to the Adair Run ecosystem. The typical rapid flushing rates characteristic of a small mountain stream, such as Adair Run, also tended to return the ash-influenced station parameters to those of the upstream reference station. Data collected indicate that in this system, complete recovery was achieved after approximately three months. In response to the complex effluent discharge from the fly ash basin, the aufwuchs community has apparently substantially changed in composition, diversity, and overall function; the downstream community has become adapted to ultilize the specific components within the basin discharge to a degree reflected in an increase in productivity and function not demonstrated by the upstream reference station. Following termination of the settling basin, the recovery exhibited by the aufwuchs community indicates the flexibility of this community, and the inherent ability to rapidly adjust structural and functional

parameters to changing environmental conditions. Dry weights and ash free dry weights demonstrated a rapid, almost immediate recovery response, and light carbon fixation rates approximated those of the reference station within two months, although chlorophyll a values remained slightly higher at the downstream station three months later.

The results of this study indicate that measurements of aufwuchs, by taking advantage of the high degree of environmental information integrated within this mountain stream community, can provide a means to monitor changes occurring within an ecosystem attributable to fly ash perturbation.

That aufwuchs measurements can serve as useful indicators of environmental changes is well documented. Williams (1969) demonstrated that raw Kraft Mill effluents enhanced heterotrophic activity in laboratory streams, while stabilized effluents stimulated autotrophy, as measured by photosynthetic and respiration rates, biomass (ash free dry weight), chlorophyll a, and species diversity. His study demonstrated the ability of the aufwuchs community to rapidly shift its specie composition and diversity, and thus other measurable parameters in response to perturbation. Economou-Amilli (1979), utilized a microfloristic "species deficit" methodology to evaluate water quality in running waters polluted by domestic sewage. The presence or absence

of individual diatom and alga species was utilized to construct an index of the relative degree of pollution at each sampling site. Cooper and Wilhm (1975), showed that in a stream receiving domestic and oil refinery effluents, the aufwuchs community exhibited spatial and temporal variations in primary productivity, species diversity, and chlorophyll content, depending upon the degree of pollution present. Patrick (1973), Patrick et al (1968, 1969), Cairns et al (1972), and Whitton and Diaz (1981) have documented numerous examples of changes in the structure and function of aufwuchs and diatom communities in response to various environmental parameters including organic pollution, nutrients, toxic substances, pH, and temperature. Guthrie et al. (1978a, 1978b), and Guthrie and Cherry (1979), reported changes in bacterial community structure and diversity in populations exposed to pH, temperature, and heavy metal loading from fly ash effluent with a relatively rapid degree of recovery in approximately two years after a new, more efficient, settling basin was operating (Guthrie et al, 1982).

A more detailed examination of the specific parameters measured during this investigation will illustrate further the changes observed between stations, and help to characterize the environmental impact of the primary producing aufwuchs community inhabiting the Adair Run ecosystem.

5.1.1 Dry Weights and Ash Free Dry Weights

Differences in values for dry weights and ash free dry weights between the upstream and downstream stations increased as the basin reached maximum operating capacity. Observed ranges for dry weights, 3.14-29.2 g m -2, and ash free dry weights, 0.26 - 3.7 g m -2 for both the upstream and downstream stations agreed closely with the published literature (Cushing, 1967; McIntire and Phinney, 1965; Kevern et al., 1966; King and Ball, 1966; McIntire, 1968b; Pavoni, 1969; Elwood and Nelson, 1972; Cattaneo and Ghittori, 1975; Mason and Bryant, 1975; Rodgers, 1977; Clark et al., 1979). That dry weights increased at the downstream station is not surprising since ash particles were observed to be adhering to the glass microscope slides as a thin gray film. The increase observed may be due either to the quantity of ash particles present, or to an increase in organic biomass and detritus. Although dry weight is far from being an absolute unit measure for comparison, since the biological material will always retain a variable quantity of residual water (Vollenweider, 1974). A similar pattern of increased ash free dry weight at the downstream station supported the probability of an increase in biologically derived material, not simply an accumulation of ash particles. Ash free dry weight, normally about 5 per cent of the total dry

weight (Soeder et al., 1975) in planktonic algae, may comprise a larger percentage in diatoms which produce massive inorganic skeletal structures. Although a detailed taxonomic survey of aufwuchs at each station was not conducted, it seems unlikely that a simple shift in species composition at the downstream sampling site occurred to favor non-diatom aufwuchs community members that would account for the higher downstream ash free dry weight values. Instead, an increase in total biomass, due to either a rise in total cell numbers or an increase in biologically produced detrital material at the downstream station, is probably the cause of higher values. Neither dry weights nor ash free dry weights can differentiate between the two above possibilities.

Because chlorophyll a is normally the most important and abundant photosynthetic pigment present in living material (Vollenweider 1974), its measurement can provide a useful estimate of algal biomass (Weber, 1974). Rieman and Ernst (1982) support the acetone extraction methodology utilized in this investigation, although different extraction methods and solvents (methanol, ethanol) might have produced slightly different results. The observed values of from 3.56 to 282 mg m -2 of chlorophyll a in the Adair Run system exhibited a somewhat broader range than any one of the following studies, but were well within the limits of the reported

values as a whole (McConnell and Sigler, 1959; Stockner and Armstrong, 1971; Cattaneo and Ghittori 1975; Rodgers, 1977; Clark et al., 1979; Rodgers et al., 1979), although McIntire et al (1964), reported somewhat higher values in laboratory streams (0.44 to 1.36 g m -2). In general the chlorophyll a data collected during the Adair Run field investigations confirmed the stimulating effect of the fly ash basin effluent to the aufwuchs community. Although measured values were lower at the downstream station until 20 June, 1980, from then until the end of the study (31 October), they remained higher than the upstream station. This indicates that either there were more photosynthetic organisms containing chlorophyll a at the downstream station, or those individuals present had acquired greater quantities of this pigment than members of the upstream community.

Values for ATP content of aufwuchs in the Adair Run system were extremely variable. Values in October and November, 1979 were the highest observed (4266 and 3480 ug ATP m -2), these values dropping to 6.27 ug/m 2 in July, 1980. No trend between upstream and downstream stations was observed, except for a decrease in values from June to October, 1980 at both sampling stations. Therefore ATP measurements appeared not to offer a viable means of detecting differences between stations in the Adair Run system, utilizing the methodology described in this manuscript.

Strong correlations between ATP and chlorophyll a contents of algae have been described by several authors (Patterson et al, 1970; Hobbie et al, 1972; Brezonik et al, 1975; Holm-Hansen, 1969), and between dry weights and ATP in bacterial biomass (Hamilton and Holm-Hansen, 1967). The lack of similar correlations in the present study was disappointing. Investigators who have utilized ATP measurements as an indicator of toxic or stimulatory effects of various compounds in natural samples and laboratory cultures (Kennicutt, 1980; Brezonik, 1975) proposed that ATP assays had definite potential for monitoring the toxicity of environmental contaminants in natural ecosystems. The results of the ATP assays in Adair Run fail to support that claim. Although the ubiquitous nature of ATP in all living matter would seem to render it a useful parameter to investigate under most circumstances, the extreme sensitivity and rapid degradation of this cellular component following cell death can introduce extraction and assay complications, leading to inaccurate estimations of actual ATP content. Problems with sample handling, transport, and temperature variations during the boiling extraction, and freezing, of aufwuchs samples are probable reasons for the inaccuracy and variability of the Adair Run ATP measurements. Glass microscope slides may have served as a thermal sink during the extraction

step, which would also introduce a source of error. Some of these problems have been addressed by Tobin et al (1978), who have proposed some solutions to the problem of replicability in ATP assay procedures. Despite the recognized problems with the ATP assay procedure, the decision was made to continue utilizing the assay protocol for the artificial stream system investigations, instead of developing a more sensitive technique. This would help to isolate and identify what specific problems were creating the high degree of variability with the procedure. ATP values were much less variable in the artificial stream system, possibly due to the lack of handling involved with aufwuchs samples, and due also to a decreased time period before ATP extraction was initiated.

5.1.2 Light and Dark Carbon Fixation Rates

The measurement of carbon fixation rates, more than any other functional parameter of the aufwuchs community, have the potential for providing information concerning the ability of the ecosystem to function under environmental stress. The measurement of this parameter in the Adair Run system provided some insights into the observed differences between the two sampling stations,which were assumed to be the direct result of effluent from the fly ash basin. While light

carbon fixation rates remained fairly constant in the upstream reference station during the study period (0.38 - 3.50 mg C m 2 hr 1), values at the downstream station reached 37.8 mg C m 2 hr -1 during the time of maximum basin capacity, and therefore maximum environmental impact observed due to the basin effluent. The pattern of measured carbon fixation rates during basin evolution was similar to those exhibited by dry weights, ash free dry weights, and chlorophyll a values, and indicated that a stimulation of the primary producing community was occurring at the downstream, ash-influenced station.

Exactly what is being measured by the 14-C tracer methodology is open to speculation. The basic principle is that a small amount of a specific radioisotope (14-carbon), added to a sample of water containing actively photosynthesizing organisms, will be assimilated at the same rate (allowing for some isotopic correction) as the corresponding non-labelled compound which occurs naturally in the water sample (CO_2) (Vollenweider, 1974). Since the tracer method measures an entirely different enzyme system than the one assayed by detecting changes in dissolved oxygen, comparisons of values for rates of primary productivity obtained by the two methods is not possible (Hunding and Hargrave, 1973; Andersen and Sand-Jensen, 1980). Measured rates in the Adair

Run system were well within values obtained by Rodgers (1977), and Rodgers et al, (1979), in a similar study conducted a few years earlier.

Andersen and Sand-Jensen (1980) suggest that, in the absence of respiratory release of 14-C under light conditions, 14-C fixation rates will measure net photosynthesis when reassimilation of respired 12-C compounds is complete. Gross photosynthetic rates will be measured when no reassimilation occurs. Since reassimilation rates are highly variable in laboratory cultures of algae, and must be even more so in the heterogeneous aufwuchs community, there is probably no means of obtaining a value for either net or gross photosynthetic rates using 14-C tracer methodology. Although dark carbon fixation rates can sometimes be used to correct for the isotopic exchange of unlabelled for labelled carbon within non-photosynthesizing algae, there remains some controversy concerning the quantity of this exchange that may occur in aufwuchs communities (Rodgers, 1977). During this study dark carbon fixation rates ranged from 0.014 to 0.78 mg C m -2 hr -1 at the upstream station, and from 0.03 to 0.68 mg C m -2 hr-1 at the downstream station. No clear relationship was observed for this parameter between stations during the course of this investigation.

Despite the problems of interpretation and comparisons between methodologies designed to assess primary production rates, there is fairly wide agreement in the literature concerning the mechanisms of carbon uptake into the cellular machinery of the photosynthetic organism. One of the primary enzymes of photosynthesis and photorespiration is ribulose 1,5-bisphosphate carboxylase (RuBP), which regulates the assimilation of carbon from atmospheric or dissolved CO_2 into the metabolic pathways (reductive pentose cycle, pentose phosphate pathway, or Calvin-Benson cycle), which ultimately generate all carbon containing components and products of the cell. The important metabolic functions and gross amounts of RuBP encountered in organisms make it probably the most important controlling enzyme of biomass production in primary producers. The rate measurements of primary production are therefore estimations of the ability of the target organism or community to incorporate carbon into cellular components, a rate under the direct control of the RuBP enzyme system.

5.1.3 Sulfate Assimilation

The extremely low values observed for the measurements of sulfate assimilation in the Adair Run system are a good example of a theory failing to work in practice. Unfortunate-

ly, as with many seemingly useful ecological methods, recent findings indicate that the measurement of sulfate assimilation as an indication of the quantity of heterotrophic production within a given system suffers from some serious conceptual drawbacks. These drawbacks have been discussed previously in the literature review section. A furthur complicating factor arose when attempting to apply the sulfate assimilation methodology to the Adair Run system. Total ambient sulfate concentrations must be low enough to allow for a measurable amount of the isotope 35-S to be incorporated into cellular biomass within reasonably short incubation periods (Jordan and Peterson, 1978). The effluent from the fly ash basin contained concentrations of sulfates ranging up to 60 mg/l, which reduced the practicality and efficacy of utilizing this parameter to assess changes in the aufwuchs community. Therefore, the results of the sulfate assimilation data in this study are questionable relative to determining a potential stimulatory effect of aufwuchs communities at the Adair Run ash-influenced station.

5.2 ARTIFICIAL STREAM EXPERIMENTS

The series of artificial stream dosing experiments conducted at the Glen Lyn facility, were designed to complement, and to some extent model the field study portion of this investigation. Although it is obvious that comparisons between the natural Adair Run system and the artificially controlled environmental regime of the model streams should be approached with some caution, artificial stream studies can provide important information to the aquatic ecologist investigating aspects of pollution ecology . The ability to control inherent natural variability with a carefully constructed series of artificial streams means that one or more parameters can be manipulated to allow the extraction of information concerning environmental effects of that specific parameter, without the complications of interpretation normally encountered in the natural world.

With the Glen Lyn artificial stream system, individual components of the complex fly ash effluent could be isolated and examined for effects upon structural and functional parameters of aufwuchs communities. Instead of attempting to interpret the possible synergistic and/or antagonistic effects of higher temperatures, increased discharge, dissolved nutrients, heavy metals in solution, pH excursions, and increased total suspended solids, which enter the Adair Run

system from the fly ash basin discharge, these parameters may be examined individually, and at varying concentrations.

Some of the differences between the Adair Run and artificial stream sytems do complicate attempts to apply the results of one series of experiments to the other. Adair Run is a cold, clear, second order mountain stream, and as such demonstrates an inherently different range of environmental parameters and characteristics from the New River, the source of water for the artificial stream system (Tables A1-A5). These differences preclude the possibility that native aufwuchs communities within each system even approach taxonomic or functional similarity, and therefore would not be expected to demonstrate identical responses to introduced environmental perturbants. In light of current ecological understanding (or lack of it), of the basic responses of communities to complex effluents, the general approach of the current investigation, and attempts to compare field studies with artificial stream experiments provides a practical starting point from which ecological theory may be advanced.

Artificial stream studies will be discussed in terms of introduced fly ash, heavy metals, and thermal and nutrient loading experiments. Additionally, relationships between the field and artificial stream portions of this investigation will be examined.

5.2.1 Fly Ash Dosing Experiments

Dosing of the artificial streams with fly ash represented an attempt to simulate the particulate fraction of the fly ash basin effluent discharging into Adair Run. Paerl and Merkel (1982) report that in aquatic systems having chronically low levels of biologically available phosphorus, that particles, such as fly ash, may act as a source of, as well as a concentrating site for, microbial nutrients. Although there is no indication that the Adair Run system lacked available phosphorus, the particle enrichment effect may have contributed to the high primary productivity rates observed. Although fly ash was obtained from the same source, (Glen Lyn electrostatic precipitators), and was sluiced into the streams with New River water, the extended residence time of ash particles and complex biogeochemistry present in the fly ash basin probably altered the chemical nature of the particulate ash fraction as it entered Adair Run. In addition, the design of the fly ash basin effectively removed the larger sized fractions of fly ash, while the artificial stream dosing apparatus lacked this ability. The fly ash entering the two systems would therefore be expected to differ substantially.

5.2.1.1 Ash Dosing Experiments with Uncolonized Substrate

The results of fly ash dosing experiments with uncolonized glass slide substrates indicated that introduced fly ash at environmentally realistic levels of total suspended solids (8.0 to 40.0 ppm) have no substantial inhibitory or stimulating effect upon measured aufwuchs structural and functional parameters. Only at TSS levels of 80 to 100 ppm were depressions in chlorophyll a, ATP, and light and dark carbon fixation rates noted, and these were either slight or not significantly different. In the Adair Run system the maximum observed TSS value was 93 ppm in late June of 1980, just prior to the initiation of basin shut-down. On that date a stimulation of the aufwuchs community was observed based upon ash free dry weights and light carbon fixation rates, although chlorophyll a values were similar to those of the upstream refernce station. At high TSS levels (80-100 ppm) dosed within the artificial streams, higher dry weights reflected the adherence of ash particles to the glass slide substrates. Although ash free dry weights were not significantly different from reference stream values, depressions in chlorophyll a and light carbon fixation rates indicated a reduced photosynthetic capacity of the ash-influenced aufwuchs community. This was probably directly re-

lated to the physical smothering effect on the attached organisms.

The findings of experiments with ash introduced at medium TSS levels (25-40ppm) presented a different situation. Although all other parameters measured failed to exhibit any significant differences between dosed and reference streams, light carbon fixation rates were greater on both sampling dates in the ash dosed stream. Since this level of dosing was evaluated for uncolonized substrates on only two occasions, it is difficult to say with any accuracy that this level of ash dosing actually stimulated primary production rates. Based upon the results obtained within measurements of other structural and functional parameters it appeared that the aufwuchs community was certainly not inhibited or depressed by TSS levels of 25-40 ppm.

A similar situation existed at low TSS levels (8-10 ppm), when on one of the two sampling dates no significant differences between streams were observed. The fact that chlorophyll a and light and dark carbon fixation rates were higher in the reference stream on the other sampling date is not sufficient data to attribute an effect to the ash dosing, and probably reflected the natural variability observed between streams.

By placing uncolonized diatometers in the ash dosed artificial streams, the field situation (which also utlized uncolonized substrates) is more closely modeled. Under these circumstances the ash particles, or basin effluent (in the Adair Run system), may function as an environmental "screening agent". In this situation, there may be a selection effect for those organisms which are capable of colonizing the substrate and able to continue metabolic functioning, and possibly utilize components of the effluent. Although this study was not designed to investigate the dynamics of aufwuchs colonization of bare substrates, it is reasonable to suspect that only some species would be capable of attachment when in competition with adhering ash particles. It is also possible that the presence of ash particles would provide an advantage for organisms not normally considered as pioneer species. In any event, it seems likely that the presence of fly ash in the artificial streams, and below the fly ash basin discharge in Adair Run, would promote the development of a taxonomically and functionally different aufwuchs community than in the reference streams or upstream Adair Run Station. The discrepancies between the field studies and the results of the artificial stream ash dosing experiments with uncolonized glass slide substrates illustrate the complexity, and concurrent multiple effects upon the

aufwuchs community inhabiting Adair Run due to the fly ash basin discharge.

5.2.1.2 Ash Dosing Experiments with Colonized Substrates

The use of pre-colonized diatometer substrates was initiated to more closely monitor a field scenario which would occur when an ash basin was constructed and placed into service. In this case the native aufwuchs of the receiving system would gradually be perturbed by increasing TSS and other physical and chemical qualities, and would be expected to respond by shifts in structural and functional parameters. An experimental regime of this type also allowed for an investigation of the temporal dynamics of changes in aufwuchs parameters in response to ash dosing. By sequentially evaluating the same community on different sampling days, information could be obtained which reflected the structural and functional reshuffling of the aufwuchs community as it adjusted to the imposed environmental changes. Instead of the increased ash particle concentration functioning as an environmental screening agent for the selection of members of the aufwuchs which could colonize and continue to function in the presence of increased TSS, ash dosing experiments with pre-colonized substrates were designed to examine the potential toxic, or stimulatory, effects of fly ash due to physical smothering, abrasion, and reduced insolation.

Results of dosing experiments with high TSS (100 ppm) demonstrated two important characteristics of aufwuchs communities within the artificial stream system. The first is the dramatic changes that occurred within the reference stream during the course of the 18-day experiment. Except for dry weights, all other measured parameters fluctuated by several unit measures, reflecting the dynamic nature of the stream system despite relatively constant water chemistry parameters (Table 10). The presence of high fly ash concentrations was reflected by dry weights which were approximately three times higher in the dosed streams, compared to reference values. Depressions in values for chlorophyll a and light carbon fixation rates indicate a potential physical or chemical inhibition effect of fly ash at high TSS levels. The fact that ash free dry weights and ATP values failed to reflect this finding may be due to an increase in heterotrophic biomass, relative to the photosynthetic component of the aufwuchs community. If ash dosing does in fact depress only photosynthetic processes within the attached aufwuchs, it may be due to a smothering effect which effectively blocks the availablity of solar energy to the cells. Additionally, the layer of ash adhering to the glass slide substrates may provide a hospitable matrix for heterotrophic organisms.

At lower concentrations of ash (35 and 65 ppm) no significant differences between dosed and reference streams was demonstrated for light carbon fixation rates. Chlorophyll a values were initially depressed in the 65-ppm dosed stream, and then demonstrated a recovery to values approximating the reference stream by day 27 of the 32-day experiment. Recovery was not demonstrated at lower TSS levels (35 ppm), where chlorophyll a levels and ash free dry weights remained below reference values throughout the 24-day experiment. The effects of TSS have not been extensively examined within aufwuchs communities. In fact this study is the first known investigation to evaluate the effects of introduced fly ash to primary producers. Friant et al (1980), examined the effects of TSS as a bacterial floc, and concluded that levels of TSS from 64 to 175 ppm did not inhibit the activity of stream organisms, including the aufwuchs.

5.2.2 Heavy Metal Dosing Experiments

The immediate and severe depression of the aufwuchs community at 10-par dosing concentrations (Table 17), as evidenced by lower values for all parameters measured, demonstrates the potential toxicity of the combination of dosed metals to the primary producing community. Values for each parameter were from two to fifteen times higher in the

treated streams than in the reference streams during this experiment. Although the six dosed metals represent only a fraction of the potentially toxic elements and chemical species present within the ash basin effluent, they were chosen due to their presence in high concentrations (Tables A1 - A5). The results of the 10-par dosing experiments confirm their potential toxicity. The utilization of the selected structural and functional parameters is also validated, since without exception all parameters were capable of demonstrating a substantial reduction in biomass and function in the dosed streams when compared to reference streams. A review of the current literature concerning the relative toxicities of heavy metals to phytoplankton and aufwuchs indicates that a likely order of decreasing toxicity of the six metals at the dosed concentrations is Cu, Cd, Cr, Ni, Pb, and Zn, with Cu targeted as probably the one metal capable of producing the inhibitory effects seen at these concentrations (Hutchinson, 1973; Klass et al, 1974; Rachlin and Farran, 1974; Rosko and Rachlin, 1977; Conway, 1978; Lue-Kim et al, 1980; Hollibaugh et al, 1980). A dosing scheme of this type fails to identify and characterize any synergistic or antagonistic interactions that may be occurring in the dosing solution of heavy metals.

At more environmentally realistic heavy metal concentrations (par and 0.5-par), the same depression of the aufwuchs community was observed, although to a lesser extent, with two important differences. First , except for carbon fixation rates, no significant differences between dosed and reference streams was observed for several days following the initiation of heavy metal dosing. Secondly, following a period of depression during the middle phases of the dosing experiment, the measured parameters within the dosed stream began to increase and approach those of the reference stream. During one experiment (Heavy Metal Dosing III), values of dosed streams for ash free dry weight and light carbon fixation rates actually reached a higher value when compared to the reference stream on day 20. What is probably being reflected by aufwuchs parameters during heavy metal stress is the ability of the community to recover from environmental perturbation, and to adjust structurally and functionally within a few days. Since this pattern was not observed at high metal concentrations, it seems that the inherent ability of aufwuchs to facilitate a recovery response may be dependent upon the initial degree of perturbation. If too much community integrity or diversity is eliminated by severe stress, the rate and degree of population recovery and structural shifts may be delayed, and this will

be reflected by the structural and functional properties of the community.

One unexpected, but interesting, side-light of the heavy metal dosing experiment was the ability of the aufwuchs to concentrate heavy metals to levels of up to one order of magnitude (Cd, Ni, Zn) greater than those of reference stream organisms. This result demonstrates the assimilative capacity of the aufwuchs community, and points to a potential bioaccumulation phenomena, if and when the primary producers are grazed upon by members of higher trophic levels, and concurs with the findings of Foster (1982) who noticed a bioconcentration of Cu, Pb, and Fe, but not Zn, in lotic algal species. If a methodology could be developed to measure metal uptake by aufwuchs exposed to fly ash effluents, it would provide important information concerning the bioavailibility and potential for bioconcentration of various heavy metals with the receiving system biota. The problem is to effectively separate the ash particles from the living portion of the attached material. Thomas et al, (1980), and Hollibaugh et al, (1980) observed that a laboratory growth inhibition of natural estuarine populations of phytoplankton occurred only when levels of 5 to 10 times that expected to occur in a polluted estuary were reached. Conway (1978) demonstrated that As and Cd were taken up differently by the

diatom, Asterionella formosa , the former metal appeared to be regulated by a detoxifying mechanism, while uptake of the latter (Cd) was a complex function of ambient concentration and time. Rose and Cushing (1970), and Cushing and Rose (1971), reported that the uptake of zinc by aufwuchs appeared to be one of adsorption, rather than absorption. It is not surprising that the complex community of primary producers would respond in different manners to concentrations of specific heavy metals, and that these would demonstrate different uptake kinetics. Obviously a great deal of investigation needs to be conducted before the complex relationships of heavy metals in a discharge to receiving system biota is understood.

5.2.3 Sulfate Dosing and Thermal Discharge Experiments

Interpretation of nutrient or thermal enrichment studies has been influenced strongly by the generally accepted limiting factor concept proposed in 1840 by Liebig ("Law of the Minimum"). However, as recent modeling efforts by Goldman and Carpenter (1974), and Lane and Levins (1977) suggest, there is a real need for the development of a more holistic approach to explain the responses of a given community to nutrient enrichment. A holistic approach demonstrates several immediate results when applied to investigations of po-

tential stimulatory agents, such as sulfates, or thermal discharges: 1) the outcome of the change of nutrient to any variable in the system is a function of the whole network of variables, 2) if more than one nutrient is added simultaneously the combined effects of each may change the outcome in surprising ways, 3) a given effect may be zero or opposite to a known physiological process, 4) a nutrient input can affect a component of a system even when there is no direct physiological link between the two, or 5) the correlation in the levels of two components of a system may be positive, negative, or zero, depending on the source of variation . Because of these findings the authors feel that "the general application of Leibig's law to biological systems is unsubstantiated by ecological reality or theory" (Lane and Levins, 1977).

The artificial stream investigations with introduced sulfate, and at increased temperatures, were initiated in an attempt to gather data that would further explain the stimulatory effect demonstrated by the fly ash basin effluent upon the aufwuchs community of Adair Run. Dry weights, ash free dry weights, chlorophyll a, and, to a lesser degree, light carbon fixation rates, combined to demonstrate an increase in biomass and photosynthetic capability of aufwuchs inhabiting the sulfate dosed artificial stream system. This trend became evident after day 12 of the 28-day experiment,

which probably is an indication of the time scale required by the community before it can adjust metabolically to take advantage of an increased supply of nutrients. Despite the problems with interpretation mentioned above, it appears that sulfate concentrations, at ambient levels modelling that of the fly ash basin effluent, are capable of stimulating specific structural and functional parameters of aufwuchs communities.

Due to previously mentioned problems with the operation of the thermal stream system, and the fluctuations in temperature differentials between the heated and reference streams, it is not possible to evaluate accurately the effect of slight increases in temperature upon the aufwuchs community. This is an area that requires future investigation.

5.2.4 Structural and Functional Parameters of Aufwuchs

A non-taxonomic assessment of aufwuchs communities to determine the degree of a specific environmental impact to aquatic ecosystems has several advantages over the examination of other trophic levels, or the use of generated diversity indices utilizing the primary producing community. Since the aufwuchs are fixed in place on the substrate, exhibit rapid turnover rates, and exist in close association

with ambient water conditions, they provide a mechanism with which to closely and accurately monitor the degree to which a given perturbant may alter the entire ecosystem. The primary producing functions, coupled with assimilative capacity, nutrient cycling, decomposition activities, and energy distribution render the aufwuchs an important component of any healthy system. If these properties are compromised by damages to this community, the integrity of the entire ecosystem, throughout multiple trophic levels, may be jeopardized.

Non-taxonomic measurements allow the inexperienced taxonomist the opportunity to quickly assess a highly complex assemblage of microorganisms, and by definition purposely consider the entire community as a discreet entity, with characteristics, qualities and functions above and beyond those of each individual species present.

In both field and artificial stream studies, the lack of complete correlation between measured structural and functional parameters suggests that perturbation of structural aspects may not be concomitant with functional changes. Although structural parameters are not expressed on a rate basis, they do integrate or reflect historical events occurring within the community, and are thus related to time and function. Functional changes would appear to relate

more closely to the physiological status of organisms comprising the aufwuchs community, but a change in structure may not manifest itself in a corresponding change in functional status. It follows that protection of the structural integrity of a community would not necessarily confer protection to overall community function, and vice versa. The development of more sensitive appropriate technology to assess specific structural and functional parameters of aufwuchs should reveal more of the intricacies of the relationships of aufwuchs community structure and function. Meanwhile, it is obvious that an approach which integrates measurements of both community structure and function can provide more information than studies which incorporate only one of these types of measurements.

Chapter VI

SUMMARY AND CONCLUSIONS

1. Coal ash effluent entering the Adair Run system created a stimulatory effect upon native aufwuchs communities as measured by dry weights, ash free dry weights, chlorophyll a, and light carbon fixation rates.
2. The observed stimulation of aufwuchs in Adair Run was probably a function of increased temperatures and sulfates in the fly ash basin discharge.
3. Aufwuchs communities demonstrated a recovery response to parameter values approximating those of the up-stream reference station within a three month period following termination of ash basin discharges into Adair Run.
4. An artificial stream system facilitated assessment of the environmental effects of individual components of the complex fly ash basin effluent. Artificial streams were successfully dosed with fly ash, six heavy metals, sulfate, and thermal discharge.
5. Dosing of artificial streams containing uncolonized diatometers with fly ash at various TSS levels indicated that at low (8.0 to 12.0 ppm), and medium (25 to 40 ppm) TSS ranges, no inhibition of community

structure or function oc . At high TSS levels (80 to 100 ppm) inhibition of photosynthetic capability was demonstrated by a reduction in chlorophyll a and light carbon fixation rates.

6. Fly ash introduced into artificial streams containing pre-colonized substrates provided for an examination of aufwuchs community response over a period of up to 32 days. High TSS (100 ppm) depressed chlorophyll a and light carbon fixation rates in the dosed stream. Lower TSS concentrations (65 and 35 ppm) initially depressed the aufwuchs community, which subsequently demonstrated a recovery response after approximately 12 days.

7. Heavy metals (Cd, Cr, Cu, Ni, Pb, Zn) at concentrations of 10 times those within the fly ash basin effluent severely depressed all functional and structural parameters of aufwuchs communities when dosed into artificial streams. At concentrations approximating those of the ash basin, and one half that concentration, aufwuchs within artificial streams exhibited an initial depression of parameter values, followed by a recovery response in which values increased to approximate those of reference streams.

8. Aufwuchs demonstrated an ability to concentrate certain heavy metals to values of up to one order of magnitude above ambient water concentrations.
9. Aufwuchs communities inhabiting artificial streams dosed with sulfate exhibited a stimulation response of structural and functional parameters at sulfate concentrations modeling the Adair Run system.
10. Investigations into the effect of thermal discharges upon aufwuchs community were inconclusive due to insufficient data.
11. Selected non-taxonomic structural and functional parameters of aufwuchs proved to be a viable methodology for evaluating environmental effects of coal ash basin effluents. The examination of ash free dry weights, chlorophyll a, and carbon fixation rates proved less variable than ATP measurements. Sulfate assimilation rates were not useful in determining community response in either the Adair Run system or within artificial streams.
12. Current EPA coal ash effluent guidelines are probably adequate to protect the primary producing aufwuchs community of aquatic receiving systems.
13. This investigation revealed the complex responses of a community to an effluent with several components,

and underscores the need for an examination of several constituents of the effluent to accurately assess its impact upon the biota of aquatic receiving systems.

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APPENDIX

Table A1. Water chemistry parameters for Glen Lyn sampling stations - July - September, 1979.

	Parameters								
	pH	Sp. Cond. (mhos/cm)	Alkal. (mg/L)	Hardness (mg/L)	Nitrates (mg/L)	Sulphates (mg/L)	Phos. (mg/L)	TSS (mg/L)	Temperature °C
New River up	7.1-7.3 (7.2)	95-100 (98)	3-47 (30)	24-26 (30)	.52-.76 (.65)	9.1- 13.5 (11.5)	.09-.18 (.13)	--	25.0-26.0 (25.5)
Adair Run up	7.2-7.3 (7.3)	160-200 (180)	45-67 (57)	20-60 (41)	.23-.44 (.38)	14.3- 52.4 (29.6)	.04-.18 (.09)	--	19.0-19.5 (19.3)
Fly Ash eff.	7.3-7.7 (7.5)	250-320 (285)	25-39 (31)	28-96 (54)	.55-.90 (.73)	123.2-190.4 (162.1)	.10-.18 (.13)	--	27.5-28.0 (27.8)
Adair Run dn	8.0-8.2 (8.1)	240-250 (245)	33-57 (41)	20-100 (47)	.50-.79 (.66)	56.4-131.4 (103.8)	.09-.18 (.12)	--	24.0-25.5 (24.7)
New River dn	7.6-7.6 (7.6)	95-130 (113)	20-58 (37)	24-36 (29)	.48-.79 (.68)	18.7-14.7 (12.0)	.10-.20 (.16)	--	28.0-30.0 (28.8)

Table A2. Water chemistry parameters for Glen Lyn sampling stations - January - March, 1980.

	Parameters								
	pH	Sp. Cond. (mhos/cm)	Alkal. (mg/L)	Hardness (mg/L)	Nitrates (mg/L)	Sulphates (mg/L)	Phos. (mg/L)	TSS (mg/L)	Temperature °C
New River up	7.1-7.3 (7.2)	95-100 (98)	3-47 (30)	24- 36 (30)	.52-.76 (.65)	9.1-13.5 (11.5)	.09 -.18 (.12)	--	25.0-26.0 (25.5)
Adair Run up	7.3-7.6 (7.4)	77- 85 (81)	34-37 (36)	56- 88 (72)	.58-.85 (.72)	13.1-13.4 (13.3)	.003-.01 (.01)	1.9- 5.1 (3.5)	1.0-10.5 (5.8)
Fly Ash eff.	6.5-7.7 (7.1)	110-140 (125)	50-52 (51)	116-144 (130)	.50-.73 (.62)	39.8-87.5 (63.7)	.02-.03 (.03)	20.6-34.3 (22.5)	3.0-14.0 (8.5)
Adair Run dn	7.0-7.7 (7.4)	100-100 (100)	41-46 (44)	116-156 (136)	.55-.79 (.67)	42.4-48.8 (45.6)	.01-.02 (.01)	20.8-26.0 (23.4)	3.0-12.5 (7.8)
New River dn	7.8-8.1 (8.0)	95-96 (96)	40-52 (46)	64-116 (90)	.65-.67 (.66)	9.1-17.6 (13.5)	.01-.01 (.01)	6.9-11.3 (9.1)	5.0-15.0 (10.0)

Table A3. Water chemistry parameters for Glen Lyn sampling stations - July, 1980.

	Parameters								
	pH	Sp. Cond. (mhos/cm)	Alkal. (mg/L)	Hardness (mg/L)	Nitrates (mg/L)	Sulphates (mg/L)	Phos. (mg/L)	TSS (mg/L)	Temperature °C
New River up	7.5-8.4 (7.9)	70-100 (83)	44-59 (50)	68-100 (79)	.17- .82 (.59)	10.7-19.0 (14.0)	.08-.17 (.11)	0-27.1 (11.9)	18.0-18.0 (18.0)
Adair Run up	7.5-7.9 (7.8)	122-160 (139)	44.86 (74)	106-128 (120)	.18-1.2 (.65)	18.0-25.0 (21.1)	.07-.09 (.08)	5.2-20.7 (11.3)	17.0-17.0 (17.0)
Fly Ash eff.	9.5	278	78	176	.96	348.0	.032	27.1	-
Adair Run dn	8.1-8.3 (8.8)	130-183 (154)	65-834 (325)	106-172 (142)	.22-.64 (.43)	30.3-90.0 (59.4)	.13-.23 (.18)	51.7-51.7 (51.7)	22.0-22.0 (22.0)
New River dn	NOT SAMPLED								

Table A4. Water chemistry parameters for Glen Lyn sampling stations - August, 1980.

	Parameters								
	pH	Sp. Cond. (mhos/cm)	Alkal. (mg/L)	Hardness (mg/L)	Nitrates (mg/L)	Sulphates (mg/L)	Phos. (mg/L)	TSS (mg/L)	Temperature °C
New River up	7.4-7.9 (7.7)	94-140 (116)	50- 63 (56)	70-104 (89)	.23-.62 (.39)	4.2-22.8 (15.6)	.06-.09 (.08)	1.4-7.8 (4.6)	6.0-26.5 (21.3)
Adair Run up	7.6-7.9 (7.8)	155-193 (175)	82-101 (91)	92-120 (107)	.12-.70 (.37)	16.2-35.0 (22.5)	.07-.20 (.12)	5.3-16.2 (10.9)	18.0-21.0 (19.9)
Fly Ash eff	NOT SAMPLED								
Adair Run dn	7.5-9.2 (8.3)	170-240 (199)	75-102 (90)	108-140 (127)	.11-.60 (.30)	8.6-91.2 (44.8)	.07-.87 (.41)	13.1-102.0 (39.7)	20.0-23.0 (22.0)
New River dn	-	-	-	-	.64	24.7	.82	-	-

Table A5. Water chemistry parameters for Glen Lyn sampling stations - September - November, 1980.

	Parameters								
	pH	Sp. Cond. (mhos/cm)	Alkal. (mg/L)	Hardness (mg/L)	Nitrates (mg/L)	Sulphates (mg/L)	Phos (mg/L)	TSS (mg/L)	Temperature °C
New River up	7.4-8.0 (7.7)	111-170 (140)	49- 73 (63)	72-148 (111)	.17-.84 (.52)	16.6-33.5 (23.1)	.08-1.78 (.57)	--	15.0-15.0 (15.0)
Adair Run up	7.6-8.1 (7.8)	162-250 (206)	6-116 (79)	104-160 (138)	.05-.41 (.28)	14.7-30.5 (23.7)	.05-1.95 (.57)	--	8.0- 8.0 (8.0)
Fly Ash eff	NOT SAMPLED								
Adair Run dn	7.6-8.2 (7.9)	163-265 (223)	109-111 (110)	140-692 (283)	.03-.28 (.20)	16.0-53.0 (35.5)	.04-2.37 (.66)	--	9.0-9.0 (9.0)
New River dn	7.7-8.3 (8.0)	109-120 (113)	66-71 (68)	104-124 (116)	.21-.69 (.49)	10.7-16.9 (14.3)	.10-2.20 (.87)	--	17.0-17.0 (17.0)

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AN ASSESSMENT OF THE ENVIRONMENTAL EFFECTS OF COAL ASH EFFLUENTS USING STRUCTURAL AND FUNCTIONAL PARAMETERS OF AUFWUCHS COMMUNITIES

by

Richard B. Nicholson

(Abstract)

A site-specific artificial stream system receiving selected levels of fly ash, heavy metals, or sulfates was compared to a natural stream (Adair Run) influenced by effluent from the fly ash settling basin at Glen Lyn, Virginia. Aufwuchs communities colonizing glass microscope slides were monitored for dry weights, ash free dry weights, chlorophylls, ATP, and 14-carbon and 35-sulfate assimilation rates. Productivity appeared to be enhanced in Adair Run due to increased concentrations of sulfates (150 mg/l), and temperature (delta T=4.5 C) in the ash basin effluent. A recovery response was observed following termination of basin operation. Artificial streams receiving selected concentrations of fly ash at low TSS (8.0-25 mg/l) exhibited no inhibition for all parameters except chlorophyll a and ATP. Higher levels (80-100 mg/l) depressed all aufwuchs parameters except AFDW within six days. Six heavy metals (Cd, Cr, Cu, Ni, Pb, Zn), when collectively pumped into artificial streams at concentrations modeling the ash basin effluent effectively lowered productivity parameters. This was fol-

lowed by a slow recovery response. Aufwuchs demonstrated an ability to bioconcentrate heavy metals from ambient water. Streams dosed with sulfates demonstrated a stimulation response at concentrations modeling the Adair Run system. Current U.S. EPA effluent guidelines for fly ash (30 mg/l maximum weekly average; 100 mg/l maximum) are evaluated concerning the degree of protection afforded primary producers of aquatic receiving systems.