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Papers of the 1991 Meeting

Botany Section

Updates on the Vascular Flora of West Virginia. VII.

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Abstract

Studies at the West Virginia University Herbarium (WVA) have revealed six species of vascular plants as new or noteworthy additions to the flora of West Virginia (based on Strausbaugh and Core, 1978). Unless otherwise noted, the nomenclature and distribution information follows Fernald, 1950.

The new species are as follows:

POACEAE. *Echinochloa muricata* (Beauv.) Fern. We have twenty six specimens, so annotated in the Herbarium, ranging in time of collection from Monongalia County: C. F. Millspaugh s.n., July 30, 1890 to Greenbrier County: Eleanor Bush s. n., September 4, 1988. Twenty counties are represented, well distributed over the state. The taxonomy of *Echinochloa* has been fluid for a long time, "a controversial matter" according to Gould, 1968. Hitchcock, 1950, Gleason, 1952, and Strausbaugh and Core, 1978, do not recognize the species *E. muricata*. Radford, et al, 1968, recognize *E. pungens, E. frumentacea* and *E. muricata* as synonyms of *E. crusgalli* (L.) Beauv. Ali, 1967 and Gleason and Cronquist, 1963, recognize *E. muricata* as a species.

Our specimens were studied and annotated by Ali and Gould.

CYPERACEAE. *Scirpus ancistrochaetus* Schuyler. Berkeley County: Rodney L. Bartgis 1296, July 22, 1989. Specimens have been previously collected in New York, Pennsylvania and Vermont (Schuyler, 1962). It is in our area and is to be expected.

CYPERACEAE. *Carex rugosperma* Mack. Monroe County: William N. Grafton s.n., June 2, 1981. This taxon is native to the northeast United States and is to be expected (Mackenzie, 1940).

VITACEAE. *Ampelopsis brevipedunculata* (Maxim.) Trautv. Kanawha County: R. L. Richardson s. n., July 26, 1989. An introduction from Asia, this species is often planted and escapes to the wild.

HYPERICACEAE. *Hypericum dissimulatum* Bickn. Barbour County: Eleanor Bush s. n., September 10, 1989. We are in the midst of this plant's range and it is, therefore, to be expected.

ASTERACEAE. *Solidago gracillima* Torr. & Gray. Fayette County: T. F. Wieboldt 5264, August 25, 1984. This species is found from northern Florida to Virginia and here, the northern portion of it's range (Cronquist, 1980).

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Ecology Section

Aquatic Phycomycetes of the Kanawha River, West Virginia

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Abstract

The Kanawha River, along with its tributaries forms the largest northwardly flowing drainage system entirely within the United States. The water molds (Phycomycetes) from this river were collected on 14 different types of substrates during the period of January 1990 to December 1990. Twelve, monthly collections resulted in the identification of twenty-one species representing 15 genera, 9 families and 6 orders of aquatic Phycomycetes. Correlation of frequency of species with physical and chemical characteristics of the river water indicated that the number of species collected increased with an increase in water temperature and pH during November to May and achieved a stable peak from March to May. *Achlya americana* (100% frequency), *Pythium debaryanum* (91% frequency), *Nowakowskiella elegans* (83% frequency), *Phytophthora* sp. (83% frequency), and *Saprolegnia eccentrica* (75% frequency) were the most common species and were found on more types of substrates than any other species. High water temperature during the period of June to August apparently accounts for the poor occurrences of fungal taxa during that period.

Introduction

Aquatic phycomycetes are found in a variety of habitats but little is known concerning their ecological role. With the current interest in the development of a sound understanding of the dynamics

of ecosystems, this group of organisms needs to be fully investigated. Farr and Paterson (1974) and Paterson (1977) emphasized the role of chytridiaceous and oomycetous fungi in the breakdown of chitin and cellulose-like substances. They also reported that aquatic phycomycetes as saprophytes and parasites on aquatic plants and animals are important in the biological interactions that occur in lakes. Running water such as streams or rivers support a variety of phycomycetous fungi which have been studied by many investigators (e. g. Harvey, 1952; Cook, 1961; Cook and Bartsch, 1960).

A special type of lotic environment is that produced by the acidic drainage from coal mines. Lackey (1939) first reported fungi present in such habitats. Cook (1966) also confirmed the presence of aquatic phycomycetes from polluted streams of the Ohio and Potomac rivers. Aquatic fungi of the New River and Little Stony Creek of Virginia have been studied by Farr and Paterson (1974). However, there has been no work published on the occurrence of aquatic phycomycetes from the rivers of West Virginia.

The purpose of this investigation was to determine the phycomycetous flora of the Kanawha River of West Virginia, which at many points receives pollutants from coal mine drainage.

Study Area

The Kanawha River and its tributaries form the largest northwardly flowing drainage system entirely within the United States. The river, which is formed by the junction of the New and the Gauley rivers in southern West Virginia, flows 97 miles in a northwesterly direction and then enters the Ohio River at Point Pleasant, West Virginia, some 266 miles down stream from Pittsburgh, Pennsylvania. The climate of the river basin is the continental type, with an average annual temperature of approximately 11° C and an average annual precipitation of 22.2 cm. The river in many areas has been affected by mine drainage pollution, which is an important limiting factor for its flora and fauna. Average water pH ranges from 6.5 to 7.8 and water temperature ranges from 5° C to 26° C. The vegetation lining the stream banks is dominated by trees such as birch (*Betula* spp.), oak (*Quercus* spp.), maple (*Acer* spp.), black locust (*Robinia pseudoacacia*), pine (*Pinus* spp), yellow poplar (*Liriodendron tulipifera*), black willow (*Salix* spp.), sycamore (*Platanus occidentalis*), and sweet gum (*Liquidambar styraciflua*). In the Montgomery area the river is populated with a variety of algal species and zooplankton

which act to provide suitable substrates for various phycomycetous fungi.

Materials and Methods

Water pH and temperature were measured on each visit to sample locations. Water samples were collected from ten locations along the river in the Montgomery area at monthly intervals from January 1990 to December 1990. All the ten water samples were mixed and baited in the laboratory with different types of organic baits in sterilized Petri dishes and incubated for 2 to 3 days at room temperature (18-24°C). Organic materials used as baits were of different types viz.; leaves (grass and corn), seeds and seed halves (hemp, rice, and mustard), fruits (apple, pear, and tomato), chitinous and keratinous substances (snake skin, horse hair, and human nail), pine (pollen and twig), and fruit fly (adult and larva). These materials were sterilized and standard procedures were used to incubate the fungi. After 2-3 days these baits were washed several times with sterilized distilled water and then examined microscopically for the presence of fungi. Representative specimens were mounted in lactophenol and cotton blue. Further incubation of baits in sterilized distilled water was continued for seven or more days to observe the occurrence of Chytridiomycetes and other slow growing Oomycetes. For any one collection, species of Phycomycetes were recorded as present or absent. As used in this paper constancy refers to the number of times the species occurred in 12 collections and is expressed as percentage.

Table 1. Occurrence of species in relation to the month of collection.

Species	Constancy	Month of collection J	F	M	A	M	J	J	A	S	O	N	D
		Very Abundant Species											
Achlya americana	100	+	+	+	+	+	+	+	+	+	+	+	+
Pythium debaryanum	91	+	+	+	+	+	+	+		+	+	+	+
Phytophthora sp.	83	+	+	+	+	+	+			+	+	+	+
Nowakowskiella elegans	83	+	+	+	+	+	+	+	+	+		+	
Saprolegnia eccentrica	75	+	+	+	+	+				+	+	+	+

Table 1. Continued.

Species	Constancy	Month of collection J	F	M	A	M	J	J	A	S	O	N	D
Moderately Abundant Species													
		J	F	M	A	M	J	J	A	S	O	N	D
Rhizidium chitinophyllum	58			+	+	+	+		+	+	+		
Catenaria anguillulae	50	+	+	+	+	+							+
Olpidium sp.	50			+	+	+					+	+	+
Saprolegnia parasitica	50	+	+	+	+	+							+
Rhizophlyctis sp.	42	+	+								+	+	+
Rhipidium sp.	42	+	+	+	+	+							
Pythium debaryanum	42	+	+			+	+	+					
Occasional Species													
Achlya diffusa	33	+	+	+									
Dictyuchus monosporus	25			+									+
Karlingia sp.	25										+	+	+
Lagenidium sp.	25			+	+	+							
Phytophthora cryptogea	25				+	+					+		
Unidentified	25		+	+	+								
Chytrid													
Scarce Species													
Allomyces arbuscula	8												+
Catenophlyctis variabilis	8				+								
Pythium sp.	8										+		
Number of Species Collected Per Month		11	12	14	14	6	4	3	6	11	9		10

Results and Discussion

The total number of taxa observed during the entire year was 21; these involved representatives of six different orders of the class Phycomycetes. Five taxa were highly abundant, three were regarded as scarce, while seven were noted as being

Table 2. Water molds of the Kanawha River and the substrates on which they occurred.

Species	Substrates
CHYTRIDIALES	
Olpidiaceae	
Olpidium sp.	pine pollen
Rhizidiaceae	
Rhizophyctis sp.	snake skin, grass blades, corn leaves
Rhizidium chitinophyllum	snake skin
Megachytriaceae	
Nowakowskiella elegans	grass blades, filter paper, corn leaves
Unidentified chytrid	corn leaves, grass blades
BLASTOCLADIALES	
Catenariaceae	
Catenaria anguillulae	snake skin, pine twigs
Catenophlyctis variabilis	snake skin, pine twigs
Blastocladiaceae	
Allomyces arbuscula	corn leaves
LAGENIDIALES	
Lagenidiaceae	
Lagenidium sp.	grass blades
LEPTOMITALES	
Rhipidiaceae	
Rhipidium sp.	apples, pears, tomatoes

Table 2. Continued.

Species	Substrates
SAPROLEGNIALES	
Saprolegniaceae	
Achlya americana	hemp, rice, mustard, grass blades, nails
Dictyuchus monosporus	fruit fly (adult and larvae)
Saprolegnia eccentrica	hemp, rice, mustard, snake
Saprolegnia parasitica	hemp. snake skin, fruit fly (adult and larva)
PERONOSPORALES	
Pythiaceae	
Phytophthora sp.	apples, pears, mustard, tomatoes
Phytophthora cryptogeae	mustard
Phythium debaryanum	hemp, mustard, rice, grass
Pythium sp. 1	rice, hemp
Pythium sp. 2	pine twigs

moderately abundant. Five fungal taxa and one unidentified member of Chytridiomycetes were recorded occasionally (Table 1). The maximum number of taxa was collected during the spring months of March, April, and May when water temperature ranged widely from 11°C to 20°C and pH of the water was moderately acidic to slightly alkaline (5.1 and 7.6). A smaller number of fungi was recorded during the summer months of June and July, with a minimum of 3 recorded in August, when the temperature of the water was 26°C and pH was still acidic (Fig. 1). Grass blades, hemp, and mustard seeds were colonized by a maximum number of fungi (Table 2). Snake skin was used by members of the group Chytridiomycetes and *Saprolegnia* spp. only. *Phytophthora* sp. and *Rhipidium* sp. were associated with apple, pear and tomato. Pine pollen was the only substrate chosen by *Olpidium* species. A profuse growth of *Dictyuchus monosporus* and *Saprolegnia* parasitica was observed on the larval and adult stages of the fruit fly. It was notable that species exhibiting constancy values occurred on a wide range of substrates, while occasional and scarce groups were observed only on a limited number of baits.

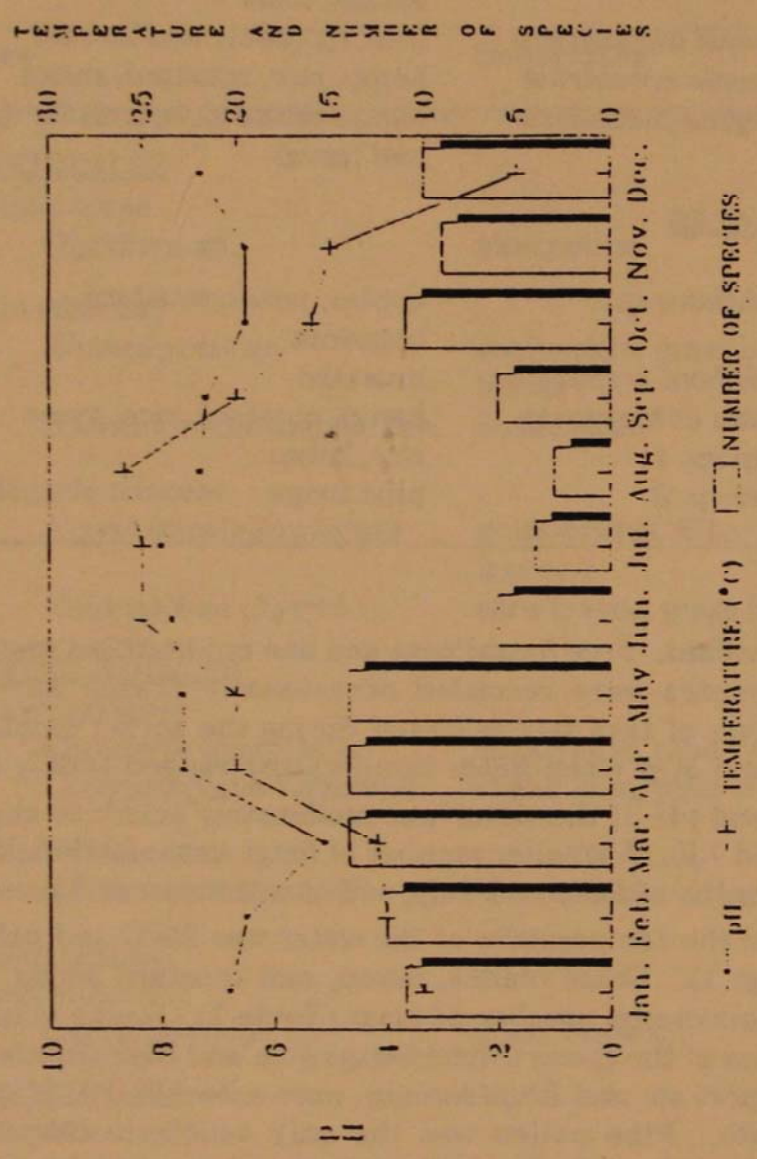

Figure 1. Monthly comparisons of temperature (°C) and pH in relation to number of species.

Maximum fungal occurrence during spring months has been reported by a large number of investigators (Coker, 1923; Forbes, 1935a, 1935b; Suzuki, 1960b and 1960f). They have also noted a winter maximum followed by a decline and eventual disappearance in summer. Dayal and Tandon (1962) explored the seasonal occurrence of aquatic Phycomycetes near the junction of two major rivers of India, the Ganges and the Yamuna, and found that most species of the Saprolegniaceae and Pythiaceae became dormant with the approach of high temperatures and germinate following low temperatures. Waterhouse (1942) attributed the disappearance of fungi in summer to the high temperatures, falling pH, and poisonous substances present in effluent, to which aquatic Phycomycetes are very sensitive. She also emphasized that aquatic Phycomycetes can play a significant role - similar to that of algae - as biological indicators of pollution in streams.

Roberts (1963) also recovered a low number of fungi in summer from both acidic and alkaline streams and concluded that temperature was a controlling factor on fungal phenology. Sparrow (1963) stated conclusively that the seasonal occurrence of fungi is intimately associated with temperature and its effects, and temperature more than any other factor is operative in determining the variations in fungal phenology. Temperature may also act indirectly to restrict competition for available substrates by other fungi.

Rooney and McKnight (1972), in a study of a subalpine lake, also noted that the fungal population was highly influenced by the abundance and quality of naturally occurring substances along with factors of the physical environment. The results of the present study would seem to indicate that the Kanawha River is richly populated by aquatic Phycomycetes which certainly play a significant role in the biodegradation of organic matter. There is also a strong possibility that some of these fungi may be of use as biological indicators of pollution, specifically acid mine drainage.

Acknowledgments

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Virginia Institute of Technology, Montgomery, WV during this study. I am also grateful to Dr. Violet Philips professor emeritus Biology WVIT for providing information about the Kanawha River.

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A Study of the Colonization of Submerged Leaves of Birch, Oak and Pine by Water-Born Hyphomycetes in the Kanawha River, West Virginia.

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Abstract

An investigation of the aquatic fungi [primarily Ingoldian Hyphomycetes] that colonize decaying submerged leaves was carried out during November and December 1990. Dead leaves of birch (*Betula* sp.), oak (*Quercus* sp.), and pine (*Pinus* sp.) were confined in fine mesh fiberglass screen cloth bags, submerged in the Kanawha River for six weeks and then returned to the laboratory and examined microscopically for aquatic fungi. Some bags contained leaves of only one type whereas others contained mixtures of all three types. Twenty three different fungal taxa were encountered, with the number recorded for birch (21) higher than those recorded for either oak (4) or pine (3). Eleven taxa were recorded from the bags of mixed leaves. Both frequency of occurrence and abundance of conidia per unit area of leaf surface were higher for birch. *Anguillospora crassa* and *Tetrachaetum elegans* were recorded as dominant forms colonizing all leaf types. The relative abundance of Ingoldian Hyphomycetes on birch leaves is apparently correlated with a faster rate of degradation. Colonization of pine needles by aquatic Hyphomycetes occurred to a greater extent than would have been anticipated, based on observations of previous workers.

Introduction

The colonization of leaf litter by aquatic Hyphomycetes is an important part of the processing required to make these substrates available to aquatic invertebrates. For example the protein content of leaf material is enhanced by fungal colonization. fungi can concentrate the inorganic nitrogen present in solution in water and with organic materials derived from their substrate can manufacture their own

biomass with an efficiency of 40 - 70 % (Harley, 1971). The amount of fungal biomass produced each year becomes very impressive when one considers that a stream receives at least a kilogram of leaves per meter of its length per year (Hynes, 1970) as a result of natural leaf fall. Fungi also possess the enzyme systems, which aquatic invertebrates lack, to soften and break leaf tissues into more easily digestible material.

Kaushik and Hynes (1971) and Triska (1970) showed that several stream detritus feeders prefer to consume partly decomposed leaves with rich microbial populations present rather than sterile or freshly fallen leaves lacking such microbial populations. Barlocher and Kendrick (1974) demonstrated that the fungal populations on leaves have a decisive influence on food selection by Gammarus and other invertebrates and the normal food preference order of these organisms can be altered by an appropriate choice of fungal inoculum.

There are several reports on the colonization of plant material by water-born Hyphomycetes from tropical and temperate streams (e.g. Sati and Tiwari, 1990 ; Manoharachari, 1990 ; Barlocher, 1990 ; Sridhar and Kaveriappa, 1984 ; Chamier and Dixon, 1982 ; Ingold, 1975 ; Willoughby and Archer, 1973 ; Nilsson, 1964) in various parts of the world. However, studies of aquatic Hyphomycetes from streams of West Virginia are generally lacking (Wolf, 1976).

In the work reported herein, the colonization of water-born Hyphomycetes on submerged leaves of several commonly occurring tree species in the Kanawha River has been investigated.

Study Area

The Kanawha River and its tributaries form the largest northwardly flowing drainage system entirely within the United States. The Kanawha River which is formed by the junction of the New and Gauley Rivers in southern West Virginia, flows 97 miles in a north westerly direction and then enters the Ohio river at Point Pleasant, West Virginia, some 266 miles downstream from Pittsburgh, Pennsylvania. The climate of the River basin is the continental type with an average annual temperature of approximately 11^0C and average precipitation of 22.2 cm. The river in many areas has been affected by mine pollution, which is the chief limiting factor for its flora and fauna. Average water pH ranges from 6.5 to 7.8 and water temperature ranges from 5 C to 26 C. The vegetation lining the stream banks is dominated by trees such as birch (*Betula* spp.), oak

(*Quercus* spp.), maple (*Acer* spp.) and pine (*Pinus* spp.). Leaves of these trees are added to the river in large amounts.

Materials and Methods

Dead leaves of *Betula* sp., *Quercus* sp. and *Pinus* sp. were sterilized with a 1.0% solution of Mercuric Chloride and then oven dried for 24 hours at 28^0C. Pieces (2 cm^2 for broad leaf species and 4 cm lengths of Pinus needles) of the leaves representing a total weight of 0.370 g for each species were sewn into bags constructed from mesh screen fiberglass cloth (mesh size 0.011 diam.). Some packs contained only leaves of a single species, whereas others contained a mixture of all three leaf types in equal quantities (1.11 g). These leaf bags were tied to submerged rocks in the stream and left in place for six weeks, from October 11 to November 25, 1990. The leaf pieces after being brought to the laboratory, were rinsed in sterilized distilled water, cut into smaller bits and incubated (at 25 $\pm$ 2°C) separately in Petri dishes containing sterilized distilled water. The Hyphomycetes that developed in each sample were identified by screening the sample every two days under a compound microscope. After screening, the water in the Petri dishes was replaced by fresh sterilized distilled water and the leaf pieces were reincubated. Observations were carried out for 15 days. The temperature and pH of river water were recorded before and after the period of sampling.

Results and Discussion

Twenty-three genera of Hyphomycetes were recorded from the four kinds of submerged bags (Table 1). Twenty-one fungal taxa (91% of the total) were found to colonize birch leaves, four (17%) were recorded from oak, and only three taxa (13%) were found on pine needles. The bag with a mixture of the three types of leaves yielded eleven taxa (48% of the total). Species such as *Anguillospora crassa* and *Tetrachaetum elegans* were the ecological dominants, as they consistently colonized leaves in all four types of bags. *Lunulospora curvula* was present on the three types of bags containing leaves of broadleaf species but did not colonize pine needles.

The maximum fungal colonization and abundant conidial growth on leaves of birch appear to be due to the more rapid softening and leaching of this leaf material when compare to oak and Pine leaves. The fact that 43% of all fungal taxa were recorded exclusively from birch leaves would seem to indicate a high degree of substrate

specificity. Only one taxon (*Ingoldiella hamata*) was restricted to the bag of mixed leaves. Colonization of oak leaves by *Anguillospora crassa* at least suggests the possibility of this species being chosen by stream invertebrates over other leaf types, although oak has been found to be a poorly colonized leaf type (Barlocher and Kendrick, 1976). Butler and Suberkropp (1986) have also demonstrated that the palatability of oak leaves to caddis fly larvae was highly influenced by the degradation potential of the fungal population on the leaf surface. The degradation potential ultimately depends upon the lipid content and the growth factors of the fungus on the one hand and on the other hand its ability to grow on and modify the leaf tissue.

Table 1. Occurrence of water-born Hyphomycetes on submerged leaves of birch (*Betula* sp.), oak (*Quercus* sp.) and pine (*Pinus* sp.) in separate and mixed bags.

Taxon	Betula	Quercus	Pinus	Mixture	Constancy
Anguillospora crassa	+	+	+	+	100
Alatospora sp.	+			+	50
Actinospora sp.	+				25
Articulospora tetracladia	+			+	50
Clavatospora sp.	+			+	50
Clavariopsis aquatica	+				25
Dendrospora sp.	+				25
Flagellospora curvula	+		+	+	75
Heliscus lugdunensis	+				25
Ingoldiella hamata				+	25
Lemmoniera aquatica	+				25
Lunulospora curvula	+	+		+	75
Speiropsis sp.	+				25
Tetrachaetum elegans	+	+	+	+	100
Tridentaria sp.		+		+	50
Triscelophorus monosporus	+				25

Table 1. Continued.

Taxon	Betula	Quercus	Pinus	Mixture	Constancy
Trinacrium sp.	+				25
Tripospermum myrti	+				25
Triposporium sp.	+			+	50
Tricladium sp.	+				25
Tetracladium marchalianum	+				25
Thallospora sp.	+				25
Varicosporium aquaticum				+	50
Number of Taxa	21	4	3	11	
Percent of Total	91	17	13	48	

The presence of *Anguillospora crassa*, *Flagellospora curvula* and *Tetrachaetum elegans* on pine needles is similar to the observation of Sati and Tiwari (1990) and Hess *et al.* (1990) who reported several species of aquatic Hyphomycetes from this type of substrate. In contrast Ingold (1975) indicated that aquatic Hyphomycetes do not colonize the leaves of Gymnosperms. Huet (1951) reported that tough conifer needles caused a lowering of the pH of streams due to the release of toxins and this in turn reduced the population of animals and algae in the streams. This factor may be responsible for the poor colonization of pine needles in bags containing only this leaf type. However, the presence of eleven taxa in the mixed bag indicates that the toxic effects of pine needles do not affect fungal colonization when several factors are acting together.

Apart from environmental factors the presence of growth inhibiting/promoting substances in leaf litter plays a major role in litter colonization by aquatic fungi (Triska and Seddel, 1976; Barlocher *et al.* 1978, 1979). Suberkropp and Klug (1976) and Sridhar and Kaveriappa (1989) suggested that abundant or poor colonization of leaves is determined by the chemical changes that take place in water due to the removal of inhibitors by leaching, along with preference, competition, different growth rates and sporulation times. Thus although aquatic Hyphomycetes exhibit substrate specificity, at least a

few genera can colonize even the toughest and slowest softened leaf material to make it palatable and degradable.

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ARTIFICIAL WATERSHED ACIDIFICATION ON THE FERNOW EXPERIMENTAL FOREST

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ABSTRACT

An artificial watershed acidification study was initiated on the Fernow Experimental Forest in December 1987. Acidification was induced by aerially applying ammonium sulfate fertilizer three times per year to a 34-ha watershed at an annual rate approximately double ambient nitrogen and sulfur inputs. A second watershed was maintained as a control. Stream water chemistry, principally during storms, and soil leachate chemistry have been monitored intensively to determine if acidification could be induced and to examine some of the biogeochemical factors involved in the acidification process. One year of pre-treatment data were collected for most parameters, and seven fertilizer applications have been made to date. Substantial information about stream chemistry responses during storms has been compiled, though stream chemistry has not been altered by the treatment. However, sulfate and nitrate concentrations in A-horizon leachate have increased since late 1989 on the treated watershed. No changes in leachate from lower horizons have been documented, probably because the B- and C-horizons are adsorbing excess anions effectively.

INTRODUCTION

West Virginia is downwind from large industrial and energy producing areas, resulting in the state receiving extremely high sulfate and nitrate loads deposited as acidic compounds (NADP/NTN 1987). Extensive environmental research which has focused upon acidic deposition during the last decade is a testament to its importance as a major nonpoint source pollutant, particularly in remote forested areas. Since 1978, the Watershed Management Research Project at the U. S. Forest Service's Timber and Watershed Laboratory in Parsons has

dedicated a significant portion of its effort to investigating the effects of atmospheric deposition on water and soil resources in north central West Virginia. As part of that work, a whole-watershed acidification experiment was begun in 1987 to examine the responses of stream water, soil water, and soil chemistry to long-term, elevated inputs of nitrogen and sulfur. Several cooperators also joined the effort to examine the effects on terrestrial salamanders, stream insects, and aquatic bryophytes and fungi, since biological organisms may respond quickly to very subtle changes that cannot be discerned as statistically significant from analyses of chemical data.

Instrumentation, sampling designs, and preliminary data analyses for the two major components of this study, stream water and soil water, are described. The data sets developed contain millions of observations, requiring extensive and time-consuming validation and verification. Consequently, extensive analyses have not been performed and the results presented in this paper should be considered extremely preliminary. However, even though they are preliminary, results are presented because they provide important information about some potential effects of acidic deposition to West Virginia and the Appalachian Mountains.

METHODS

Two adjacent watersheds on the Fernow Experimental Forest in Tucker County, West Virginia (approximately 39^0 3' 15" latitude, 79^0 49' 15" longitude) were studied. They are in the unglaciated Allegheny plateau of the Appalachian Mountains and are characterized by steep slopes and shallow soils derived from acidic sandstones and shales. Watershed 4 was a control, and Watershed 3 was treated to induce acidification. Other than stand age and composition, both watersheds have very similar physical characteristics (Table 1). Though these vegetative differences do affect hydrologic budgets and chemistry, their differences are accounted for using paired watershed analyses.

Watershed 4 has been undisturbed since about 1905 when the area was heavily cut, but not clearcut. Dead American chestnut (*Castanea dentata* (Marsh.) Borkh.) was salvaged from the watershed in the 1940s, though the volume removed was not recorded. Current dominant vegetation is approximately 85 years old, but some residual trees, believed to be about 200 years old, are scattered throughout the watershed. Watershed 3 was clearcut to 2.5-cm dbh between July 1969

and May 1970 except for a 2.99-ha buffer strip along the stream channel, in which a light selection cut was made. In November 1972, the buffer strip was clearcut and all debris in the channel and within 2.5 m on either side of the stream channel was manually removed, effectively eliminating all stream channel shade. Natural revegetation followed each cutting.

The streams draining each watershed are small second-order headwater streams, which occasionally dry up between late August and mid-September. Both watersheds have been gauged using 120^0 V-notch weirs in combination with FW-1 water-level recorders since May 1951. Average annual and daily flows based upon 39 years of records are given in Table 1.

Table 1. Physical characteristics of Watersheds 3 and 4.

	Watershed 4	Watershed 3
Area (ha)	38.8	34.7
Max Elevation (m)	870	860
Min Elevation (m)	750	735
Average Slope (%)	20	20
Aspect	SSE	S
Bedrock	Hampshire Formation	Hampshire Formation
Soil Type	Calvin and Berks	Calvin and Berks
Soil Depth (m)	0.6-2	0.6-2
Stand Age (yr)	85	21
Dominant Species	*Acer saccharum* Marsh. *Quercus rubra* L. *Prunus serotina* Ehrh. *Betula lenta* L.	*Prunus serotina* Ehrh. *Acer rubrum* L. *Betula lenta* L. *Fagus grandifolia* Ehrh.
Average daily flow (L/s)	7.8	7.2
Average annual flow (L/s)	2852.5	2635.7

Stream water was sampled weekly and during storms, though most emphasis was placed on the latter component. Grab samples were collected every Tuesday morning from a permanently marked location just upstream from each weir. Storm samples also were collected just upstream from the weirs using ISCO model 2700 automatic sequential samplers housed in specially-designed shelters (Kochenderfer and Edwards 1990) for protection. The ISCO samplers were triggered automatically by a rise in the water level in the weir ponds at the onset of storms. Subsequent samples were taken at preset time intervals thereafter until manually discontinued. From about October to May, samples were taken hourly on the rising limb of the storm and just past the peak. On the remainder of the falling limb samples were collected on 2- to 3-hour intervals. From about June to September, samples were taken on 15- or 30-minute intervals on the rising limb and just past the peak, and on 1-hour intervals during the falling limb. About 9-12 storms per year were sampled.

In situ stream pH, electrical conductivity, and temperature were measured continuously using an electrochemical instrument called a Minimonitor, developed by the U.S. Geological Survey. Readings were converted from electrical signals to chemical or physical measurements and recorded on Omnidata Easy Logger data loggers. Five-minute averages, determined from minute readings, were recorded on the data logger storage packs. Minimonitors and data loggers also were housed in the shelters.

Soils in both watersheds were mapped using rigorous protocols (Lammers et al. 1987), so that lysimeters could be installed within all major soil series, slope classes, etc. On each watershed, 39 lysimeters were installed across 15 sites, at 2 or 3 depths per site. Gravity-drained leachate was collected from these zero-tension pan lysimeters monthly or after major storm events to minimize overflow, and then chemically analyzed.

Watershed acidification was simulated by applying granular ammonium sulfate fertilizer to Watershed 3 at a rate double annual ambient N and S inputs in throughfall, which approximates the combined inputs of wet and dry deposition. Ambient deposition varies seasonally, with more than one-half of the annual sulfur in wet deposition occurring from May to August. Therefore, to better mimic natural seasonal variations of S and N inputs, and to avoid overwhelming the system with a single large annual dose of S and/or N, three applications per year were made. With the exception of the first application on Jan. 31, 1989, subsequent applications were made

March, July, and November of each year. The applications for successive Marches, Julies, and Novembers were double the average historical deposition rates for the periods January - April, May - August, and September - December, respectively. Thus, the March and November applications each were 33 kg ha^{-1}, corresponding to 8.1 kg ha^{-1} S and 7.1 kg ha^{-1} N, respectively, and the July applications were 101 kg ha^{-1}, or 24.2 kg ha^{-1} S and 21.3 kg ha^{-1} N, respectively. The fertilizer was applied with a helicopter using north-south and east-west flight lines to ensure even coverage across the watershed.

All water samples were analyzed at the U.S. Forest Service's Timber and Watershed Laboratory in Parsons, WV, using EPA-approved protocols, holding times, and quality assurance/quality control procedures.

Results and Discussion

Data validation and verification currently are on-going, so statistical analyses have not been completed, and the results presented here are preliminary. However, the intensive monitoring associated with this study has provided substantial information about stream responses, particularly during storms. In addition, soil leachate data suggest that the acidification treatment already may have caused chemical changes in Watershed 3.

During most storm or melt episodes, pH depressions and concentration increases of major stream water ions are experienced. The timing of the maximum peak and minimum depressions is strongly related to the timing of peak flow; pH depressions coincide almost exactly with the time of peak flow (Fig. 1), while the other ionic constituents usually peak just before peak flow (Fig. 2). Stream chemistry changes associated with the rising limb of the hydrograph are rapid, while the return to baseflow concentrations is delayed (Figs. 1 and 2). The similar lag to baseflow conditions demonstrated for flow and chemistry indicates that subsurface flow is a significant influence in these watersheds.

Soil leachate data are extremely variable so identifying and quantifying precise changes are not easy. However, from the limited data analyses done to-date, acidification appears to have had some effect on sulfate and nitrate soil leachate chemistry. Mean A-horizon responses across the 15 lysimeter locations on each watershed suggest that the application of ammonium sulfate increased mobile sulfate

concentrations on Watershed 3, beginning in late 1989 or early 1990 compared to Watershed 4 (Fig. 3). Since no increase occurred in the B- or C-horizons or in stream water, most of the mobile sulfate probably was adsorbed in these lower horizons. Nitrate behavior is more erratic, but some A-horizon increase is evident (Fig. 4). Again no response was found in leachate from the B- and C-horizons. The sink for mobile nitrate has not been identified, but vegetation may have been a primary sink during growing seasons, though adsorption in lower horizons also may have been significant, especially during the dormant season.

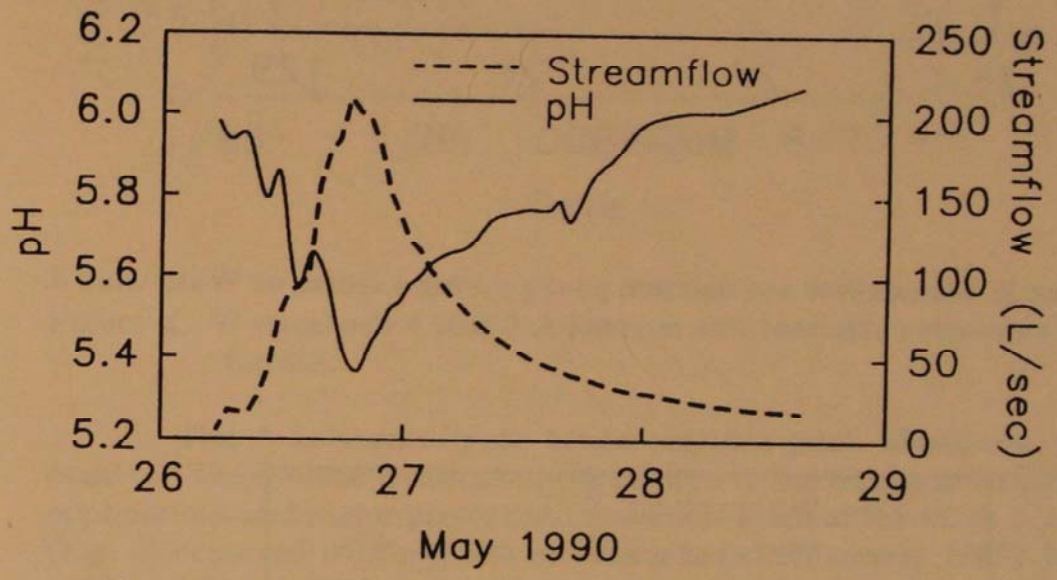

Figure 1. Streamflow peak and pH depression (determined from Minimonitor) during a storm on Watershed 3.

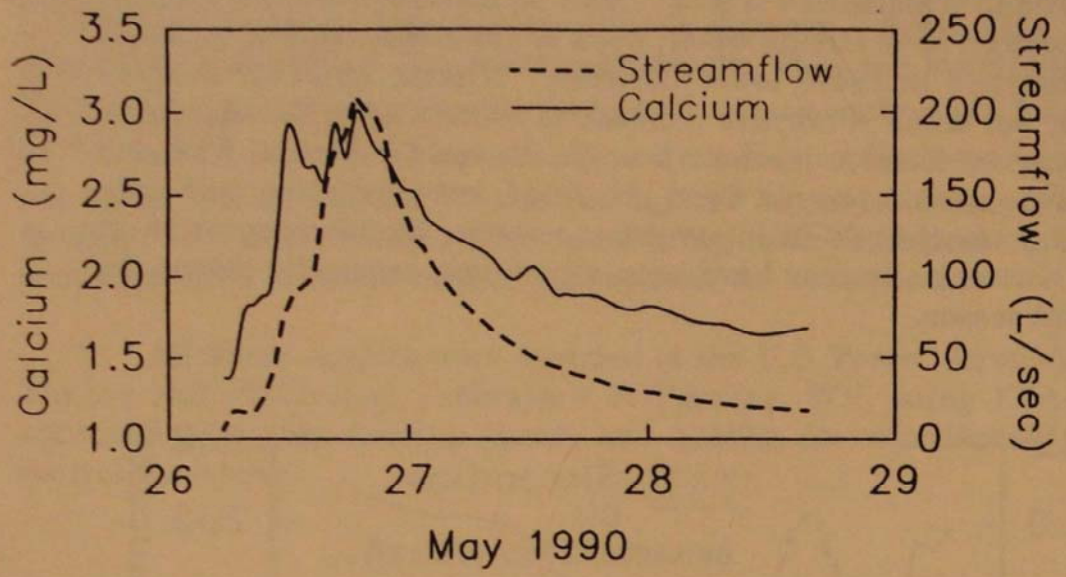

Figure 2. Streamflow and calcium peaks during a storm on Watershed 3.

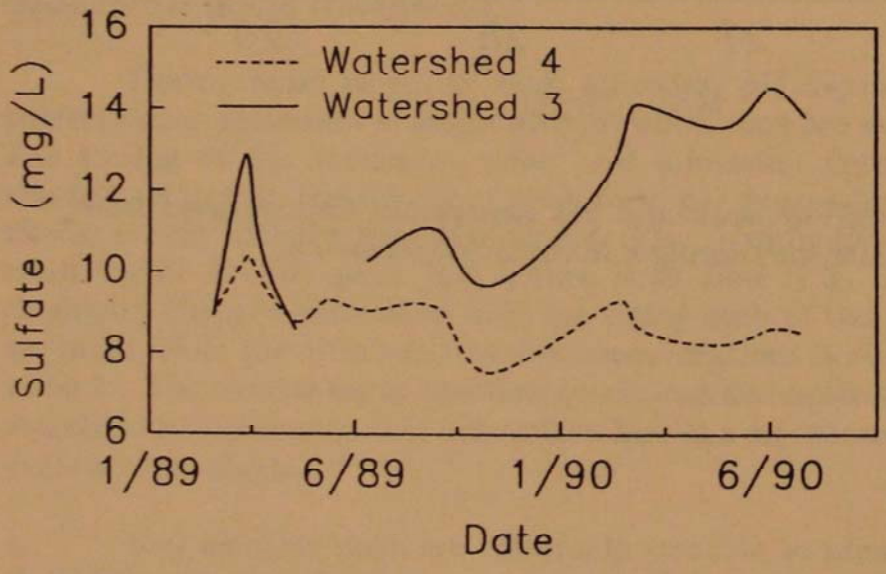

Figure 3. Watersheds 4 and 3 A-horizon soil leachate responses for sulfate.

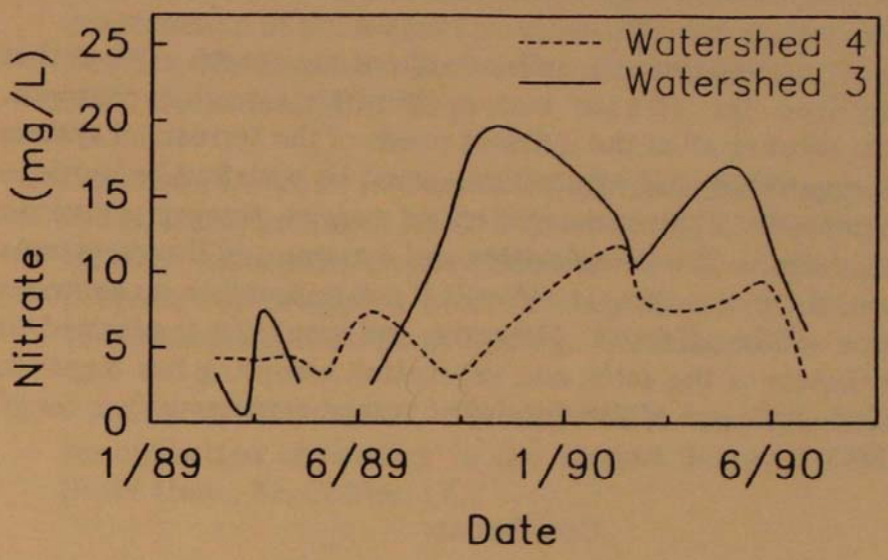

Figure 4. Watersheds 4 and 3 A-horizon soil leachate responses for nitrate.

The A-horizon nitrate concentrations peak about every 6 months. The cyclical pattern may be related to the timing of fertilizer applications and major precipitation events. Each of the three peaks (Fig. 4) occurred within a few months after the January 1989, July 1989, and March 1990 applications so that nitrification of the applied ammonium probably would have been complete. A large storm or an extended series of moderate to large storms ranging from 3.3 to 7.6 cm of precipitation occurred just a few days before each of the lysimeter collection periods. Evidently, much of the available nitrate was not assimilated or immobilized after the fertilization, allowing it to become mobilized as large fluxes of water moved through the soil, into lower soil horizons. No peak was seen after the November 1989 application even though a significant amount of rainfall preceded the collection. However, the peak may not have been resolved individually because the effects of the large July application still were controlling leachate chemistry.

Validation of cation data for the lysimeters has not been begun, so no results are presented. But because anions and cations must pair as they move through the soil, concentrations of one or more cations also must have increased. Soil leachate and stream water

chemistry from other watersheds on and near the Fernow indicate that calcium most frequently pairs with anions in these watersheds (Edwards and Helvey 1991, Helvey et al. 1989, Edwards, unpublished data), so similar pairing is expected in this study.

No estimations have been made about the length of time that will be required for stream water chemistry to be changed. Theoretically, most or all of the nutrient needs of the terrestrial system -- soil, microorganisms, and vegetation -- must be satisfied before ions can be exported to stream systems, except during extreme precipitation events. The complexities and dynamics of these systems are enormous, thereby making it difficult if not impossible, to estimate when streams will be affected. However, soil sampling is planned to study the behavior of the soils, and vegetation sampling has begun to quantify the importance of the dominant tree species as a sink for N and S.

Conclusion

The induced acidification is influencing soil leachate chemistry faster than expected at the onset of the study; however, the effects currently are limited to the A-horizon. The lower horizons have sufficient anion adsorption capacities to retain excess sulfate and nitrate in the soil, thereby controlling many of the negative consequences associated with accelerated anion leaching to streams, such as solubilization and mobilization of toxic metals. But before such extreme changes occur, stream water probably first will experience accelerated base cation exports and perhaps increases in peak nutrient discharges during storm events. This study is being continued to monitor the development and progression of these changes. The results then will be used as a barometer against which regional responses can be compared.

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Effect of Water Pollutants on the Rate of Oxygen Uptake of Three Aquatic Fungi from India

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Abstract

Effect of detergent, DDT, sewage, and high chlorine content on oxygen uptake of *Catenaria anguillulae*, *Pythium tenue* and *Phytophthora erythroseptica* was studied. These pollutants and fungi are common in bodies of water in the Jabalpur area. All three fungi exhibited an increased rate of oxygen uptake in the presence of sewage water which coincides with their capacity to withstand and compete with bacteria in polluted water. Water with DDT also increased the respiratory rate of the three fungi, thus, supporting their role in the accumulation and degradation of DDT. There was a gradual decline in the rate of oxygen uptake of *Catenaria anguillulae* in the presence of water containing detergents and also water containing excess chlorine.

Chlorinated water reduced the respiratory rates of *Pythium tenue* and *Phytophthora erythroseptica* to the minimum values. Extreme sensitivity of Blastocladiales (*C. anguillulae*) to such conditions can be correlated with sensitivity to detergent and the same is true of Peronosporales (*P. erythroseptica*) with the presence of high levels of chlorination.

Introduction

Aquatic environments in India are subjected to various types of pollutants, i.e., detergent from domestic practices, pesticides from agricultural and industrial sources, excessive chlorination from water filtration plants and sewage from surrounding drainage systems.

Presuming aquatic fungi to be highly aerobic, it is interesting to note their rate of oxygen uptake under the influence of these pollutants as there is much evidence that respiration of fungi play a significant role in the accumulation and degradation of many organic pollutants present in water (Kallman and Andrews, 1963). The present investigation deals with the study of the effect of sewage, detergent, excess chlorination, DDT (Dichlor-diphenyl-trichloroethane) and sterilized tap water on the rate of oxygen uptake of three aquatic fungi viz. *Catenaria anguillulae* (order Blastocladiales), *Pythium tenue* and *Phytophthora erythroseptica* (order Peronosporales). These three genera are commonly distributed in the aquatic habitats of Jabalpur city and the last two genera were recorded as constant species (Gupta and Dubey, 1981).

Materials and Methods

Measurements of oxygen uptake were made by the direct method of Warburg on a Braun Warburg apparatus. Braudie's fluid was used as manometric fluid (Umbreit et al., 1964).

Fungal mycelia were harvested from one week old cultures of three fungi to prepare the mycelial suspension in 0.01 M phosphate buffer at 6.5. Aliquots of mycelial suspensions were added to the reaction vessel flask. The center well of the flasks contained 0.2 ml of 10% KOH and filter paper skirts. Side arm contained specific pollutant dissolved in water. Sewage water was collected from a pond rich in sewage effluent.

Forty ppm solutions of detergent, chlorine and DDT were prepared in sterilized distilled water. Sterilized tap water was used for the control. The final volume of fluid was adjusted for thermobarometric variations and recorded at 30 minute intervals for two hours. All experiments were conducted at room temperature. The respiratory data were qualified on the basis of total nitrogen content of the tissue based on method of Thompson and Morrison (1951).

Final corrected values represent an average of replicate treatment and are calculated on the basis of microliters (μ liter) of oxygen consumed per mg. of nitrogen (O_2N). Oxygen uptake was determined by taking the difference in pressure in millimeters (h). Exact volume of oxygen consumed (x) at N.T.P. was calculated as follows:

$x = hk$ K = flask constant (Predetermined)

Table 1. Effect of different types of pollutants present in water on rate of oxygen, uptake of starved mycelium of three aquatic fungi.

Type of Pollutant	μL of O_2 uptake of Mycelium				Rate of Respiration
	Time in Minutes				
	30	60	90	120	μL O_2/mt/g.
	Catenaria anguillulae				
1. Sterilized tap water	17.4	20.7	23	27.6	.3525
2. Sewage	20.7	29.9	32.2	34.5	.4584
3. Detergent	9.2	6.9	2.3	00	.1692
4. Chlorination	16.1	13.8	11.5	6.9	.2378
5. DDT	11.5	18.4	36.8	57.5	.3986
	Pythium tenue				
1. Sterilized tap water	138	184	230	345	3.2742
2. Sewage	184	460	690	920	7.2832
3. Detergent	115	230	460	690	4.6383
4. Chlorination	230	460	345	185	5.1770
5. DDT	115	230	345	460	3.8333
	Phytophthora erythroseptica				
1. Sterilized tap water	113.5	141.5	155.6	176.9	2.9257
2. Sewage	212.3	265.3	283.07	353.8	9.3977
3. Detergent	80.7	155.6	185.3	233.5	2.3219
4. Chlorination	70.7	80.7	120.3	116.9	1.6275
5. DDT	155.6	176.9	212.3	233.5	3.1098

Results and Discussion

Observations revealed the sewage water as most favorable among all five treatments for the rate of oxygen uptake of three fungi under consideration (Table 1).

C. anguillulae was highly sensitive to detergent solution, *P. tenue* to DDT and *P. erythroseptica* to chlorine.

Lowest rate of respiration was noted for *P. erythroseptica* in the presence of chlorinated water. Low respiratory rates of three genera in the presence of sterilized tap water indicated their inability to grow in a medium without nutrients and substrate.

There was a gradual decline in the rate of oxygen uptake by *C. anguillulae* under the influence of detergent and chlorination with increase in time, although it was minimal in detergent.

P. tenue and *P. erythroseptica* on the other hand showed a continuous increase in rate with the increase in time in the presence of detergent, but chlorination accelerated the respiratory activity for the first 60 minutes followed by gradual decrease in 60 more minutes. Finally, *P. tenue* showed the best response for these two factors among the three genera.

Enhanced respiratory rates of three fungi in presence of sewage water indicates their ability to compete with other microbes present in water. This also confirms their significance in decomposition of nitrogenous organic substances richly present in sewage effluents. Willoughby and Collins (1966) also noted the presence of *Saprolegnia farax* exclusively in the vicinity of sewage treatment plant effluent. Respiratory activity of *C. anguillulae* was very different under the influence of detergent when compared to the observations of Farr and Paterson (1974) who observed in another chytrid *Rhizidium* sp. a greater degree of tolerance for surfactants than a filamentous fungus *Achlya caroliniana*. An increase in pH of the medium due to the stearic acid salts of sodium and potassium present in detergent, seems to be responsible for the poor rate of respiration of *C. anguillulae*.

Pagan (1970) reported a concentration of 25 ppm of chlorine as sufficient to destroy many human pathogenic fungi including *Candida albicans*. This leads to the conclusion that these phycomycetous fungi can exist in high concentration of chlorine where a limited number of microorganisms can grow.

DDT like other pesticides is an economic poison and has been detected in the surface waters or bottom sediments of different water bodies (Hindin et. al., 1966; Bridges et. al., 1963). It produces serious biological effects in non target organisms. The persistence of DDT in natural ecosystems has been attributed to the inability of microorganisms to degrade chlorinated hydrocarbon molecules.

The response of three aquatic fungi toward DDT are encouraging and indicate their possible importance in degradation of this pesticide. Matsumura and Boush (1968) noted 83% of decomposition of DDT in three days by fungi. Dalton and Smith (1971) and Ko and Lockwood (1968ab) compared the accumulation and degradation of DDT by many aquatic fungi. *Heliscus submersus* showed a greater rate of degradation than *Clavariopsis aquatica*. Madeline Hodkinson (1976) studied the effect of DDT on fungal respiration and found a linear relationship between oxygen consumption of *H. submersus* and DDT concentration up to a concentration of 35 ppm. He also noted an increase in respiratory quotients of various sugars and a corresponding increase in the anaerobic fermentation process. Kallman and Andrews (1963) further confirmed the involvement of respiration in the accumulation and degradation of DDT, as living fungal cultures take up more insecticides than dead ones.

These interpretations lead to the conclusion that Phycomycetous general although slow in growth can thrive in extremely polluted environments where only a limited number of microbes can grow. Perhaps their wide range of pollutant tolerance enables these fungal species to become the constant species of aquatic environments (Gupta and Dubey, 1981) and thus to contribute as a significant component to the energy flow and homeostasis of the ecosystem.

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Effects of artificial Lake Destratification on the Macrobenthos in Beech Fork Lake, West Virginia

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Abstract

A study was conducted at Beech Fork Lake, Wayne County, West Virginia, in 1987 to compare the effects of first-year operation of artificial destratification fans upon the macrobenthos with pre-destratification samples taken in 1985. Benthos and water quality samples were taken from two stations at 1.53 and 4.58 m depth and from 4.58 m at two additional stations during the summer and fall.

The analyses of macrobenthos and water chemistry showed significant changes during both seasons in first-year operation based on MANOVA ($p<0.05$), t-test ($p<0.05$) and step-wise discriminant analysis ($p<0.01$). Significant post-destratification increases occurred in number of individuals, diversity, taxa richness, and temperature The overall trophic structure showed a slight trend toward the resident detritivores and away from the predators. Based on Wilks' Criterion, significant ($p<0.05$) overall increases occurred in benthic population density during both seasons. No significant change in dissolved oxygen was found following destratification. Water quality changes that occurred between 1985 and 1987 due to operation of the destratification fans appear favorably for macrobenthos.

INTRODUCTION

Impounding a water shed area has many impacts on the surrounding environment, including water quality (Symons, 1969; Baxter and Gaulde, 1980). Sufficient depth and a long water retention time provide conditions conducive to thermal stratification. In eastern North America thermal stratification during the summer months, coupled with high nutrient levels, may produce nuisance algal "blooms" and a high biological oxygen demand that may ultimately cause a depletion of oxygen in the hypolimnion. Lake production levels are ultimately related to the hypolimnetic oxygen deficit (Hutchinson,

1957; Charlton, 1980) and provide a potential for further deterioration of the water quality through chemical transformations (Pastorok et al., 1982).

Artificial circulation is one of the most frequently used methods to help manage water quality problems associated with thermal stratification and hypolimnetic oxygen depletion in reservoirs (Toetz et al., 1972; Fast, 1979; Pastorok et al., 1980; Raman and Evans, 1984). Personnel from the U. S. Army Corps of Engineers, Huntington District, installed four artificial destratification fans in Beech Fork Lake, Wayne County, West Virginia, in May 1987 in an attempt to alleviate the problems associated with summer thermal stratification. Historically, during the summer thermal stratification period at Beech Fork Lake, conditions of severe oxygen depletion, low pH, high levels of conductivity, manganese, iron, and nitrogen were measured in the hypolimnetic water (U. S. Army Corps of Engineers, pers. comm.).

Initiation of the artificial lake destratification project provided a unique opportunity to monitor the effects on water quality and macrobenthos. Objectives of this study were: 1) to determine if any significant changes occurred in water quality or macrobenthos that could be attributed to first-year operation of the fans; 2) to determine if the seasonal changes of macrobenthos were significant; 3) to determine which group(s) of macrobenthos were most affected. This was accomplished by comparing data bases from pre-destratification (1985) and post-destratification (1987) conditions.

DESCRIPTION OF STUDY AREA

Beech Fork Lake, a U. S. Army Corps of Engineers project, was authorized by the Flood Control Act of 1962 (Public Law 87- 874). The reservoir has minimum and essential net pool areas of 182 and 291 ha, respectively. During maximum pool, lake elevation is 181 m; mean depth, 3.9 m; and shoreline length, 50 km.

MATERIALS AND METHODS

Water Quality

During the study periods, dissolved oxygen (mg/L), pH, and temperature (C) were measured biweekly at four stations (1BBF20001, 1BBF20123, 1BBF20124, 1BBF20125) in Beech Fork Lake (Table 1). A HYDROLAB Model 6 surveyor surface unit was used to measure all

parameters. At each station, data were collected at the surface and every 0.6096 m (2 ft) to the bottom of the lake.

Macrobenthos

Macrobenthos were collected from the four stations in Beech Fork Lake using Hester Dendy multiplate samplers. Samplers were set in triplicate at each station with each sampler having an area of 0.0929 m^2 (1 ft^2) for a total of 0.2787 m^2 (3 ft^2) per station. A small float was attached to one end of the samplers. Samplers were then anchored in place on the lake bottom at the particular station by SCUBA divers. At two stations (1BBF20124, 1BBF20125), multiplate samplers were installed (triplicate) at depths 1.53 and 4.58 m (5 and 15 ft) and were allowed to be colonized for approximately six weeks. At two additional stations (1BBF20123, 1BBF20001), multiplate samplers were installed at 4.58 m (5 and 15 ft) and were allowed to be colonized for approximately six weeks. All samples were colonized during the following time periods: 23 July - 13 September 1985, 28 September - 2 November 1985 (pre-destratification), 8 August - 19 September 1987, 25 September - 30 October 1987 (post-destratification). At the end of the sampling period, SCUBA divers retrieved the samplers *in situ* by carefully enclosing each sampler in a zip-lock water tight bag and bringing them to the surface. Benthic samples were preserved in the field in 10% formalin and returned to the laboratory for sorting. Identifications were made to the lowest possible taxonomic category.

Data Analysis and Statistics

The Shannon-Weaver diversity index $\left(\bar{d}\right)$ and the mean number of organisms per sample were calculated for each station (Lloyd and Ghelardi, 1964; Lloyd et al., 1968). Mode of existence (clingers, sprawlers, swimmers, divers) and functional feeding groups (collector-gatherers, collector-filterers, and predators of macrobenthos) were also determined (Merritt and Cummins, 1984). A multivariate analysis of variance (MANOVA) was used to test for significant ($p<0.05$) differences in the parameters that may have resulted from destratification. Annual means of each parameter (mean density/m^2, diversity index, number of taxa, pH, temperature, and dissolved oxygen) at each sampling station were used for statistical comparisons. Wilks' Criterion was used to determine if there was an overall significant difference ($p<0.05$) in each of the two seasons (summer and fall) since operation of the destratification fans. A t-test (Lin, 1986) was

utilized to determine if a significant difference ($p<0.05$) existed between 1.53 m and the 4.58 m depths for each parameter within each sampling period. Step-wise discriminant analysis (Dixon, 1974) was employed to determine the extent of variation between seasons (axis 1) and before and after artificial destratification (axis 2). This analysis also helped identify those parameters which were influenced most by change in the season or artificial destratification.

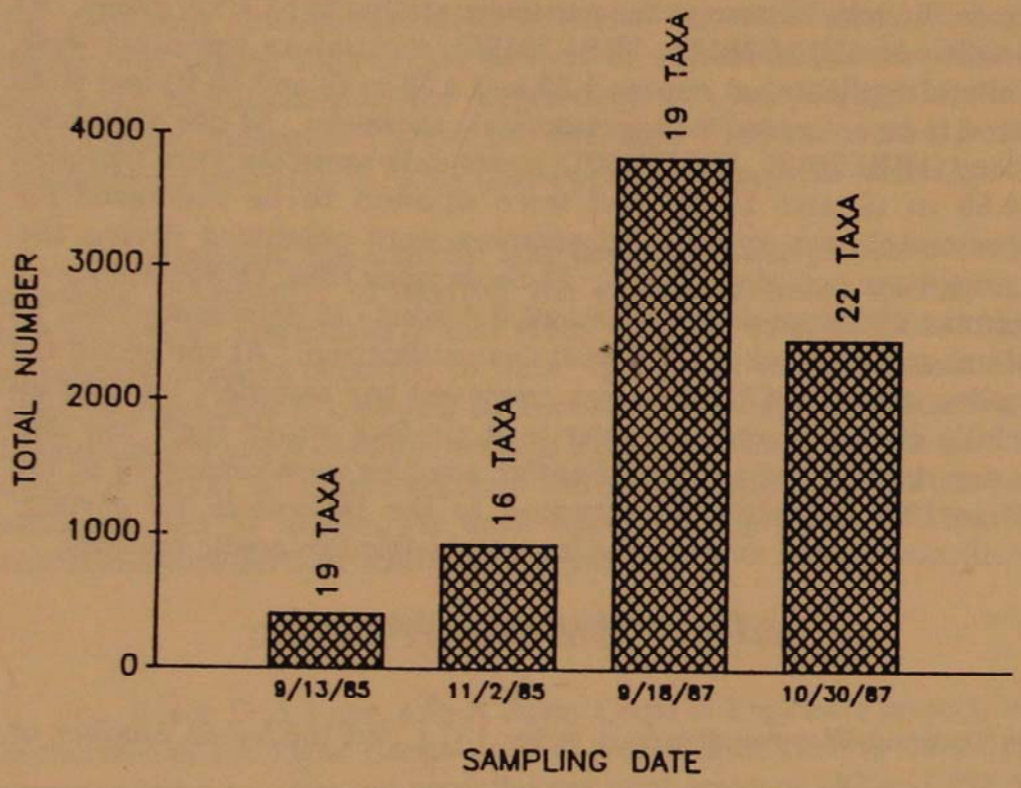

Figure 1. Total number and total taxa collected from all stations at 1.53 and 4.58 m, during 1985 and 1987.

RESULTS

Summer Season - During pre-destratification (1985), 405 organisms were collected from a total of eighteen Hester Dendy samplers located at Stations 1BBF20001, 1BBF20123, 1BBF20124, 1BBF20125 at 1.5 and 4.6 m during the sampling period of 23 July - 13 September 1985 (Figure 1). Nineteen taxa representing nine families and 19 general were identified from the samples (Table 1).

Table 1. Summary of benthic macroinvertebrates collected from Hester Dendy multiplate samplers at 1.53 m and 4.58 m, 13 September 1985 (*), 2 November 1985 (**, pre-destratification), 18 September 1987 (+), 30 October 1987 (++, post-destratification), Stations 1BBF20001 (=001), 1BBF20123 (+= 123), 1BBF20124 (= 124), 1BBF20125 (= 125).

Nematoda (*) (124,125)
Platyhelminthes
- Turbellaria
  - Planaridea (*, **, +, ++) (001, 124, 125)

Annelida
- Oligochaeta (*, **, +, ++) (001, 124, 125)

Arthropoda
- Insecta
  - Ephemeroptera
    - Baetidae
      - *Cloeon* (*, **, ++) (125)
    - Caenidae
      - *Caenis* (8, **, +, ++) (001, 124, 125)
    - Heptageniidae
      - *Stenacron* (*, **, +, ++) (123, 124, 125)
      - *Stenonema* (*, **) (124)
  - Plecoptera
    - Perlidea
      - *Acroneuria* (+) (001, 125)
  - Tricoptera
    - Hydroptilidea
      - *Hydroptila* (+, ++) (124, 125)
    - Polycentropodidae
      - *Neureclipsis* (*, **, +, ++) (001, 123, 124, 125)
  - Diptera
    - Ceratopogonidae (+) (124)
    - Chironomidae (*, **, +, ++) (001, 123, 124, 125)
      - *Chironomus* (*, **, +, ++) (001, 123, 124, 125)
      - *Cryptochironomus* (+, ++) (124, 125)
      - *Dicrotendipes* (*, **, +, ++) (001, 123, 124, 125)

Table 1. Continued

		Endochironomus (*, +, ++) (123, 124, 125)
		Glyptotendipes (*, **, =, ++) (001, 123, 124, 125)
		Parachironomus (*, **, +, ++) (001, 123, 124, 125)
		Tanytarsus (*, +, ++) (001, 124, 125)
		Coelotanypus (*, +) (001,124)
		Ablabesmyia (*, +, ++) (001, 123, 124, 125)
		Larsia (*, **, +, ++) (001, 123, 124, 125)
		Tanypus (*, +, ++) (001, 124, 125)
	Tipulidae	
		Tipula (**)
Odonata		
	Coenagrionidae	
		Argia (*, +, ++) (001, 124, 125)
	Macromiidae	
		Macromia (++)
Mollusca		
Gastropoda		
	Planorbidae	
		Gyraulis (**, ++)
Pelecypoda		
	Corbiculidae	
		Corbicula (++)
Collembola		
	Entomobryidae	
		Harlomillsia (**)

Unidentified larval chironomids ranked first in total number (114), percent composition (28.2), and mean density (0.59/m^2). Larval *Glyptotendipes* ranked second in numbers (89) and percent composition (22.0); the mean density was 0.46/m^2.

Based on mode of existence, burrowers ranked first in total number (107) and percent composition (26.4) (Figure 2); the mean

density was 0.55/m^2. Clingers ranked second in total number (103) and percent composition (25.4); the mean density was 0.53/m^2.

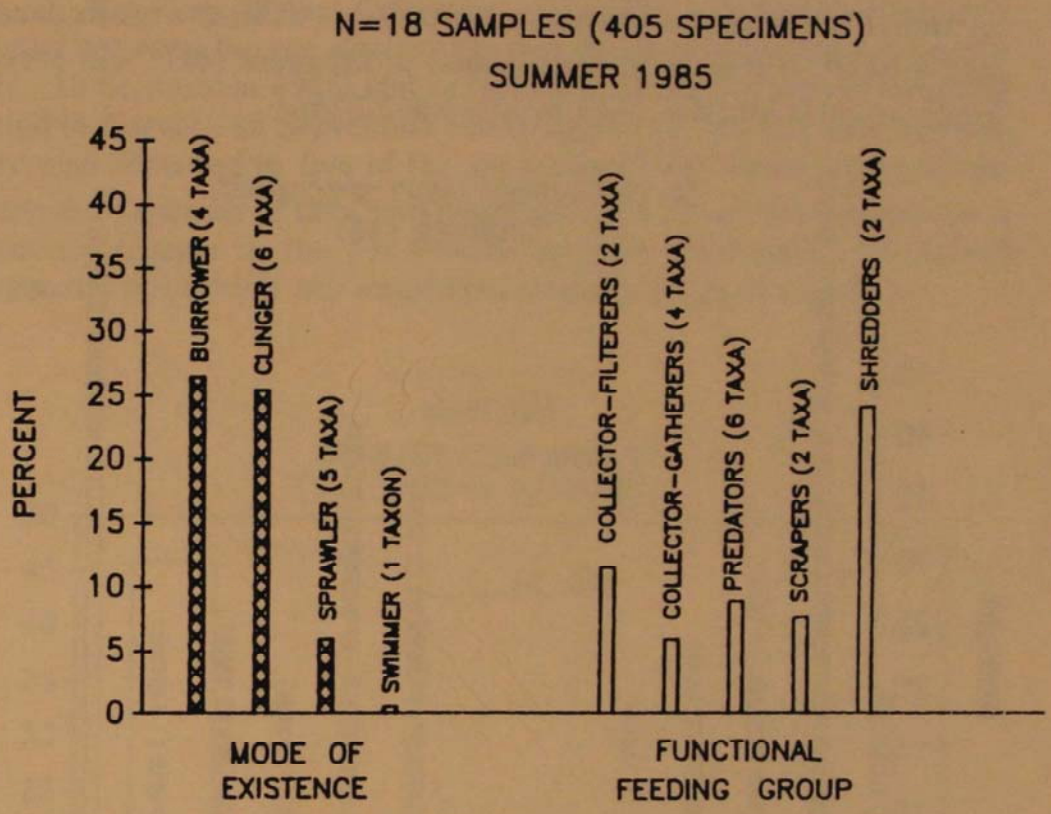

Figure 2. Percent composition of classified ecological types identified from all stations (23 July - 13 September 1985).

Based on functional feeding groups, shredders ranked first in numbers (98) and percent composition (24.7) (Figure 2); the mean density was 0.50/m^2. Collector-filterers ranked second in numbers (47) and percent composition (11.6); the mean density was 0.24/m^2.

Overall, mean density was 2.09/m^2 for the summer collecting period (1985). The overall taxa diversity was 3.2.

During post-destratification (1987), a total of 3827 organisms was collected from eighteen Hester Dendy samplers located at all stations at 1.5 and 4.6 m during the sampling period of 8 August - 18 September 1987 (Figure 1). Nineteen taxa, representing nine families and 19 genera, were collected from the samples (Table 1). Larval *Glyptotendipes* ranked first in number (1225) and percent composition (32.0); the mean density was 6.3/m^2. Unidentified larval chironomids

ranked second in numbers (1205) and percent composition (31.5); the mean density was 6.2/m^2.

Based on mode of existence (Figure 3), burrowers ranked first in numbers (1549) and percent composition (40.5); the mean density was 8.0/m^2. Clingers ranked second in numbers (497) and percent composition (13.0); the mean density was 2.57/m^2.

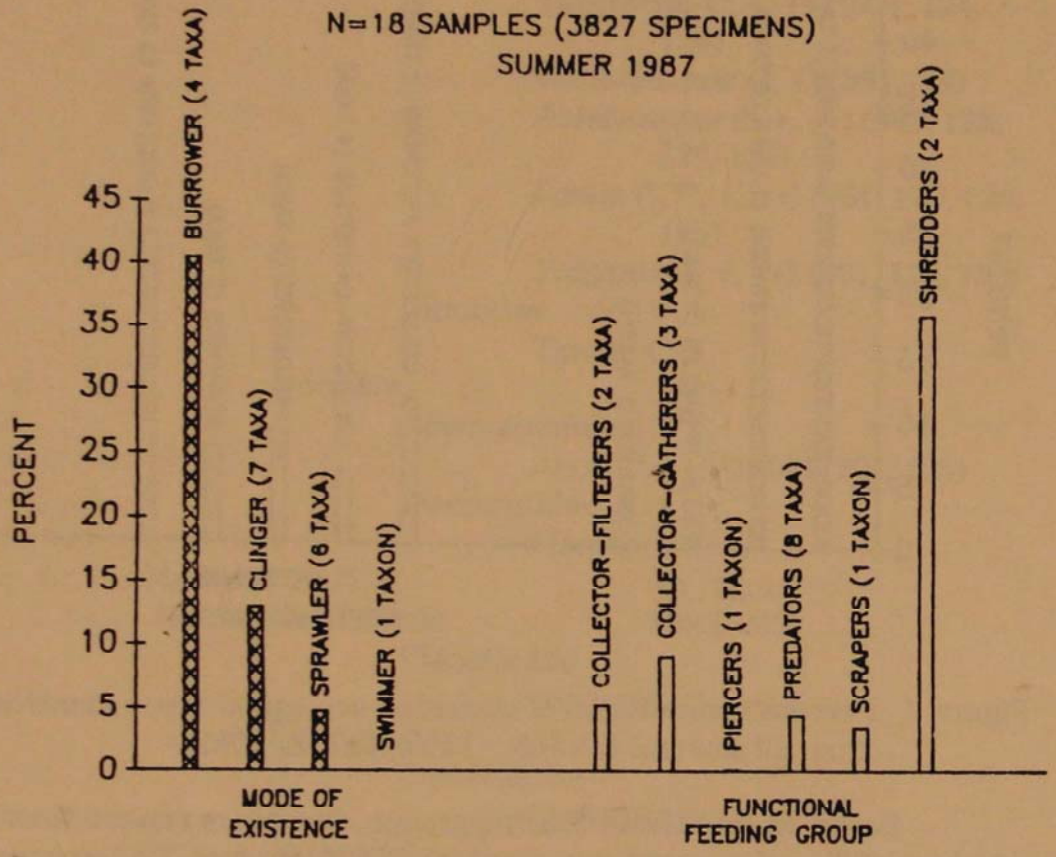

Figure 3. Percent composition of classified ecological types identified from all stations (8 August - 18 September 1987).

Based on the functional groups, shredders ranked first in numbers (1377) and percent composition (36.0) (Figure 3); the mean density was 7.10/m^2. Collector-gatherers ranked second in numbers (348) and percent composition (19.3); the mean density was 0.85/m^2.

Overall, the mean density was 19.76/m^2 for the summer collecting period (1987); taxa diversity was 2.7.

Based on Wilks' Criterion, a significant difference ($p<0.05$) existed between the pre-destratification summer sampling season (23 July - 13 September 1985) and post-destratification summer sampling period (8 August - 18 September 1987) (Figure 4). Significant increases were also observed in five of the six parameters: temperature, mean density/m^2, number of taxa, and diversity index. Most noticeable was a dramatic change in the pH values between each year. Dissolved oxygen did not exhibit any significant overall change (Table 2).

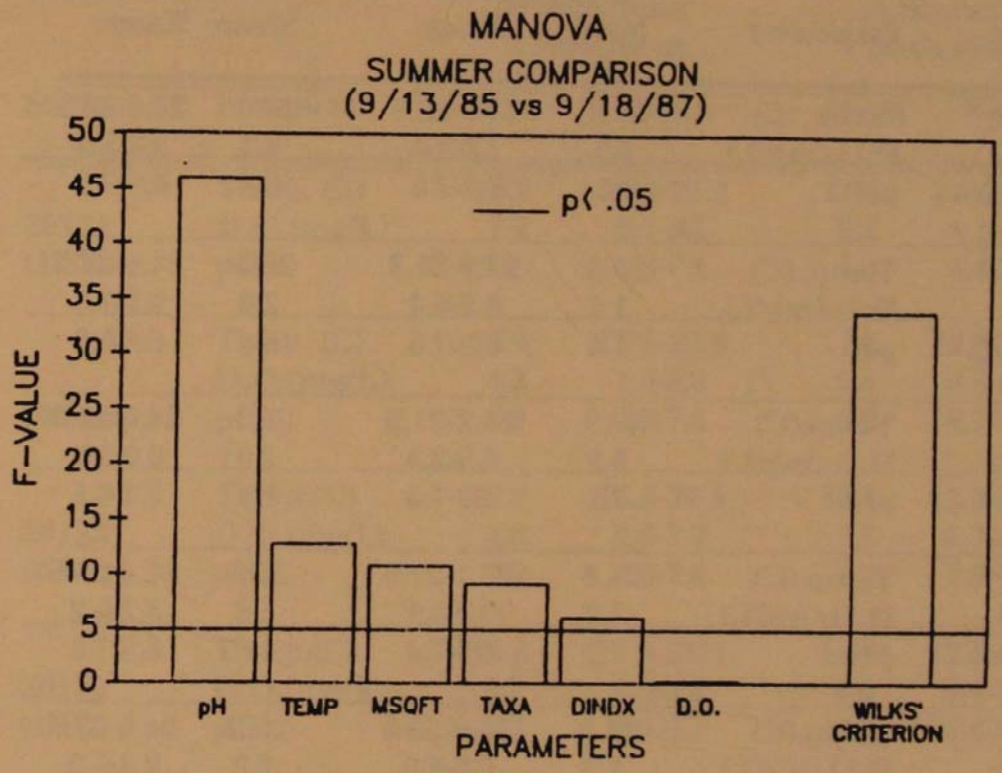

Figure 4. Significance of parameters (pH, temperature, mean number/square foot, number of taxa, diversity index, dissolved oxygen) of the summer season MANOVA.

Fall Season - During pre-destratification, 824 organisms were collected from eighteen Hester Dendy samplers located at Stations 1BBF20001, 1BBF20123, 1BBF20124, and 1BBF20125 at 5 ft and 15 ft

(1.5 and 4.6 m) during the sampling period of 28 September - 2 November 1985 (Table 1, Figure 1). Sixteen taxa , representing seven families and at least nine genera, were collected from the samples (Table 1). Oligochaete worms ranked first in numbers (482) and percent composition (52.2); the mean density was 2.49/m^2. Larval *Glyptotendipes* ranked second in numbers (137) and percent composition (14.8), and mean density (0.71/m^2).

Table 2. Seasonal water quality parameters, Beech Fork Lake, Wayne County, West Virginia.

Station	Parameter	Summer 1985 23 July - 13 September Mean	Range	Summer 1987 8 August- 18 September Mean	Range
	Temp. (C)	25.8	24.0-26.8	26.6	24.6-28.5
20124	D.O.(mg/L)	8.1	7.8-8.6	4.3	3.2-5.6
(1.53m)	pH	7.4	7.1-7.6	6.8	6.6-7.0
	Temp. (C)	20.0	17.9-21.3	26.2	24.0-27.6
	D.O.(mg/L)	1.4	0.5-5.4	2.0	0.1-3.8
(4.58m)	pH	7.4	6.9-7.6	6.6	6.4-6.8
	Temp.(C)	19.9	18.2-21.9	26.1	24.0-27.3
20123	D.O.(mg/L)	0.9	0.5-2.4	2.0	0.0-3.0
(4.58m)	pH	7.1	6.9-7.3	6.5	6.4-6.5
	Temp.(C)	25.8	23.1-27.0	26.6	24.4-28.6
20125	D.O.(mg/L)	8.0	7.4-8.1	4.8	3.2-6.2
(1.53m)	pH	7.2	6.9-7.4	6.8	6.5-7.0
	Temp.(C)	20.2	18.0-22.5	26.2	24.0-27.8
	D.O.(mg/L)	1.3	0.5-6.0	2.2	0.2-5.2
(4.58m)	pH	7.2	6.9-7.4	6.6	6.4-6.8
	Temp.(C)	19.9	17.6-21.8	26.2	24.0-27.8
20001	D.O.(mg/L)	0.9	0.4-3.6	1.2	0.2-3.2
(4.58m)	pH	7.0	6.7-7.4	6.5	6.4-6.7

Table 2. Continued.

Seasonal Means for Summer 1985:	Temp.	= 21.9 C
	D. O.	= 3.4 mg/L
	pH	= 7.2
Seasonal Means for Summer 1987:	Temp.	= 26.3 C
	D. O.	= 2.8 mg/L
	pH	= 6.6

20124 = Station 1BBF20124; 20123 = Station 1BBF20123; 20125 = Station 1BBF20125; 20001 = Station 1BBF20001

		Fall 1985 28 September - 2 November		Fall 1987 25 September- 30 October	
Station	Parameter	Mean	Range	Mean	Range
	Temp. (C)	18.7	16.8-21.4	17.9	12.6-23.0
20124	D.O.(mg/L)	7.7	5.7-9.1	6.6	4.1-8.2
(1.53m)	pH	7.2	6.8-7.4	7.0	6.8-7.1
	Temp. (C)	17.8	16.5-20.6	17.8	12.5-23.1
	D.O.(mg/L)	4.3	1.8-7.3	6.4	3.9-6.2
(4.58m)	pH	6.9	6.6-7.4	6.9	6.7-7.1
	Temp.(C)	17.8	16.5-20.8	15.8	12.3-20.2
20123	D.O.(mg/L)	4.0	0.6-7.7	7.6	6.7-8.4
(4.58m)	pH	7.0	6.6-7.4	7.0	7.0-7.1
	Temp.(C)	18.8	16.5-21.4	18.0	12.7-23.3
20125	D.O.(mg/L)	7.7	5.9-9.4	6.4	3.4-8.2
(1.53m)	pH	7.3	6.8-7.7	7.0	6.8-7.2
	Temp.(C)	17.8	16.5-21.0	17.9	12.6-23.2
	D.O.(mg/L)	4.4	0.6-6.7	6.1	3.2-8.0
(4.58m)	pH	6.9	6.5-7.4	6.9	6.7-7.1
	Temp.(C)	18.0	16.3-21.4	17.8	12.5-23.2
20001	D.O.(mg/L)	6.2	1.2-6.7	6.4	4.2-8.2
(4.58m)	pH	6.9	6.5-7.5	7.0	6.8-7.2

Table 2. Continued

Seasonal Means for Fall 1985	Temp.	= 18.2 C
	D. O.	= 5.7 mg/L
	pH	= 7.0
Seasonal Means for Fall 1987:	Temp.	= 17.5 C
	D. O.	= 6.6 mg/L
	pH	= 7.0

20124 = Station 1BBF20124; 20123 = Station 1BBF20123; 20125 = Station 1BBF20125; 20001 = Station 1BBF20001

Based on mode of existence, burrowers ranked first in numbers (218) and percent composition (23.6) (Figure 5); the mean density was $1.13/m^2$ and most were dominated by larval *Glyptotendipes* (137). Sprawlers were second in numbers (56) and percent composition (6.0); the mean density was $0.29/m^2$.

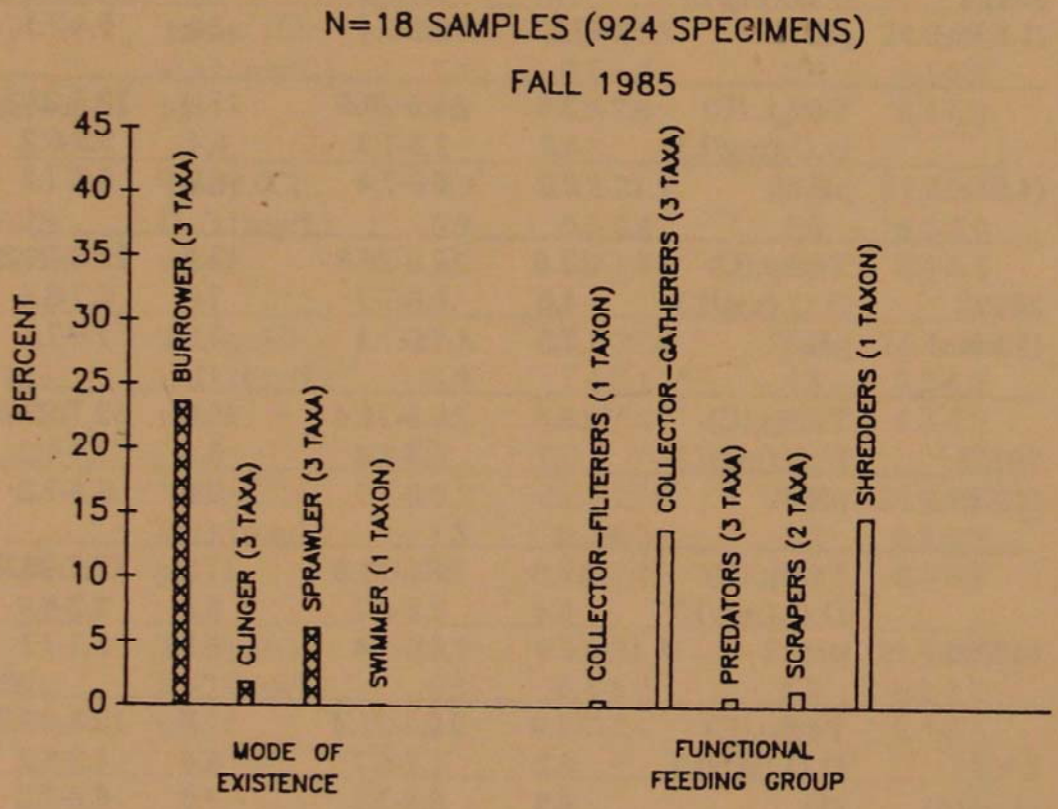

Figure 5. Percent composition of classified ecological types identified from all stations (28 September-2 November 1985).

Based on functional feeding groups, shredders (larval *Glyptotendipes*) ranked first in numbers (137) and percent composition (14.9) (Figure 5); the mean density was 0.72/m^2. Collector-gatherers ranked second in numbers (129) and percent composition (13.9); the mean density was 4.77/m^2; the composited, overall taxon diversity was 2.3.

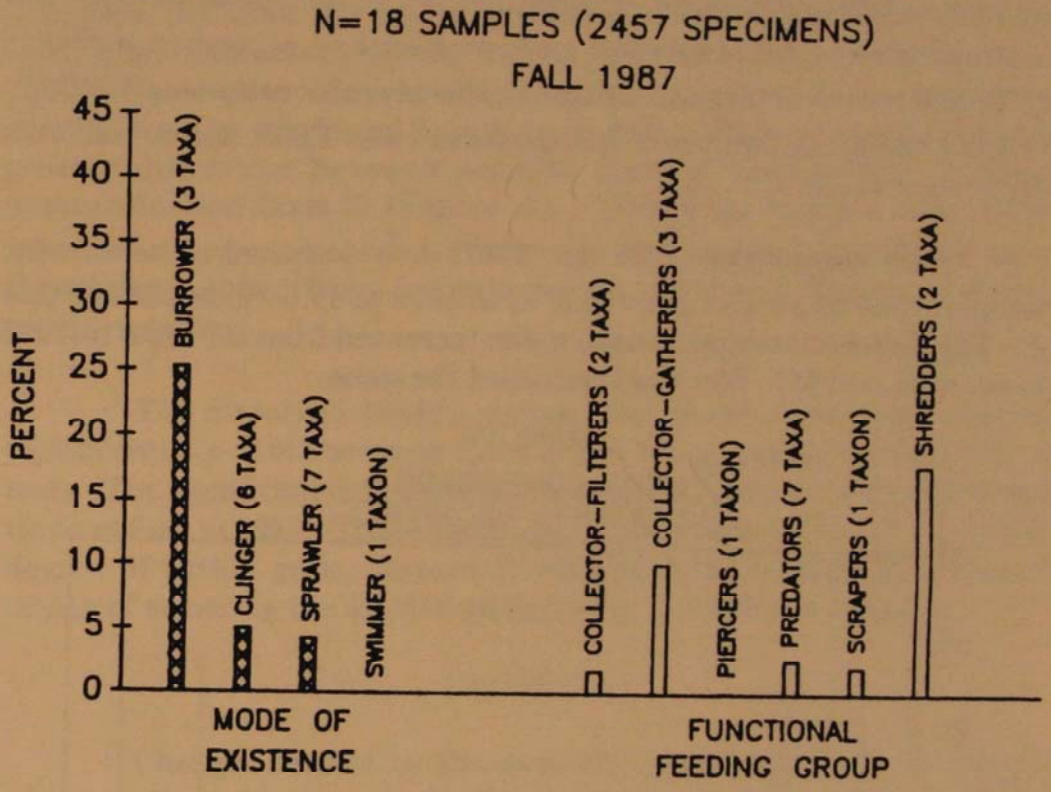

Figure 6. Percent composition of classified ecological types identified ftrom all stations (25 September 30 October 1987).

During post-destratification (1987), 2457 organisms were collected from eighteen Hester Dendy samplers located at Stations 1BBF20001, 1BBF20123, 1BBF20124, and 1BBF20125 at 1.5 and 4.6 m during the sampling period of 25 September - 30 October 1987 Figure 1). Twenty-two taxa, representing 12 families and 22 genera, were identified from the samples (Table 1). Oligochaete worms ranked first in numbers (774) and percent composition (31.5); the mean density was 4.0/m^2. Larval *Glyptotendipes* ranked second in numbers (540) and percent composition (22.0); the mean density was 2.79/m^2.

Based on mode of existence, burrowers ranked first in numbers (620) and percent composition (25.2); the mean density was 3.20/m^2 (Figure 6). Clingers ranked second in numbers (123) and percent composition (5.0); the mean density was 0.63/m^2.
from all stations (25 September-30 October 1987).

Based on functional feeding groups, shredders ranked first in numbers (439) and percent composition (17.9); the mean density was 2.27/m^2 (Figure 6). Collector-gatherers ranked second in numbers (249) and percent composition (10.1); the mean density was 1.29/m^2. Overall, the mean number of specimens/m^2 was 12.69; the overall taxa diversity was 2.9.

In comparing 1985 to 1987, the seasonal mean water temperature decreased from 18.2 °C in 1985 to 17.5 °C in 1987 (Table 2). The dissolved oxygen season mean increased from 5.7 mg/L in 1985 to 6.6 mg/L in 1987. Mean pH remained the same.

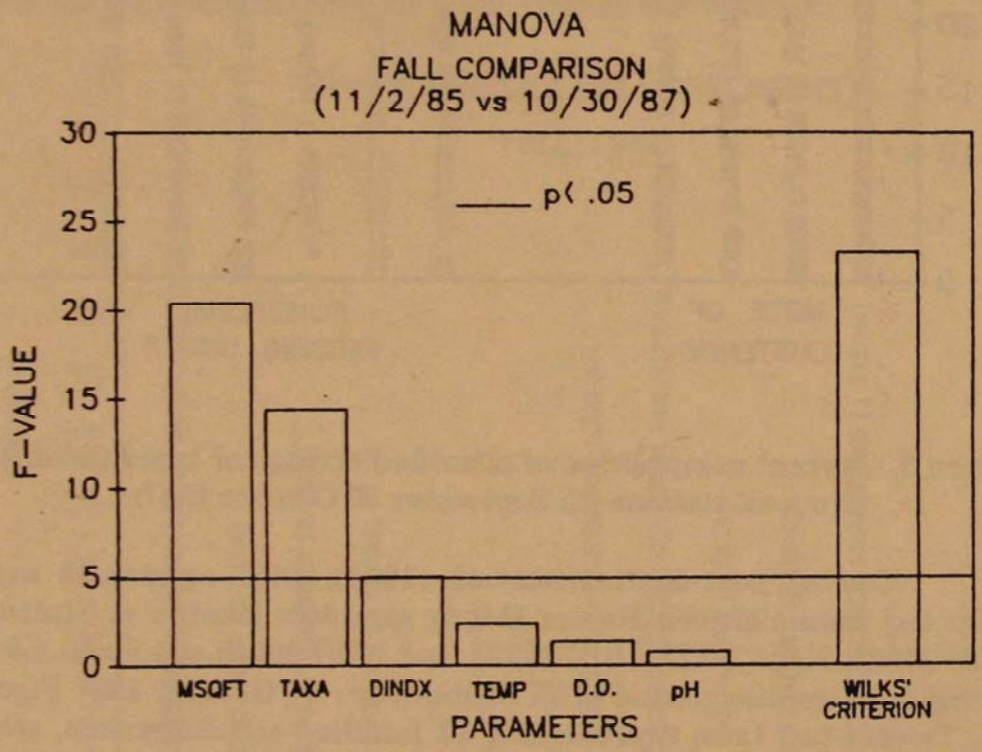

Figure 7. Significance parameters (mean number/square foot, number of taxa, diversity index) of the fall season MANOVA.

Wilks' Criterion showed that significant differences occurred between the fall seasons of 1985 and 1987 for the mean density/m^2,

number of taxa, diversity index, and temperature (Figure 7). Dissolved oxygen and pH were not significantly different (Figure 7).

Step-wise discriminant analysis showed (as plotted on the first two canonical axes) significant differences ($p<0.01$) among all four sampling dates (Figure 8). The first two canonical axes accounted for 99.67% of the total separation. Based on coefficients of canonical variables, pH, diversity index, and temperature were the three most important characters in separating the sampling periods when comparing seasons. Separation of sampling periods before and after destratification was based primarily on pH. There appeared to be a greater difference between seasons (axis 1) than before or after destratification (axis 2) (Figure 8). Dissolved oxygen and mean number of organisms/m^2 did not pass the tolerance test and were therefore excluded from analysis due to their high degree of within group variation.

The dissolved oxygen values and mean density/m^2 varied significantly ($p<0.05$) between the 1.5 and 4.6 m depths as shown by t-tests. The significant difference was noted in all four t-tests of 1985 and three of four in 1987. These significant differences account for the high degree of within group (internal) variation due to the experimental design of sampling the 4.6 m depths during the summer months.

Discussion

Changes caused by the destratification fans have an overall positive impact on macrobenthos at Beech Fork Lake. These improvements are shown through increases in faunal density and number of taxa present following operation of the fans. Reports from other lakes undergoing artificial destratification (Kothandaraman et al., 1978; Wilhm et at., 1978) have shown that changes occurring in the benthic macroinvertebrate populations are usually consistent with an increase in numbers, biomass, and diversity of those taxa present prior to treatment. The same situation has occurred at Beech Fork Lake, where dominant taxa present after treatment (e.g. chironomids, oligochaete worms and caddisflies) were the same as before treatment. Chironomids before and after comprised about 65 - 80% of the total organisms present, with larval *Glyptotendipes* ranging from a low of 14.8% in the fall of 1985 to a high of 32.0% in the summer of 1987. Oligochaete worms remain second in ranking, ranging from 14.7 in the summer of 1987 to 31.5% in the fall of 1987. Larval *Neureclipsis*,

planariid worms, and *Stenacron* also remain important although their numbers changed. This agrees with reports of Kothandaraman et al.

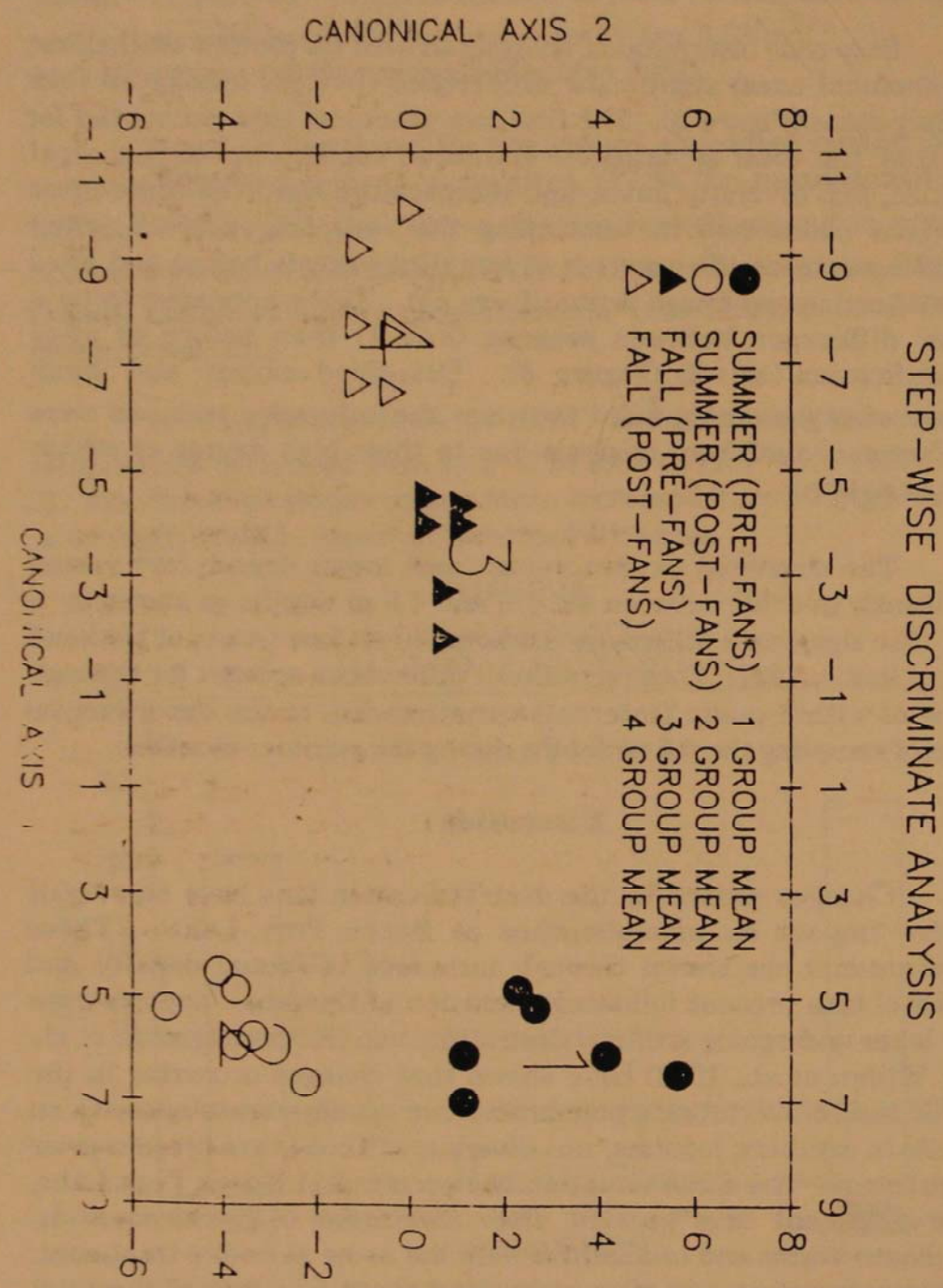

Figure 8. Plot of the first two canonical axes (axis 1 = seasons and axis 2 = destratification) for each station (sampling date). Each symbol represents a composite of 6 parameter means or the group mean.

(1978) and Wilhm et al. (1978) that quantitative, not qualitative, changes usually occur as a result of destratification.

Barnes (1977) concluded that destratification techniques allow migration of macrobenthos from littoral zone areas down into the previously uninhabitable profundal zone, and this situation is obviously occurring at Beech Fork Lake at all 4.58 m sampling depths. Pastorok et al. (1980) reported that colonization of previously uninhabited profundal zones may occur by: 1) passive transport of animals by currents, 2) active migration from other areas, and 3) colonization by sedimentation of eggs. All of these are probably occurring at Beech Fork Lake since all of the stations are located within the destratified area of the lake. The presence of the destratification fans allowed the organisms to survive where previously unfavorable water quality parameters existed.

The trophic structure of lakes undergoing destratification may shift in such a way that predatory larvae decrease in abundance as resident detritivores increase (Pastorok et al., 1980). At Beech Fork Lake the predators in the summer season decreased from 8.9% in 1985 to 4.4% in 1987. A shift in trophic structure did not occur in the fall season, though, as predators increased from 0.8% to 2.7%. A slight increase in predators may be due to increased numbers of all groups, reducing competition and providing predators with a greater food source in the fall. Overall, during the fall the percent of predators was below summer levels. Shredders, which represent the dominant resident detritivores, increased in importance in both seasons between 1985 and 1987. A possible reason for the increase in shredders may be due to the increase of available detritus. The action of the fans creates currents that retard sedimentation of suspended solids and debris, allowing an increase of favorable periphyton and photoplankton for use by macrobenthos.

Increases in seasonal temperature in the summer is a major factor in the disruption of the thermocline. In Beech Fork, heating surface waters from the spring through late fall allows a temperature and density gradient to set up through the water column. The pre-destratification thermocline would usually occur at 3.05 m at Beech Fork Lake based on lake profile data from 1985 and past data from the U. S. Army Corps of Engineers. The lack of mixing and long water retention time coupled with high nutrient levels may have caused "blooms" of nuisance algae and a high biological oxygen demand that may ultimately have resulted in a depletion of oxygen in the hypolimnion (Hutchinson, 1957). All stations at the 4.58 m sampling

depths during the summer months experienced low concentrations of dissolved oxygen prior to destratification. Another problem in the hypolimnion may be that increased turbidity prevents light penetration and thus heat absorption (Wetzel, 1955).

Stratification of Beech Fork Lake in 1985 caused great differences in temperatures above 1.53 m and below 4.58 m. Disruption of the thermocline through operation of the fans allowed Beech Fork Lake to become isothermic between the 1.53 and 4.58 m sampling stations. Temperatures during two fall seasons (1985 vs 1987) were not significantly different.

T-tests indicate a significant difference ($p<0.05$) between the dissolved oxygen values at 1.53 and 4.53 m for the summer season for both years at Stations 1BBF20124 and 1BBF20125. Hypolimnetic waters typically experience an oxygen depletion in the summer due to epilimnetic algal blooms, high biological oxygen demand in the hypolimnion caused by decomposing and chemotropic bacteria, and low levels of mixing during stratification. The destratification process during 1987 prevented algal blooms from occurring. Input of oxygen by increased photosynthesis may have also contributed to the increase of dissolved oxygen during daylight hours. The lowering of the 1.53 m dissolved oxygen values can be attributed to a mixing and an averaging effect on the water column, i. e., the range of the oxygen values in 1987 was less extreme than that of 1985.

Summer season pH values decreased from a mean of 7.2 in 1985 to 6.6 in 1987 and is probably due to reduced dissolved oxygen: pH remained unchanged in the fall season. When carbon dioxide enters the water a small portion of it is hydrated to form carbonic acid, resulting in a lowering of the pH. This probably is the case at Beech Fork, but the degree of difference in the summer (pH = 7.2) (1985) compared to 1987 (pH = 6.6) cannot be totally explained by this. Possibly, there are some other sources of acids associated with the release of nutrients and organics from the sediments caused by the mixing action of the fans. Release of methane, hydrogen sulfides, and sulfates previously locked in the sediments may also be occurring, thereby lowering the pH. During the fall turnover, no differences were shown in the mean pH values.

ACKNOWLEDGMENTS

We would like to thank the U. S. Army Corps of Engineers, Huntington District, for funding this study. Our thanks go to the many

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Tardigrade fauna (Phylum: Tardigrada) in mosses and liverworts from Seneca Rocks in the Monongahela National Forest, Pendleton County, West Virginia

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Abstract

Twelve species of tardigrades, including one new species, were collected from mosses and liverworts from Seneca Rocks, a 1,000-foot quartzite formation located in the Monongahela National Forest in northeastern West Virginia. The quartzite rises above the North Fork Valley at the Mouth of Seneca, West Virginia. Three state records, *Diphascon oculatum* Murray, *Isohypsibius bakonyiensis* (Iharos), and *I. pappi* (Tharos) are recorded in this study. Additionally, the following nine species were found in mosses and liverworts on the ground, rocks and trees: *Diphascon* n. sp, *Hypsibius convergens* (Urbanowicz), *H. dujardini* (Doyere), *Isohypsibius macrodactylus* (Maucci), *Macrobiotus hufelandi* Schultze, *M. richtersi* Murray, *Milnesium tardigradum* Doyere, *Minibiotus intermedius* (Plate), and *Pseudechiniscus suillus* (Ehrenberg)

Introduction

Tardigrades (water bears) are found in marine, freshwater, and terrestrial microhabitats throughout the world. More than 500 species have been described, mainly in Europe. Despite their cosmopolitan distribution, the tardigrade fauna of the United States is poorly known. Regionally, tardigrade species have been reported from eastern Tennessee, western North Carolina, southeastern Virginia,

and West virginia (Nelson, 1975; Tarter et al., 1989). The primary objective of the investigation was to report the first records of tardigrades from Seneca Rocks.

Study Area

Seneca Rocks is a 1,000-foot quartzite formation of Silurian Age located in the Monongahela National Forest in Pendleton County, West Virginia. The formation rises above the North Fork Valley at the Mouth of Seneca.

Materials and Methods

Mosses and liverworts were collected from the ground, logs, rocks, and trees at Seneca Rocks during the summer of 1990. The samples were returned to the laboratory for identification and removal of tardigrades. They were soaked separately in a stoppered funnel in tap water overnight. Following the soaking process, the mosses and liverworts were removed and squeezed over a beaker to remove the remaining water and tardigrades. After the debris settled, the top layer of water was decanted and a small aliquot of the bottom layer was placed in a petri dish and searched for active tardigrades. If the sample was positive for tardigrades, the entire sample was fixed by the addition of hot ethanol. An Irwin loop (200 µm X 500 µm) was used to transfer the tardigrades from petri dishes to slides. Finally, tardigrades were mounted in Hoyer's medium and oriented under a small coverslip (No. 1) for identification under an Olympus BH phase contrast compound microscope. Depending on the abundance of tardigrades in the sample, a representative number (usually 5-15) was removed from each sample. Tardigrades were identified mainly with keys and descriptions in Ramazzotti and Maucci (1983).

Results and Discussion

Twelve species of tardigrades, including one new species, representing seven genera (*Diphascon, Hypsibius, Isohypsibius, Macrobiotus, Milnesium, Pseudechiniscus*) were identified from Seneca Rocks (Table 1). Three species, *Diphascon oculatum, Isohypsibius bakonyiensis*, and *I. pappi*, are state records. The most common species were *Macrobiotus richtersi* and *Minibiotus intermedius*.

The liverwort *Porella platyphylloidea* contained three species of tardigrades (Table 1) and all 12 species of tardigrades were found in the following mosses: *Anomodon attenuatus, Dicranum scoparum,*

Endodon cladorrhizans, Hedwigia ciliata, Hypnum curvifolium, Leucobryon albidum, Minium cuspidatum, and *Thuidium delicatulum.*

Table 1. Checklist and habitats of tardigrades from Seneca Rocks, Pendleton County, West Virginia.

Taxon	MOSS	LIVERWORT
Class Heterotardigrada		
Order Echiniscoides		
Family Echiniscidae		
Pseudechiniscus suillus (Ehrenberg, 1853)	X	X
Class Eutardigrada		
Order Parachela		
Family Macrobiotidae		
Macrobiotus hufelandi Schultze, 1833	X	X
M. richtersi Murray, 1911	X	
Minibiotus intermedius (Plate, 1988)	X	
Family Hypsibiidae		
Hypsibius convergens (Urbanowicz, 1925	X	
H. dujardini (Doyere, 1840)	X	
Isohypsibius bakonyiensis (Tharos, 1964)	X	X
I. macrodactylus (Maucci, 1978)	X	
I. pappi (Tharos, 1966)	X	
Diphascon oculatum Murray, 1906	X	
D. n. sp.	X	
Order Apochela		
Family Milnesiidae		
Milnesium tardigradum Doyere, 1840	X	

Based on this investigation at Seneca Rocks and the records of Tarter et al. (1989), 19 species of tardigrades have been reported from West Virginia.

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Protein patterns in salt-stressed sandalwood (*Santalum album* L.) callus

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Abstract

Effects of salt stress on changes in protein patterns were investigated in sandalwood calli. Four-week calli growing on Murashige-Skoog medium were transferred to a medium containing 2 - 8 g/L NaCl. After 24 to 60 h of exposure to varying salt concentrations, a 30 to 40% reduction in callus volume was observed. One-dimensional polyacrylamide gel electrophoresis revealed exposure to high salt concentrations produced both qualitative and quantitative reductions in overall protein patterns.

Introduction

Higher plants typically exhibit significant morphological and metabolic changes in response to perturbation of their normal environment. Many of these changes are believed to be adaptive responses which enable organisms to cope with the altered environment. In many cases, the phenotypic changes are complex and difficult to evaluate, thus making it difficult to establish a quantitative relationship between the environmental parameter and phenotypic response. In addition, precise control over environmental parameters and uniformity of response of plant tissue can be difficult to achieve. Such difficulties can be greatly reduced by the use of cultured plant cells.

East Indian sandalwood (*Santalum album* L.) has been known for over 2000 years and has been prized by the cultures of the East. Sandalwood attracted attention because of the fragrance of its yellow brown wood, and because the heartwood is capable of resisting the dreaded white ants of the tropics. The wood is extremely valuable to handicraft industry because it is close-grained and resembles ivory or ebony in its ability to be worked to a fine finish. Since 1974 there has

been a gradual decline in sandalwood plantations because of the devastating effects of spike disease, drought and salt stresses (Rao and Muniyappa, 1987).

We have been interested in understanding the altered expression of genes in response to salt and drought stress which may play a role in increased survival or growth of sandalwood. Our objective is to establish a correlation between level of different gene products *i.e.* mRNA or protein and the degree of adaptation to salt stress, which may be analogous to drought stress (Valluri, et al., 1990). In the present study, we examined the levels and changes of cellular proteins in callus tissue exposed to varying levels of salt stress.

Materials and Methods

Cell culture

Sandalwood (*Santalum album* L.) callus was obtained from The Indian Institute of Science, India. To establish callus tissue capable of prolific growth, calli were maintained on MS medium (Murashige and Skoog 1962) supplemented with 2,4-D and two different cytokinins viz. kinetin (Kn) and benzyladenine (BA). Callus formation was profuse on 2,4-D (1 mg/L) and BA (1 mg/L) compared with callus growing on 2,4-D (1 mg/L) and Kn (1 mg/L). The growth of callus was perpetuated by transferring the tissue every two weeks to fresh medium. Callus cultures were maintained at 25 ^{o}C and 16 h photoperiod.

NaCl-induced stress

Four week old calli pieces (25-50 mg each) were placed on Heller supports in test tubes containing liquid MS medium appended with varying levels of NaCl concentrations. For low stress level treatment, test tubes containing NaCl concentrations ranging from 1 g/L were established. For high stress level treatment, NaCl concentrations ranging from 10 g/L to 25 g/L were established. Sandal calli were harvested at varying time intervals ranging from 1 to 48 h.

Callus volume measurement

The change in callus volume relative to the changes in salt concentrations was determined by an air comparison pycnometer as described by Treat, et. al. (1988).

Extraction of proteins

At the end of each incubation period callus tissue was homogenized in ice-cold 50 mM Tris (hydroxymethyl) aminomethane (pH 8.65), containing 20 mM KCl, and 10 mM $MgCl_2$. The slurry was centrifuged at 10,000 g for 15 min and the supernatant was collected. Estimation of total protein was determined spectrophotometrically according to Bradford (1975).

Gel electrophoresis of proteins

Proteins were analyzed by one-dimensional electrophoresis on 12% polyacylamide gels containing 0.1% SDS as described by Laemmli (1970). Visualization of proteins was by silver staining (after equal amounts of protein were loaded onto each lane). Proteins were fixed in 50% methanol.

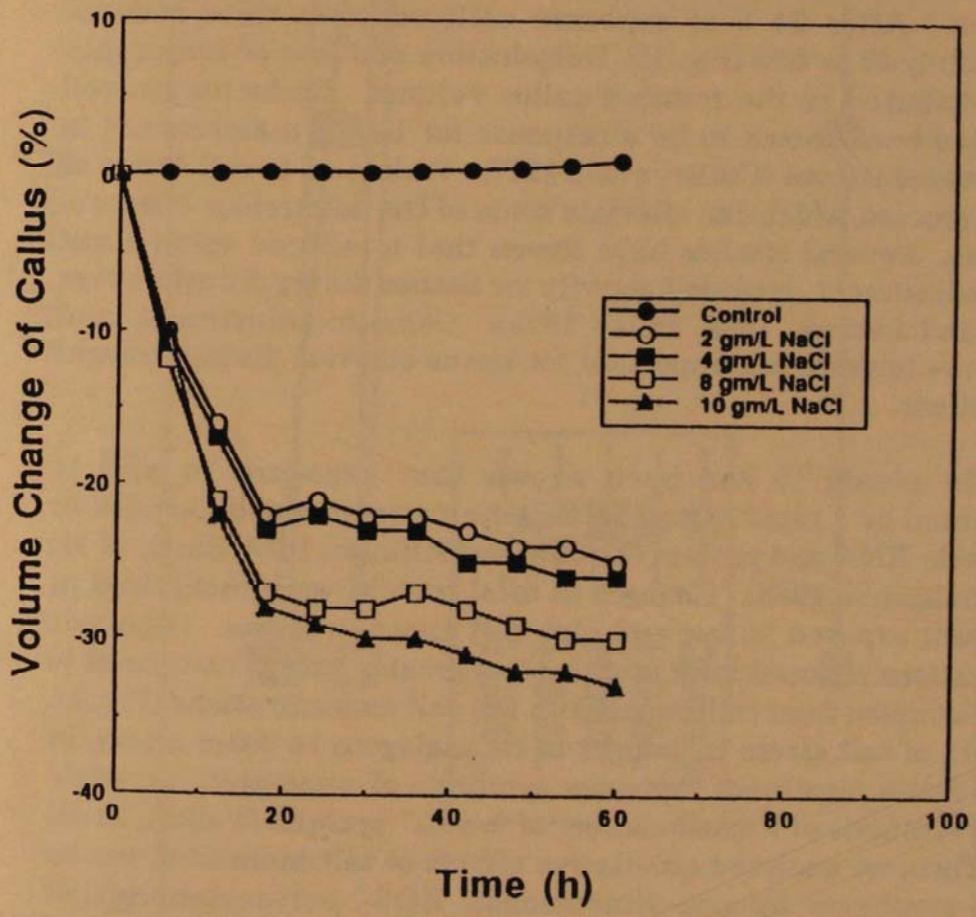

Figure 1. Reduction in calli volume at varying salt stress levels.

Results and Discussion

The effects of salt stress on the growth of sandal callus tissues were examined over a 7 day culture period. Calli exposed to high stress levels grew slowly with visible changes in morphology. Calli appeared to be healthy during the first day of salt stress but showed browning by the second day. Browning of calli was greater in cultures exposed to high salt concentrations (10 g/L to 25 g/L). However under low salt stress conditions, no browning was observed, but calli exhibited reduced levels of extractable protein. Three tissues appeared healthy and showed a shift in protein patterns compared to the unstressed tissue.

Volumes of calli relative to varying salt concentrations (2, 4, 8, 10 g/L) were measured over a 60 h period. Reduction in calli volumes were rapid at 8 and 10% salt levels for the first 24 h and leveled thereafter. After 24 h of exposure calli volumes were reduced approximately 30 to 40% (Fig. 1). Dehydration and loss of turgor may have contributed to the reduced callus volume. Reduction in cell volume has been shown to be a response for turgor maintenance in water stressed tissues (Cutler, et al,. 1977). Reduction in cell size is an adaptive process, which can alleviate some of the deleterious effects of salt stress. Several studies have shown that a reduced volume and osmotic adjustment provide longevity for tissues during drought stress (Flower and Ludlow, 1986, Hsiao 1973). Osmotic adjustment and turgor maintenance are important for tissue survival during drought and salt stress.

In plants it has been shown that exposure to salt is accompanied by a rapid alteration in gene expression and changes in translatable RNA and protein (Ericson and Alfinito, 1984; Singh *et al.*, 1985, Ramagopal, 1988). Changes in total protein were monitored in sandal calli exposed to low and high salt concentrations. High salt concentrations reduced total proteins to a greater extent compared to proteins sampled from calli exposed to low salt concentrations (Fig. 2). The effect of salt stress is thought to be analogous to water stress, in that the stress treatment represses synthesis of some proteins, while inducing synthesis of a small number of "stress" proteins (Valluri, *et at.*, 1989). Thus, we analyzed qualitative effects of salt-induced stress on protein synthesis by one-dimensional, SDS- polyacylamide gel electrophoresis. High salt stress levels resulted in total suppression of protein synthesis. Gels revealed only very low molecular weight proteins, most likely protein fragments and short polypeptide chains.

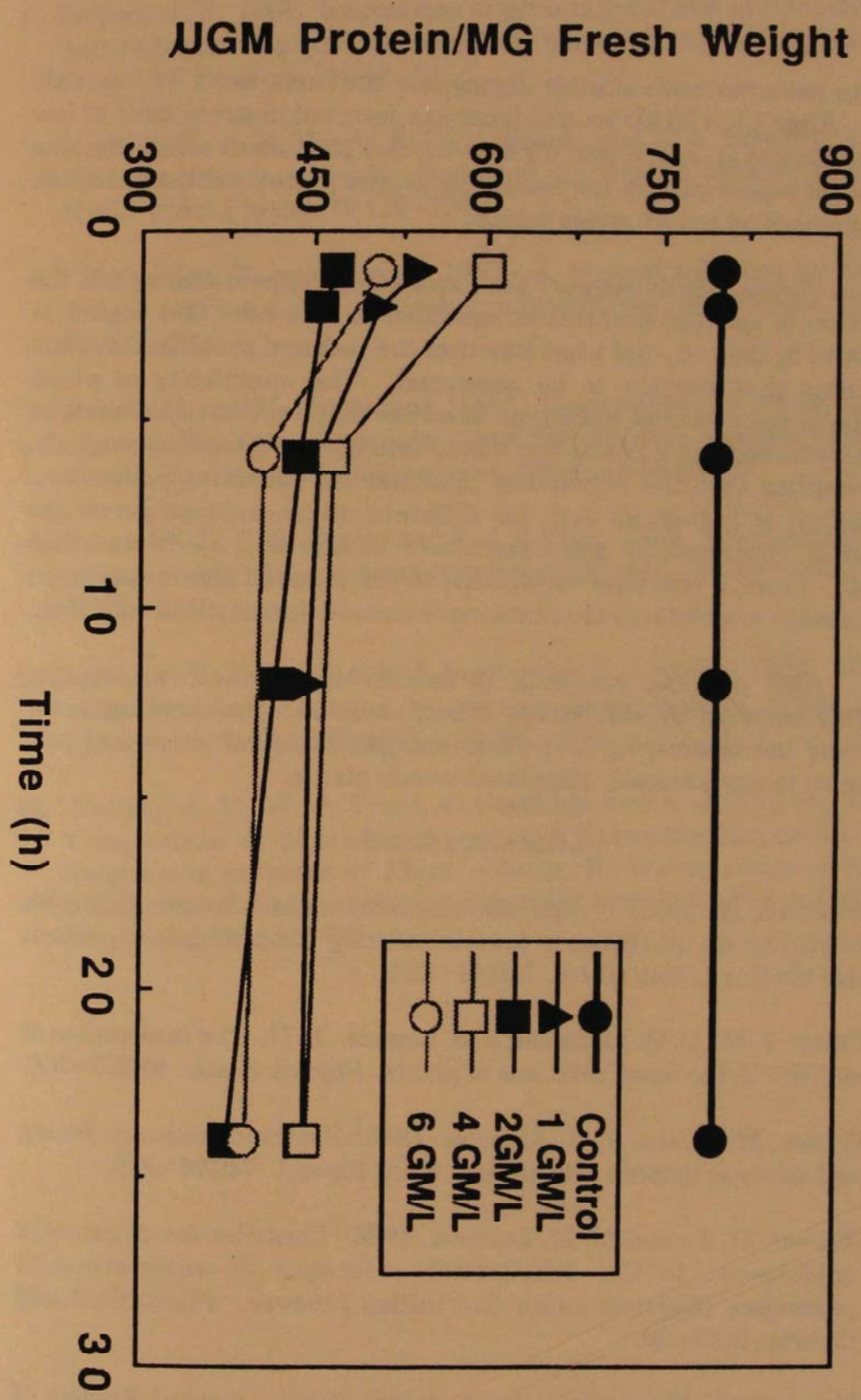

Figure 2. Bradford assay of total protein at low salt stress.

Protein patterns were similar during the first one hour of low salt stress. After 1 h, a 55 kD protein band was detected in stress calli at low levels of stress (1, 2, 4, 6, and 8 g/L). Another prominent alteration was the initial suppression of low molecular weight proteins followed by an enhancement after 12 h stress period.

These results suggest that salt stress appears to signal the induction of specific proteins in sandalwood but how this signal is perceived by the cell, and what function the induced proteins have are questions that remain to be answered. The specificity of plant metabolic responses to each type of stress indicates that patterns of stress-induced gene function are called into action by specific signals. This implies that the regulatory information, including promoters, enhancers, or regulatory loci, for different stress-induced genes are mutually independent and responsive to specific environmental signals. From a practical viewpoint, stress-induced gene expression may provide a useful way to create more stress-tolerant plant varieties.

Our ongoing research is mainly to characterize unique proteins induced by salt stress which seem to offer prospects for resolving the underlying molecular and genetic mechanisms of salt tolerance in economically-important woody plants.

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Bryophyte habitats of the Upper Shavers Fork Basin

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Abstract

Bryophyte populations were sampled in 45 study sites and 18 habitat types of the Upper Shavers Fork Basin of West Virginia during the spring and summer seasons of 1990. A total of 109 species of mosses and 24 species of hepatics were collected. Twelve bryophyte species of the study area are classed as rare in West Virginia based upon their reported occurrence in three or fewer counties. Six of these species occurred only in intermediately disturbed areas while three were restricted intensively disturbed areas.

The majority of bryophytes in the study area occur in intermediately disturbed habitats of the northern hardwood and spruce forests. The occurrence of most of these species in only one or two habitat types indicates a narrow degree of ecological tolerance. This suggests that intensive disturbance of the northern hardwood and spruce forest will result in a substantial reduction of species richness in the study area. Only six moss species demonstrate broad ecological tolerance by their occurrence in ten or more habitat types.

Introduction

Human activities such as mining and timbering produce conspicuous changes in the physical and biotic environments of mixed communities of bryophyte and vascular plants (Weaks and Creekmore, 1981; Engelmann and Weaks, 1985). These changes can be expected to generate quantitative and qualitative changes in bryophyte flora. Coal mining alone has destroyed vast amounts of prime habitat for plants. A staggering 322,700 ha have been ripped by uncontrolled strip-mining in Appalachia prior to 1966 (Udall, 1966). While no accurate estimate of comparable unregulated mining practices in the Appalachian region have been made available in more recent years, it seems reasonable to assume that the area subjected to destructive mining practices has continued to increase with time. A substantial loss of bryophyte flora

may have resulted from these activities paralleling those occurring in other parts of the world. For example, Barkman (1958) reported that within the last century the Dutch flora has lost 15% of the terrestrial bryophytes and 13% of the epiphytic species as a result of human disturbance.

Some bryophytes have been shown to benefit from disturbance (Carvey *et al.*, 1977). For example, a number of species of mosses have been found to have the ability to grow and develop on substrates that have been highly acidified by the presence of industrial wastes (Rao and LeBlanc, 1967). Carvey *et al.* (1977) reported that highly acid mineral substrates of coal spoils present unusual opportunities for bryophyte colonization. In a somewhat different study, Bayfield (1976) demonstrated that mixed populations of bryophytes can effectively colonize bare soil more efficiently than single species.

Little is known of the ecological tolerances of most species of bryophytes and few definitive studies are available on the distribution of these plants in relation to environmental stress. But several papers dealing with the study of bryophyte flora in relation to ecological factors have been published. These include studies by Sharp (1939), Bryant *et al.* (1973), Bird and Hong (1975), Weaks and Creekmore (1981), and Engelmann and Weaks (1985).

This paper presents the results of studies of bryophytes and their habitats in the Upper Shavers Fork Basin. Purposes of the study were: (1) to establish species composition and richness within habitat types, (2) to evaluate the ecological tolerances of the various species within these habitats, and (3) to identify rare species occurring within the study area.

The Study Area

The study area lies within the phytogeographic province and section known as the Appalachian Plateau Province, Allegheny Mountain Section and is located at latitude 380 32' 45", longitude 790 54' 45". The highest elevation within the area is Bald Knob (1475 m elev.) on Back Allegheny Mountain, and the lowest elevation is at Cheat Bridge (1073 m elev.). The area is drained by two watershed systems, the Upper Shavers Fork of Cheat River which flows north to join with other forks of the Cheat River, and a small, high elevation drainage, Leatherbark Run, which drains the south-facing slope of Bald Knob, winding its way east through a steep cut in Back Allegheny Mountain,

past the town of Cass, into the Greenbrier River. The basin is predominantly forested by two physiognomically distinct types. These are described by Core (1966) as the northern evergreen forest type on the highest slopes and the northern hardwoods forest type at low elevations. The dominant canopy tree of the Northern Evergreen Forest is red spruce (*Picea rubens* Sarg.), and within the Northern Hardwood Forest the canopy dominants include yellow birch (*Betula alleghaniensis* Britt.), sugar maple (*Acer saccharum* Marsh.), and wild black cherry (*Prunus serotina* Ehrh.). Frequent breaks in the forest cover are evident and represent both reclaimed and orphan strip-mines. Selective timbering and clear-cutting, both of which have been conducted over a period of many years, have also contributed to intensive habitat disturbance within the study area.

The extreme geological complexity of the Upper Shaver's Fork region is due chiefly to a complicated system of faulting, folding, and overthrusting. The rock types of the study area are of the Hinton, Bluestone, and Princeton Formations of the Mauch Chunk Group (Mississippian) and the New River and Kanawha Formations (Pennsylvanian). These units are largely composed of beds of sandstone, shale, siltstone, and coal. Soils are medium-textured, of medium depth and are extremely rocky throughout the general area.

Methods

A general reconnaissance of bryophyte flora and distribution in the Upper Shavers Fork region was conducted in the spring of 1990. Based on these preliminary surveys, forest community information available, and topographic maps, the following eighteen habitat types were designated and selected for study:

BS - birch stand
FHF - floodplain hardwood forest
FSF - floodplain spruce forest
GSM - grass sedge marsh
LC - logging clear-cut
NHF - northern hardwood forest
OF - old field
OFM - open floodplain meadow
OW - open water of large stream
OWB - open water of beaver pond
RBS - road banks and shoulders
RR - railroad cut
RSM - reclaimed strip-mine
SB - *Sphagnum* bog
SBS - scoured banks large streams
SF - spruce forest
SM - strip-mine
SS - shrub swamp

Observations and collections of bryophytes from 45 study sites were made during the spring and summer seasons of 1990. Species richness (S) and species composition were used to evaluate intermediately and intensively disturbed habitat types. Intensive disturbance within the basin resulted primarily from the widespread physical removal of logs and overburden, construction of haul roads, and damage to immature trees. No undisturbed habitat types were located in the study area. Species richness was based solely on the bryophytes present at sampling sites and was simply the number of species in the study area.

Voucher specimens are deposited in the herbarium at Marshall University. Duplicate specimens are housed in the herbarium at the West Virginia Division of Natural Resources (Natural Heritage Program), Elkins, West Virginia. Nomenclature conforms to Crum and Anderson (1981) for the true mosses, McQueen (1990) for *Sphagnum* and Conard and Redfearn (1979) for hepatics.

Results and Discussion

The majority of moss species occurred in intermediately disturbed forested habitats of the Upper Shavers Fork Basin (Table 1). Of the total of 109 species found to be present, 51.4 percent and 30.3 percent were collected from the northern hardwood forest and spruce forest, respectively (Table 1). Species richness (S) was likewise high for the birch stand (25 species). The percentages of the total number of species, for intermediately disturbed habitats, ranged substantially higher than those for intensively disturbed habitats. While species diversity was not determined for habitat types, the species richment values suggest an agreement, in part, with the intermediate disturbance hypothesis of Whittaker (1970) and Connell (1978). This hypothesis predicts that species diversity will be higher when disturbance is intermediate on the scale of frequency and area. The harvesting of timber within the study area as well as strip-mining are infrequent activities that can rarely be classed as intermediate disturbances. Therefore, this hypothesis predicts that intensive disturbance resulting from such activities will cause a decrease in bryophyte species diversity.

Of the intensively disturbed habitats, S was found to be highest for road banks and shoulders. The removal of competing vegetation during road construction, coupled with the exposure of a nutrient poor

Table 1. Distribution of moss flora in habitats of the Upper Shaver's Fork Basin.

Extent of disturbance and habitat types	Number species	Percent of total number species (109)	in category	samples (319)
Intermediate disturbance		(percent of 83)		
Northern hardwood forest	56	51.4	67.5	17.6
Spruce forest	33	30.3	39.8	10.3
Birch stand	25	22.9	30.1	7.8
Floodplain spruce forest	25	22.9	30.1	7.8
Grass sedge marsh	24	22.0	28.9	7.5
Shrub swamp	19	17.4	22.9	6.0
Floodplain hardwood forest	18	16.5	21.7	5.6
Open floodplain meadow	11	10.1	13.3	3.4
Sphagnum bog	6	5.5	7.2	1.9
Open water of large streams	2	1.8	2.4	0.1
Intensive disturbance		(percent of 61)		
Road banks and shoulders	24	22.0	39.3	7.5
Old fields	19	17.4	31.1	6.0
Reclaimed strip-mines	16	14.7	26.2	5.0
Strip-mines	14	12.8	23.0	4.4
Scoured banks of large streams	13	11.9	21.3	4.1
Railroad cuts	13	11.9	21.3	4.1
Open water of beaver ponds	9	8.3	14.8	2.8
Logging clearcuts	5	4.6	8.2	1.6

substrate, would appear to make road banks and shoulders suitable substrates for colonization by certain opportunistic mosses. These habitats in the Upper Shavers Fork Basin were generally densely shaded by overhanging trees and located near seeps and/or received high amounts of water from surface runoff. Patterns of hepatic habitat distribution were similar to those for mosses.

Most hepatic species likewise occurred in intermediately disturbed forested habitats (Table 2). Of a total of 24 species found to be present within the study area, 62.5 and 54.2 percent were collected

Table 2. Distribution of hepatic flora in habitats of the Upper Shaver's Fork Basin.

Extent of disturbance and habitat types	Number species	Percentage of total number species (24)	in category	samples (58)
Intermediate disturbance			(percent of 22)	
Northern hardwood forest	15	62.5	68.2	25.9
Spruce forest	13	54.2	59.1	22.4
Floodplain spruce forest	5	20.1	22.7	8.6
Floodplain hardwood forest	4	16.7	18.2	6.9
Shrub swamp	3	12.5	13.6	5.2
Birch stand	2	8.3	9.1	3.4
Grass sedge marsh	2	8.3	9.1	3.4
Sphagnum bog	1	4.2	4.5	1.7
Open floodplain meadow	1	4.2	4.5	1.7
Open water of large streams	0	0.0	0.0	0.0
Intensive disturbance			(percent of 11)	
Road banks and shoulders	3	12.5	27.3	5.2
Scoured banks of large streams	2	8.3	18.2	3.4
Strip-mines	2	8.3	18.2	3.4
Reclaimed strip-mines	2	8.3	18.2	3.4
Old fields	1	4.2	9.1	1.7
Open water of beaver ponds	1	4.2	9.1	1.7
Railroad cuts	1	4.2	9.1	1.7
Logging clearcuts	0	0.0	0.0	0.0

from the northern hardwood forest and spruce forest, respectively. The percent of the total number of species was low for all intensively disturbed habitat types. The percent of the total number of samples (58) was, likewise, low for this category of disturbance. As was the case for mosses, S was higher for road banks and shoulders than for other intensively disturbed habitat types.

The majority of moss species occurred in only one or two habitat types suggesting a narrow range of ecological tolerance (Table

3). However, six moss species demonstrated broad ecological tolerance by their occurrence in ten or more habitat types. One species of this latter group was collected from sixteen of the eighteen habitat types studied. This moss, *Hypnum curvifolium* Hedw. commonly forms smooth, dense, interwoven mats over substrata such as logs or large rocks. A similar species, *Hypnum cupressiforme* Hedw. occurred in eleven habitat types. Those mosses that were collected in nine or more habitat types were all large species. Of the mosses that occurred in multiple habitats, *Hypnum* spp., *Dicranum* spp., and *Thuidium* spp. are increasingly exploited in West Virginia by commercial moss collectors. In some parts of the state, any large weft, mat, or turf type moss is harvested for use by the florist and nursery industries. While population levels of some of these mosses are generally high in West Virginia and are not presently endangered, other large species are particularly susceptible to stress from massive harvesting because of their very slow growth and narrow ecological tolerance. Two of these species, *Hylocomium splendens* Hedw. and *Pleurozium schreberi* (Brid.) Mitt. were observed in one and three habitats, respectively. Population levels were low in the study area for both of these species of feather mosses. *Ptilium crista-castrensis* (Hedw.) De Not., another large frondose moss that occurs in extensive mats, was expected to occur in the study area but was not observed. These three feather mosses grow exclusively at high elevations within the state and are regarded as choice specimens by moss harvesters.

Chapin *et al.* (1978) calculated that in a spruce forest where feather mosses (mostly *Hylocomium* and *Pleurozium*) constituted only 6 percent of the total plant biomass, they contained 17 percent of the total plant phosphorus. However, because their annual production was 49 percent of the total, the phosphorus contained in the annual growth of the mosses represented 75 percent of the total annual accumulation by all plants present. Such calculations suggest that bryophytes may constitute both an important temporary reservoir of phosphorus and may have a significant impact on nutrient cycling in this habitat.

The relatively broad ecological tolerance of a few species of mosses of Upper Shaver's Fork Basin was not matched by the hepatics (Table 3). Apparently hepatics of the study area are more sensitive to high environmental stress than moss species. The majority of hepatic species collected (70.8 percent) occurred in not more than two habitat types. Only one, *Bazzania trilobata* (L.) S. Gray, occurred in more than five habitat types.

Table 3. Habitat restrictions of bryophytes of the Upper Shaver's Fork Basin

Number of species	Number of habitats where occur	Percent of total number of species
Mosses		(109 species)
53	1	48.6
23	2	21.1
11	3	10.1
9	4	8.3
3	5	2.8
3	6	2.8
2	7	1.8
3	8	2.8
2	9	1.8
2	10	1.8
2	11	1.8
1	12	0.9
1	16	0.9
Hepatics		(24 species)
12	1	50.0
5	2	20.8
1	3	4.2
3	4	12.5
2	5	8.3
0	6-8	0.0
1	9	4.2

Twelve bryophytes occurring within the Upper Shaver's Fork Basin are rare species within West Virginia (Table 4). Six of these species were found to occur only in intermediately disturbed areas while three were restricted to intensively disturbed areas. Three other species were collected from both intermediately and intensively disturbed habitat types.

Table 4. Bryophytes of the Upper Shaver's Fork Basin that are rare in West Virginia.

	Reference or Collection							
	Ammons*	Aurelio**	Gray*	M. U. Herb.	Millspaugh*	Sheldon*	# counties	Habitattypes
Mosses								
Clasmatodon parvulus (Hampe) Hook. & Wils. ex Sull.			x				1	SM
Dichelyma capillaceum (With.) Myr.			x	x			1	NHF
Encalypta ciliata Hedw.		x					1	RBS
Fabronia ciliaris (Brid.) Brid.	x		x				1	RBS
Hygrohypnum ochraceum (Turn. ex Wils.)			x	x			3	GSM NHF OF
Schwetschkeopsis fabronia (Schwaegr.) Broth.		x		x			3	FSF SF
Sematophyllum adnatum (Michx.) Britt.				x	x		2	NHF
Taxiphyllum deplanatum (Bruch & Schimp. ex Sull.) Fleisch				x		x	2	NHF SM
Tortella tortuosa (Hedw.) Limpr.			x	x	x		3	NHF
Trichostomum tenuirostre (Hook. & Tayl.) Lindb.			x			x	2	NHF

Table 4. Continued.

	Reference or Collection							
	Ammons*	Aurelio**	Gray*	M. U. Herb.	Millspaugh*	Sheldon*	# counties	Habitat types
Hepatics								
Anastrophyllum helleranum (Nees) Schust.		x					1	NHF SM
Lophocolea minor Nees		x		x			2	SS

* Ammons (1934)
** Aurelio (1974)

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Educational Psychology Section

A Statistical Method for Determining STS Activities in Science Classrooms

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Abstract

During the 1988 - 89 school year, a successful program called Global Issues Chemistry produced statistical results at the 95th percentile on the Comprehensive Tests of Basic Skills, (CTBS), subsection: Science, form 4T. At the end of the school year, the Global Issues Survey (GIS) was administered as a post test item. Based on the growth that occurred from pre to post on both the CTBS and GIS, validity was established in our situation.

This instrument was utilized as a pre test for Chemistry II and Chemistry I students. Chemistry I students were used as a control to determine if there was a difference in the knowledge and placement of global issues. Spearman Rank Order Coefficient and a follow up "t" test was used to determine if there was a difference in students knowledge and placement of global issues in terms of priority. Ratings by both students correlated highly $r = 0.714$ and "t" = 3.16, $p < 0.001$ for global issues.

In order to determine if the knowledge and priority placement between the students who had the Global Issues Chemistry course, and the current Chemistry II students are the same, priority ratings were compared. A correlation, $r = 0.31$, $p > 0.10$ and "t" = 1.18, $p > 0.05$ indicated that the priority ratings were not correlated. Because of the low correlation between the two classes, the listing of all the STS

materials supplied to the Global Issues Chemistry class were assigned to the current class and at the same rate of 20%. It appeared that a reliability factor could be generated and on subsequent testing of the statistical model, amounts and levels of the STS activities to be used might be determined. As a result, activities were truncated in March rather than in May as in the previous post GIS. The results indicated a $r = 0.45$, $t = 1.65$, $df = 14$, $p < 0.05$. While this value is close to the cut off value for $p < 0.05$, further application of these articles would suggest even better results.

The Project

Students in the chemistry classes at Jefferson High School were pretested regarding their ratings of the twelve global issues (Table 1) during the school year, 1990 - 1991. The following information was provided to the students as they prepared to respond to survey.

Table 1. Pretest-posttest data on present priority rating of the twelve global issues 1990 - 1991 and 1987 - 1988.

	Pre 1990 - 1991		Post 1987 - 1988	Post 1990 - 1991
	ChemII	ChemI	Chem II	Chem II
Global Issue	Pres	Pres	Pres	Pres
The Atmosphere	1	1	4	1
Human health and disease	2	2	1	4
World hunger and food resources	3	7	2	3
Water resources	4	5	6	7
Nuclear reactors	5	9	12	11.5
Hazardous substances	6	4	7	6
Energy shortages	7	6	11	2
Land use	8	11	8	11.5
Extinction of plants	9.5	12	3	8
War technology	9.5	12	3	8
Population growth	11.5	3	9	10
Mineral resources	11.5	10	5	5
N	18	17	17	16

In question in this course is the status of twelve issues that have been identified as global. This is because they affect everyone. The

study of chemistry II may contribute to how you think these global issues should be priority rated. Your preliminary view of these issues will be measured by pretest.

In the columns listed below are the twelve major global issues. They are randomly placed there which means they are in no particular order. Next to the listing of the global issues are two columns which are marked present and future. In the first column marked present, rate the global issues on the basis of priority. That is, assign the value of "1" to that which is the highest priority and "12" to the lowest priority. Other items between these two points are rated appropriately. The last column is marked future and it simply is a rating of how you feel these issues will be in terms of importance ten years from now.

The values were listed on a tally sheet as they were marked by each participant. The values were then summed and the smallest numerical total was rated as priority "1" by the class. Each succeeding priority was assigned to the next smallest number until twelve was reached. The same process was used to rate the future items, however, they are not included in this paper.

The issues and ratings are shown in Table 1.

Once the issues were priority rated, students were periodically supplied current articles which dealt with the issues that could be directly linked in some way to the chemistry course objectives. This meant that nine of the twelve issues were addressed in at least one article during the course of the school year. No real discussion followed any of the articles read, unless the topic was brought up by the students. It was intended that students make independent decisions based purely on what was read and the relationship that might have inadvertently been brought up either in connection with a lab or lecture-discussion. War technology, population growth and land use were omitted from specific article presentations while they may have been mentioned, they were not emphasized. A note should be made, however, that with the Iraq situation that war technology and articles related to population sizes that have appeared in the newspapers may have provided enough coverage on these issues, if they were read. With the current status as I wrote this material National Guard troops were being called up for service. So the impact of knowledge about the populations and concerns with war technology may well have been treated moreso than the article treatment in the classroom. Land use may have also received considerable attention because issues locally relate to zoning for new growth and building in the communities surrounding the

school and throughout the county. A strong potential growth has been projected for Jefferson County in the next ten years. In some manner each of the issues have been treated but in no particular order other than how it might be related to the topics being logically taught in the Chemistry II classes. Eighteen seniors not in Chemistry II were selected and administered the pretest. They were compared to the senior Chemistry II students in an effort to determine if there was a difference in knowledge, simply because the students were in Chemistry II. The posttest for Chemistry II students was held off as much as possible to the presentation date and was administered March 6, 1991. This was an earlier date than the posttest for the 1987 - 88 students which was given in early May, 1988. This provided an opportunity to determine if the materials given out to date were effective up to that point. It also would provide a gauge for determining the amounts and kinds of STS materials that would be useful in effecting change with students attitudes toward the twelve issues and potentially the academic growth along with it. However, this comparison is a starting point for future exploration with this instrument.

The procedure for dealing with rank order data, as was used in this comparison, is well known and established for use by Popham (1). The data were treated with the non-parametric statistics Spearman Rank Order Correlation, which basically is the Pearson Product Moment Statistic applied to rank orders. Correlation was determined by a formula which was designed for $n = 4$ to 30, followed by a "t" test.

When seniors in Chemistry I and II were compared on the pretest, a correlation of 0.82 was found and a "t" = 4.59, $p < 0.001$. This indicated that the seniors in each course basically had the same priority ratings and they were not influenced on global issue rating by having taken Chemistry I or any other science courses. The seniors who took Chemistry II in 1987 - 88 made significant growth from the 83rd percentile to the 95th percentile on the Comprehensive Tests of Basic Skills, subsection, Science, Form 4T. They also post-tested on the Global Issues Chemistry survey as shown in Table 1. Based on this great growth that occurred on the CTBS, it was felt that the changes that transpired with the academic growth would also occur with the Global Issues Survey. This established construct validity (4) with the instrument used and the results of the testing. It should be also noted that the reliability and validity had been established by Bybee (2) in 1975. When compared to the post-test results for the 1987 - 88 year, the data from the pretest for Chemistry II students did not correlate well and the "t" test was not significant as reported as follows: $r = 0.32$, $t = 1.18$, $p > 0.05$. This indicates that the students in Chemistry II needed to

explore these issues in some form. The form chosen was the same as that chosen for the 1987 - 88 Chemistry students.

Articles were selected which emphasized the global issues which include the atmosphere, human health and disease, world hunger and food resources, nuclear reactors, energy shortages, extinction of plants, and mineral resources. Land use, population growth, and war technology were not emphasized in separate articles but were mentioned in some of the articles to some degree as they related to the articles emphasized. In early March, 1991, the post-test was administered to the current Chemistry II class. The results are shown in Table 1. When the current class was compared to the post-test results for the 1987 - 88 class, a correlation of $r = 0.45$ was found with $t = 1.65$, $p < 0.05$. This indicated that the materials that were supplied for student reading along with the materials used in class were sufficient for changing students attitudes as measured by the Global Issues Survey. As a result, an instrument coupled with appropriate readings related to the issues, is enough to measure changes in the rank order of items in the survey. It suggests that the instrument may be used to selectively measure student changes in attitudes about specific issues.

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The Effect of Hindsight on Subjects' Judgments of Blameworthiness and Suggested Length of Criminal Sentence

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Abstract

Fifty-six subjects (28 female, 28 male) read descriptions of a homeowner who surprised, then shot and killed, an unknown intruder in his home. Descriptions varied in terms of intruder gender and intruder criminal record, neither of which were known to the homeowner at the time of the shooting. Analyses of variance showed significant hindsight effects; subjects rated the homeowner significantly more blameworthy for the shooting, and liable to a significantly sterner punishment, if the intruder did not have a criminal record. Subjects rated the homeowner significantly least blameworthy in the shooting of a female with a criminal record.

Introduction

Many events and behaviors seem much more explainable when considered after the fact than when considered at the time they occurred (Wood, 1979). More specifically, judgments about a person's responsibility for his or her actions often are based on information which becomes available only after the incident; this is known as hindsight (Alicke and Davis, 1989). In one early study, Fischoff (1979) tested the effects of hindsight on sentences meted out by judges. The study showed that many judges used hindsight information to make final judgments without realizing people they were judging often did not have the same information available at the time they acted.

In a second study of hindsight, Alicke and Davis (1989) presented subjects with a scenario about one person shooting another. Subjects who received hindsight information that the shooting victim was a criminal blamed the shooter significantly less than did subjects who received hindsight information that the shooting victim was an innocent person. The present study extends the Alicke and Davis

findings by exposing subjects to hindsight information about a shooting victim's gender as well as the shooting victim's criminal record.

Materials and Methods

Subjects were 28 female and 28 male undergraduate students between the ages of 18 and 22 recruited from a liberal arts college in the upper Ohio Valley. Students volunteered for participation in the study in order to receive extra credit in a psychology course. Each subject individually was given a scenario to read. All scenarios described a homeowner who came home from work early one day to find an unknown person in his daughter's room, going through her possessions. The homeowner got a licensed gun he owned, then returned to his daughter's room. The intruder turned quickly, and appeared to be holding a gun. The homeowner fired, killing the intruder.

The final paragraph of each scenario contained hindsight information. In the final paragraph, the shooting victim was described as a convicted felon or a neighbor, and as a female or a male.

After reading a scenario, each subject provided responses on Likert-type scales designed to measure: how much actual danger the homeowner thought he was facing (1 = no danger at all, 10 = extreme danger); how blameworthy the homeowner was for his action (1 = not at all blameworthy, 10 = very blameworthy); if brought to trial for the shooting, what sentence the homeowner should receive (1 = life imprisonment without parole; 10 = no sentence at all).

Results

Separate 2 (subject gender) by 2 (victim's criminal record or lack of criminal record) by 2 (victim's gender) analyses of variance were conducted for each of the Likert-type scales described above. For the first item - how much actual danger the homeowner thought he was facing - there were no statistically significant ($p < 0.05$) main or interactive effects. Hindsight information apparently did not affect these evaluations.

For the second item - how blameworthy the homeowner was for his action - subjects found the homeowner significantly more blameworthy if the victim did not have a criminal record, $F(1,48) = 21.39$, $p < 0.001$. Newman-Keuls posttests on a significant victim gender by criminal record interaction, $F(1,48) = 4.14$, $p < 0.05$, showed

that the homeowner was evaluated significantly least blameworthy when the intruder was a female with a criminal record. Thus, on this item there were significant hindsight effects.

Table 1. Mean blameworthiness scores as a function of intruder's gender and criminal record.

Gender	Criminal Record Yes	No
Female	2.43	6.29
Male	4.29	5.79

The analysis of variance for the final item - if brought to trial, what sentence the homeowner should receive - also showed significant evidence of hindsight. Subjects suggested the homeowner should receive a significantly more severe punishment when the victim had no criminal record, $F(1,48) = 9.34$, $p < 0.01$. In no analysis was there a significant subject gender main or interactive effect.

Discussion

This experiment shows strong hindsight effects for two of the three items included, and shows those effects equally strongly for male and female subjects who responded to the scenarios. Subjects assigned different amounts of blameworthiness and suggested different punishments by relying on information that was not available to the homeowner at the time of the shooting. Additionally, this study shows that subjects were less concerned about the shooting of a female with a criminal record than about shooting a male with a criminal record.

Table 2. Mean homeowner punishment severity as a function of intruder's criminal record.

Criminal record Yes	No
8.61	6.43

The strong hindsight effects obtained may be the result of using a very dramatic scenario - one involving a killing. Future research should explore whether hindsight effects would be as strong with less dramatic scenarios. Also, future studies in this area should try to establish why killing a female who has a criminal record is seen as less blameworthy - by female and male subjects - than killing a male with a criminal record. Perhaps the reason is that a female who is a criminal is doubly liable, not only for being a criminal but also for adopting an "unfeminine" role; certainly this finding merits future study.

The present study, and the other studies cited, address the issue of hindsight use by those not directly involved as perpetrators or victims. Future studies might also address the use of hindsight information by actual perpetrators and victims after an incident has occurred.

Finally, more detailed hindsight research must be done to try to tease out why and how subjects use hindsight information. The present study shows that subjects indeed use hindsight information, but does not show details about the possible mechanisms for processing that information. These additional findings could be vitally important, for example, for those attempting to understand more clearly what judges and juries do in actual criminal trials - or, for that matter, what parents do every day in dealing with their children, or teachers do in dealing with their students.

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Mathematics Section

A Nonlinear Ordinary Differential Equation in Electron Beam Dynamics

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Abstract

The author studies the nonlinear ordinary differential equation $y''+\alpha\phi(x)y = 2\alpha K y^{-3} + \beta y^{-1}$, which occurs in the theory of periodic magnetic focusing of cylindrical electron beams of high density. The constants α, β, and K are ≥ 0 (with $\alpha > 0$), taking values usually ≤ 1.5. The function $\phi(x)$ is supposed bounded and continuous, with intergrable derivative. A class of regular solutions is defined, which would appear to include all solutions of physical interest. It is shown that no solution of that class can vanish anywhere (settling an open question). The author also conjectures (for reasons which are given) that the equation has no unbounded solutions when $\phi(x) = 1 + \cos 2x$, a physically important case.

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Introduction

There are several kinds of high-vacuum devices which employ a cylindrical beam of electrons as active element (especially at microwave frequencies). Typically, in such a device, the electrons of the beam are emitted from a thermionic cathode in the presence of one or more beam-forming electrodes, upon emerging from which the beam enters a region in which it drifts at about constant velocity (in the direction of its axis) over a considerable length (from a few inches to a few feet). When the beam is of high density, the mutually repulsive Coulomb forces between electrons cause the beam to spread out -- to diverge from the desired cylindrical shape -- whence measures must be applied to compensate for such forces. One method which is sometimes applicable for this purpose is *periodic magnetic focusing*, in which a static magnetic field is applied, which is periodic in the distance along the beam axis. (Some further technical details may be found in various places, including the literature to be mentioned below.)

Several researchers in periodic focusing have employed substantially the same equation of motion with which to study the trajectories of individual particles in such a beam. In cylindrical coordinates, (r, ϕ, z) suppose the beam enters the domain of the periodic magnetic field $B_z = B_0 \cos\frac{2\pi z}{L}$ at $z = 0$ (B_0 = const. > 0, L = period of B_z) with radius r_0, and charge density p = const. $\neq 0$. Let $r = r(t)$ be the radius of an electron as a function of the time t, which at $z = 0 = t$ is on the edged of the beam: $r(0) = r_0$. Some considerable analysis leads to the equation

$$\frac{d^2r}{dt^2} + \left(\frac{\eta B_z}{2}\right)^2 r - \left(\frac{\eta^B c}{2}\right)^2 \frac{r_c^2}{r^3} - \frac{\eta\rho}{2\varepsilon}\frac{r_0^2}{r} = 0 \qquad (1)$$

(Such equations are usually derived from the general equation of motion obtained by combining Newton's second law with the Lorentz force.) In (1), $\eta = |e/m|$ = charge-to-mass ratio of an electron; B_c is the magnetic flux density (if any) penetrating the region $z < 0$ out to some pre-entrance radius r_c ; and ε is the permittivity of vacuum. If we define the quantities (in which u is the constant drift velocity of all the electrons in the beam along the z-axis, in the direction $z > 0$)

$$x = \frac{2\pi ut}{L} \quad ; \quad y = \frac{r}{r_0} ; \quad K = \left(\frac{\sqrt{r_c}}{r_0}\right)^4 \left(\frac{B_c}{B_0}\right)^2 ;$$

$$\alpha = \frac{1}{2}\left(\frac{L}{2\pi u}\right)^2 \left(\frac{\eta B_0}{2}\right)^2 ; \quad \beta = \frac{1}{2}\left(\frac{L}{2\pi\mu}\right)^2 \frac{\eta\rho}{\varepsilon}$$

then -- upon multiplying through by $\frac{1}{r_0}\left(\frac{L}{2\pi\mu}\right)^2$ -- (1) takes the less tedious form

$$\frac{d^2y}{dx^2} + \alpha(1+\cos 2x)y = \frac{2\alpha K}{y^3} + \frac{\beta}{y} \qquad (2)$$

in which independent variable x is proportional to distance along the beam. (The constants α, β and K take moderate values; a large range of practical situations is included in the values $0 < \alpha \leq 1$; $0 \leq \beta \leq 0.5$; and $0 \leq K \leq 0.8$.) In (2), α is a measure of the maximum value of the periodic magnetic field; K measures the quantity of flux threading the "pre-entry" region; and β measures the space-charge force, proportional to the beam charge density. For an electron entering the drift space on the edge of the beam, and parallel to the beam axis, the initial conditions accompanying (2) are thus $y(0) = 1, y'(0) = 0$.

Eq. (2) was considered (with $K = 0$) by Mendel, Quate and Yocom (1954), and by Clogston and Heffner (1954). Subsequently (with $K \neq 0$) (2) was studied by Harker (1955) and somewhat later by Seunik (1964) and Linn, Pöschl and Veith (1964). (There seems to be little, if any, more recent work.)

If $K = 0 = \beta$, then (2) reduces to the special *Mathieu's equation*

$$y'' + \alpha(1 + \cos 2x)y = 0 \qquad (3)$$

solutions of which (as is well-known) are stable or unstable according to the value of α. (See, for example, Maclachlan (1947) and/or Blanch (1964).) In studying the literature cited in the last paragraph, one soon realizes that the authors believe (at least implicitly) that solutions of (2) must exhibit the same stability-instability phenomena as (3). And not

without reason: the measurements of Mendel, Quate and Yocom (*op. cit.*) seemed to lend support to such a view. However, when Harker (*op. cit.*) compared his family of analog computer solutions of (2) with their results, his solutions seemed to be stable over a wider range of values of α than is given by (3). He concluded that the nonlinear terms y^{-1} and y^{-3} in (2) "... due to space charge and finite flux [= B_c] at the cathode prevent the edge electron from crossing the axis [i.e., prevent y from being zero], thereby eliminating the mechanism by which [solutions of (3) may become unbounded], and [thus] extending the zone of stability ..." (*op. cit.*, p. 14). One sees that Harker none the less believes such zones of stability and instability for (2) do exist. These remarks of Harker's (and others of like character in the literature above-mentioned), while interesting and suggestive, of course do not constitute a rigorous argument. And, as a matter of fact, we can readily imagine that (2) *might* have a solution y which approaches zero as x approaches some value p (say), in such a way that y" becomes unbounded, and thus (2) would remain satisfied in the limit at p.

Formulation and Analysis

Let us reformulate our problem as follows, beginning with a differential equation somewhat more general than (2), *viz.*,

$$y''+\alpha\phi(x)y = 2\alpha K y^{-3} + \beta y^{-1} \qquad (4)$$

with initial conditions

$$y(x_0) = y_0, y'(x_0) = m_0 \qquad (5)$$

where the initial values $x_0 \geq 0$, $y_0 > 0$ and initial slope m_0 may have either sign, but is finite; α, β and K are as given above in (2); and, for the present, we suppose that the function $\phi(x)$ is bounded and continuous on $x_0 \geq 0$. We seek information concerning the solutions of (4) on the domain $x > x_0$ and satisfying the initial conditions (5).

We want to notice, first, that it is possible to apply the standard existence and uniqueness theory to (4) + (5) – actually, to an equivalent

first-order *system*. If we set $X_1 = y$, $X_2 = y'$ then (4) + (5) is equivalent to

$$\dot{x_1} = x_2$$

$$\dot{x_2} = -\alpha\phi(x)X_1 + 2\alpha K X_1^{-3} + \beta X_1^{-1} \qquad (*)$$

$$\left(X_1(x_0) = y_0, X_2(x_0) = m_0\right)$$

However, the function on the right in (*) satisfies a merely local Lipschitz condition, and then only on a domain of points (x, X_1, X_2) for which $X_1 = y \geq$ const. > 0 (or < 0). One thus obtains only *local* existence and uniqueness, leaving the most interesting questions virtually untouched. (The theory to which we have just alluded is discussed in many texts; we shall only mention here that of Kaplan (1958).) We are thus obliged to -- tentatively -- assume the existence of certain classes of solutions to (4) + (5), with specified properties, and then ask whether such properties are consistent with what may be deduced directly from (4) itself.

Let us say that a solution of (4) is *very smooth* (on the domain $x \geq x_0$) if $y''(x)$ is continuous there. For such solutions, the conjecture of Harker, quoted above, may be verified immediately:

> *Theorem 1*. If $K \neq 0$, or if $K = 0$ and $\beta \neq 0$, then a very smooth solution of (4) cannot have an isolated zero.

Proof: Suppose, on the contrary, that x_1 is such a zero, and let I be a closed interval containing x_1. Then, for all x in I - $\{x_1\}$, multiply (4) through by y^3, obtaining

$$y^3 y'' + \alpha\phi(x)y^4 = 2\alpha K + \beta y^2; \qquad (6)$$

letting $x \to x_1$, we obtain $2\alpha K = 0$, a contradiction, since we always have $\alpha > 0$. In the same way, the second alternative leads to the equation

$$yy''+\alpha\phi(x)y^2=\beta$$

leading to the contradiction $\beta = 0$, concluding the proof.

Some slightly stronger results of this same kind are not difficult to obtain, but such theorems do not really bear on the physical problem. The fact is that we are not justified *a priori* in making any such strong assumption concerning the second derivative. As we shall now show, such an assumption is not needed.

Let us say that $y = y(x)$ is a *regular solution* of (4) and/or (6) if (a) y satisfies the given initial conditions (5); (b) $y'(x)$ is continuous on $x > x_0$; and (c) y'' is Lebesgue-integrable on every bounded subinterval of the domain $x > x_0$. For such a solution, multiply (4) through by $2y'(x)$, obtaining

$$2y'\,y''+\alpha\phi(x)(2yy')=-2\alpha K\left(-2y^{-3}y'\right)+2\beta\frac{y'}{y}$$

or

$$\frac{d}{dx}\left[(y')^2+\frac{2\alpha K}{y^2}-2\beta\log|y|\right]+\alpha\phi(x)\frac{d}{dx}\left(y^2\right)=0 \qquad (7)$$

Since y'' is integrable, so is $2y'y''$, and integration of (7) gives

$$(y')^2+\frac{2\alpha K}{y^2}-2\beta\log|y|-m_0^2-\frac{2\alpha K}{y_0^2}+2\beta\log y_0$$
$$+\alpha\int_{x_0}^{x}\phi(t)d\left[y^2(t)\right]=0 \qquad (8)$$

If we assume, further, that $\phi(x)$ has an everywhere integrable derivative, then the last term in (8) may be integrated by parts to give the integro-differential equation,

$$\left(\frac{dy}{dx}\right)^2 + \frac{2\alpha K}{y^2} + \alpha\phi(x)y^2 - 2\beta \log|y|$$

$$= C_0 + \alpha\int_{x_0}^{x} \phi'(t)(y(t))^2\,dt \qquad (9)$$

in which

$$C_0 = \text{const.} = m_0^2 + \frac{2\alpha k}{y_0^2} + \alpha\phi(x_0)y_0^2 - 2\beta \log y_0 .$$

Now it is clear that if (9) has a solution y with continuous second derivative y'', then it satisfies (4), i.e., it is then a very smooth solution. However, (9) itself is quite free of y'', so that we shall revise our definition of regular solution slightly, replacing (c) by (c'): $y = y(x)$ satisfies (9).

Theorem 2. Let $K \neq 0$ (or $K = 0$ and $\beta \neq 0$), and let $\phi'(x)$ be integrable. Then no regular solution can vanish anywhere.

Proof: Multiplying (9) by y^2, we have

$$\left(y\frac{dy}{dx}\right)^2 + 2\alpha K + \alpha\phi(x)y^4 - 2\beta y^2 \log y$$

$$= C_0 y^2 + \alpha(y(x))^2 \int_{x_0}^{x} \phi'(t)(y(t))^2\,dt \qquad (10)$$

and suppose that $y = 0$ at x_1. Letting $x \to x_1$, (10) again implies $2\alpha K = 0$, a contradiction. For the case $K = 0$, $\beta \neq 0$, we may write (9) in the form

$$\left(\frac{dy}{dx}\right)^2 + \alpha\phi(x)y^2 + \beta \log\frac{1}{y^2} = C_0 + \alpha\int_{x_0}^{x} \phi'(t)(y(t))^2\,dt \qquad (11)$$

Taking absolute values in (11), the absolute value of the left-hand side cannot exceed

$$|C_0| + \alpha\left|\int_{x_0}^{x} \phi'(t)(y(t))^2\,dt\right| \tag{12}$$

Again let $x \to x_1$; if $\phi'(x)$ is actually continuous, then the integral in (12) cannot exceed

$$\int_{x_0}^{x_1} |\phi'(t)|(y(t))^2\,dt \le M\int_{x_0}^{x_1} (y(t))^2\,dt$$

(M = const. > 0 being a bound on $\phi'(x)$) which is therefore bounded. If $\phi'(x)$ is merely integrable, we may apply Schwarz's inequality to the integral in (12) and conclude again that the latter is bounded. The left-hand side of (11) is thus dominated on $[x_0,x_1]$ by a bounded quantity, and hence is itself bounded. But since $y \to 0$ as $x \to x_1$, the term $\beta \log\frac{1}{y^2}$ is unbounded. This contradiction concludes the proof.

Let us notice that we have nowhere made use of any periodicity property of $\phi(x)$, invoking only the quite mild properties that $\phi(x)$ be bounded and continuous with integrable derivative.

Since the terms of (10) are functions of y^2, we see that if we set $u = y^2$ (whence $yy' = u'/2$), the equation takes the slightly simpler form

$$\frac{1}{4}\left(\frac{du}{dx}\right)^2 + 2\alpha K + \alpha\phi(x)u^2 - \beta u \log u$$
$$= C_0 u + \alpha u(x)\int_{x_0}^{x} \phi'(t)u(t)\,dt \tag{13}$$

Having shown that no regular solution of (10) (hence of (13)) can vanish, we naturally wonder -- at the other extreme -- whether (13) has any solutions which are unbounded as $x \to \infty$. (A regular solution certainly cannot be unbounded in the neighborhood of any finite value of x; such behavior would entail loss of continuity of y', contrary to

property (b).) There is some reason to conjecture that (13) has no unbounded solutions *in the periodic case*, $\phi(x) = 1 + \cos 2x$. Then (13) may be written

$$\frac{1}{4}\left(\frac{du}{dx}\right)^2 + 2\alpha K + \alpha\phi(x)u^2$$

$$= u(C_0 + \beta \log u) - 2\alpha u(x)\int_{x_0}^{x} u(t)\sin 2t\,dt \quad (14)$$

Suppose that $u \to \infty$ as $x \to \infty$. The left side of (14) remains always ≥ 0 (strictly > 0 if $K \neq 0$), while the integral term on the right evidently experiences fairly regular changes in sign. In fact, the successive maxima and minima of this term -- of opposite sign -- appear to grow in absolute value at a rate comparable to u^2, whereas the other term, $u(C_0 + \beta \log u)$, only grows slightly more rapidly than linearly. It would seem then that, for all sufficiently large x, the right-hand side of (14) oscillates in sign, whereas the other side never changes sign. However, we have not yet succeeded in reducing these considerations to a rigorous proof. Such a theorem would show that there is no close connection between the solution spaces of (2) and (3).

Summary and an Acknowledgment

In the first case of Theorem 2, the result may be strengthened slightly: as $x \to x_1$, suppose that (instead of being bounded) $y' \to \pm\infty$ but in such a way that yy' remains bounded, say $yy' \to M$. Then (10) yields $M^2 + 2\alpha K = 0$, a contradiction. We thank Prof. George Carlson of the WVIT physics department for pointing this out.

In summary, we remark that a nonlinear ordinary differential equation arising in the theory of periodic magnetic focusing of electron beams has been our subject of study. In particular, it is found that no solution of a certain class can vanish -- a fact hitherto only conjectured. The class in question would appear to include all solutions of physical importance.

It remains to thank the reviewer for his careful reading of the manuscript of this paper.

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Zoology Section

Autorhythmometry in a Teenage Population

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Abstract

Seventeen students, fourteen to seventeen years old, participated in a study to measure their own circadian rhythms (autorhythmometry). The existence of rhythms in one's body was a new fact for many of the students. Every three hours for a period of twenty-four hours each teenager took his/her oral temperature, pulse rate, and blood pressure. They also performed two tests to determine the degree of eye-hand coordination and measured their peak expiratory flow and grip strength. The students graphed their results to see individual circadian rhythms. The peaks, troughs, and the amplitudes were noted. The means for male students and female students were calculated and compared by Student's t-test.

Temperature, pulse rate, and the "bean jar coordinometer" test showed marked circadian rhythms. The mean peak temperature and

the mean low temperature for female students and male students were approximately the same. With regard to the phasing of the temperature curves, there was a 3 hour phase difference in both the peak and the trough, with female students lagging that of the male students. The mean pulse rate had a broad peak during the day and a low level during the night. In the "bean jar coordinometer" test, the fastest mean time for the male students was at 1630 and for the female students it was at 1930. The slowest mean time for both male students and female students occurred at 0430. Overall the female students performed this task faster than the male students did. Various reasons may exist for the students not finding rhythms in blood pressure, the other eye-hand coordination test, peak expiratory flow, and grip strength.

Introduction

Homeostasis is generally understood as a state of equilibrium in which the internal environment of the body remains relatively constant, around an unchanging physiological set point. The area of chronobiology, a quantitative study of biological functions that fluctuate over a period of time, recognizes variation in many processes over a period of approximately 24 hours, and thus the rhythm is called a circadian rhythm. It is believed that this rhythmic variation is fundamental to life and it offers a key to new understanding of health (Glascow et al., 1982). Circadian rhythms shape not only our physiology and our behavior, but also our sports performance (Eichner, 1989) and our job performance (Moore-Ede et al., 1982).

In an effort to enhance the education of American youth, several projects have been undertaken to teach students to monitor their own health (Glascow et al., 1982; Halberg et al., 1972; Halberg et al., 1974; Levine et al., 1974; Rabatin et al., 1977; Smolensky et al., 1977). This concept of self-measurement of rhythms is called autorhythmometry (Glascow et al., 1982). Thus, the primary purpose of this study was to educate the students so that they would understand more about their circadian rhythms. Furthermore, it was anticipated that additional data would be obtained on the rhythmic characteristics of physiological parameters in teenage students.

Materials and Methods

During the summer of 1990, seventeen high school students from the ages of 14 - 17 participated in a study to measure circadian rhythms of their bodies. This project was part of the chronobiology

studies conducted during the National Science Foundation Young Scholars Program at Bethany College. Each student was instructed on the proper method to perform each measurement or task and they practiced doing so frequently for a period of 2 days. A supervised trial run was conducted and the results were recorded at 0730 of the day the study took place. Thereafter, every 3 hours for a period of 24 hours each student took his/her oral temperature, radial pulse rate and blood pressure. In addition, each student performed 2 tests involving eye-hand coordination and measured his/her peak expiratory flow and grip strength in both right and left hand. Each student graphed his/her results, making note of the time when the peaks and troughs occurred as well as the amplitudes of the curves. Seven male students and ten female students participated; all students were right handed. All the tasks performed were similar to those described by Dennis R. Glascow and his colleagues in the Little Rock public high schools (Glascow et al., 1982) as modified by Dr. Lawrence E. Scheving (University of Arkansas College of Medicine).

The students were divided into 4 groups of 4 or 5 students to perform the tests at the same time. Each student had a work area equipped with his/her personal oral thermometer, a stop watch, an aneroid sphygmomanometer, and a stethoscope. A "bean jar coordinometer", "ring apparatus", peak flow meter, and a common dynamometer (MY-GRIPPER) had to be shared between 2 or 3 students. Each person used the same equipment for the entire study. As each group completed the tests, the next group began the tests. All results were entered on a data sheet provided. For convenience, the 1030, 1330, 1630, and 0730 trials were held in the biology laboratory while the 1930, 2230, 0130, and 0430 trials were held in the dormitory. All sessions were supervised by adult teachers. Following the test period, the data were entered into Microsoft ®Works, version 1.1; mean data for males and females were calculated, graphed and compared. The Student's t-test was applied to compare the high and low mean of each test to determine statistical significance. The existence of circadian rhythms was tested by mean cosinor analysis, a widely accepted standard of determining rhythmicity in data by fitting the data to a cosine curve (Halberg et al., 1972).

Test Descriptions

ORAL TEMPERATURE: At the beginning of each session, the thermometer was checked and if the mercury column was not below 96°F, it was shaken down. The thermometer then was placed as far

back under the tongue as comfortable and the temperature was taken for five minutes. The value was recorded to the nearest 0.1 °F. Following the 24 hour trial period, all temperatures were entered into Microsoft ®Works, version 1.1 and converted to degrees Celsius.

RADIAL PULSE: During the time that the temperature was being taken, the radial pulse was taken at the wrist with the index and middle finger for a period of 1 minute. This was recorded in the appropriate space on the data sheet. The data from only 6 of the male students were used because the seventh male student had a pacemaker and thus he would not have a circadian rhythm for heart rate.

BLOOD PRESSURE: Directly after temperature and pulse were taken, blood pressure was taken with an aneroid sphygmomanometer. Some students had difficulty getting a blood pressure reading. In these cases, the supervisors assisted with the blood pressure reading.

EYE-HAND COORDINATION: During this experiment, the subject was required to hold a stop watch, face down, in the non-dominant left hand, and using the right hand transfer 30 small beans, one at a time, from a small styrofoam plate into a coordinometer. The coordinometer was a small plastic jar with a screw top. A hole had been drilled through the top and a short piece of copper tubing placed through it. The styrofoam plate was to the right of the coordinometer. Five extra beans were available on a second plate, so that several beans could be accidentally dropped without running out of beans before the test was completed. The subject was not allowed to hold the coordinometer. With the right hand on the first bean, the stop watch was started with the left hand and thirty beans were transferred, one at a time, into the coordinometer. After all thirty beans had been transferred, the watch was stopped and the elapsed time recorded to the nearest 0.01 second.

NUT-THREADING RING TEST: This test used a ring about nine centimeters in diameter constructed of a 2 mm diameter metal wire with a 1.2 cm portion made of the stem of a 1/4 inch threaded bolt. While the left hand gripped the ring just below and to the left of the threaded portion, the right hand brought three nuts, one at a time, from the bottom of the ring to the top of the ring and screwed them over the threaded portion. A second person timed this test, starting the stop watch when the subject grasped the first nut at the bottom of the ring

and stopped it when the third nut was completely screwed over the threaded portion.

PEAK EXPIRATORY FLOW: Standing with feet about one foot apart, the subject held the peak flow meter in both hands with the indicator set at zero. After taking a deep inhalation, the subject exhaled forcibly into the peak flow meter. The expired air was recorded in liters per minute. The process was repeated once and the two scores were averaged.

GRIP STRENGTH: A common dynamometer (MY-GRIPPER) was used to test the grip strength of each student by squeezing it three times in the dominant hand and three times in the non-dominant hand and then averaging the scores for each hand separately. The dynamometer was calibrated in kilograms of pressure. In order to perform the test, the subject stood with eyes forward, shoulders back, feet about one foot apart. The dynamometer was held in the test hand with the arm extended at about a 30^{o} angle from the body.

Results and Discussion

ORAL TEMPERATURE: The mean peak temperature (Fig. 1), although approximately the same ($37.3 \pm 0.12\ ^{o}C$ for male students and $37.2 \pm 0.79\ ^{o}C$ for female students) showed a three hour phase difference (peak at 1630 for male students and at 1930 for female students). The mean low temperature ($36.7 \pm 0.27\ ^{o}C$ for male students and $36.4 \pm 0.16\ ^{o}C$ for female students) also differed in phase (trough at 0130 for male students and 0430 for female students). According to the Student's t-test, both sets of data showed differences between peaks and troughs that were significant (male students: $0.025 < p \leq 0.05$; female students: $p \leq 0.0005$). However, the circadian variation for the male students did not fit a cosine curve whereas the circadian variation for the female students did ($p = 0.004$). The difference between the Student's t-tests and the lack of a fit to a cosine curve for the data for the male students might be accounted for by the fact that the data were not symmetrical as is a cosine curve, or more likely, because of the small number of subjects (7).

The 3 hour phase difference may be explained by the fact that oral body temperature peaks and troughs vary. Peak oral temperatures have been reported in both the late afternoon, 1608 (Smolensky et al., 1977), and the early evening, 1930 (Rabatin et al.,

1977). Researchers have attempted to link the peak variations to personality types in which "introverts" tend to be "larks", having earlier peak temperatures, and "extroverts" tend to be "owls", having later peak temperatures (Palmer, 1983; Winger et al., 1985). Sports performance, especially swimming, running, and soccer, has also been linked to peak temperature times (Eichner, 1989; Winget et al., 1985). However, it has been suggested that rather than a relationship of cause and effect, it is a relationship in which both body temperature and sports performance are controlled by a common oscillator (Winget et al., 1985).

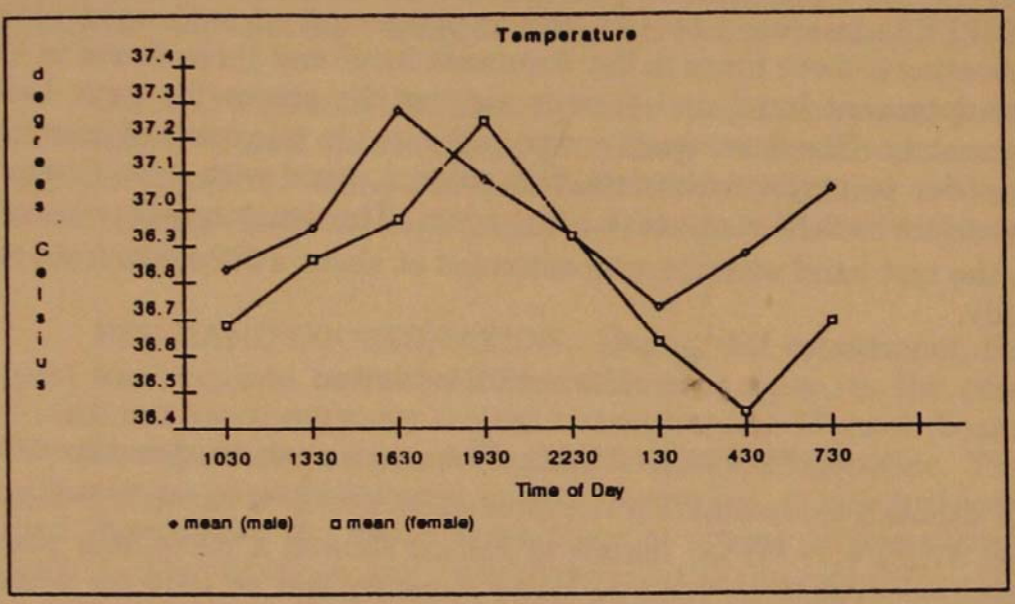

Figure 1. Changes in mean temperature during the 24 hours for female students and male students. A significant rhythm was found for only the female students, cosine, $p = 0.004$.

RADIAL PULSE: The mean radial pulse for the female students (Fig. 2) was significant for both the Student's t-test ($0.01 < p \leq 0.025$, peak vs. trough) and the data fit a cosine curve ($p = 0.007$). The mean pulse rate had a broad peak in the afternoon (77.4 ± 3.62 beats per minute at 1330 to 77.5 ± 2.98 beats per minute at 1630) and a low level (64.8 ± 3.99 beats per minute) at 0130. The data from the 6 remaining boys did not show a circadian rhythm although the Student's t-test did show a significant probability ($0.005 < p \leq 0.01$) of a difference between the peak and trough. The lack of a statistically significant circadian rhythm for the male students may be accounted for by the extra trough that occurred at 1330. The peaks and the main trough of the curves for

both male students and female students occurred at the same time (peak at 1630; trough at 0130).

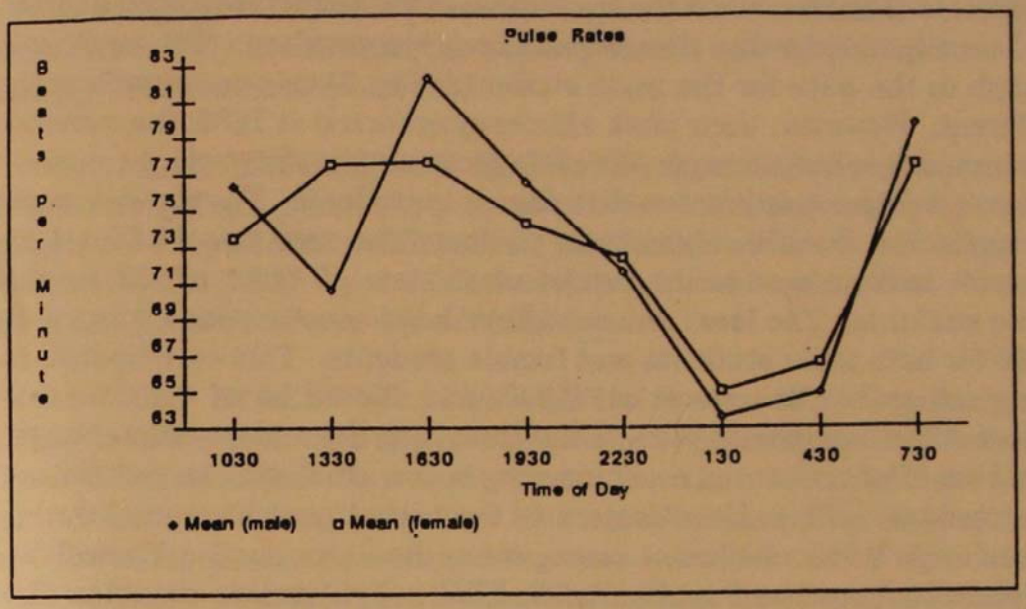

Figure 2. Changes in mean pulse rate during the 24 hours for female students and male students. A significant rhythm was found for only the female students, cosine, $p = 0.007$.

Heart rate (pulse) and body temperature tend to show similar circadian patterns with the fastest heart rate in the late afternoon or early evening (Palmer, 1986). This is supported by the data for male students but not that of the female students. The peak and trough for the pulse rate of the female students precedes the peak and trough for the mean temperature of the male students by 3 hours.

BLOOD PRESSURE: Blood pressure also was monitored but no significant circadian pattern was found. Inexperience by the students in using a sphygmomanometer may account for the lack of rhythms in blood pressure data. Blood pressure is lowest at about 0300 and highest at 1500 (Dewey, 1971), however, neither Smolensky et al. (1977) nor Glascow et al. (1982) found significant circadian rhythms in blood pressure. Circadian rhythmicity of systolic blood pressure is not as pronounced as either temperature or pulse, and diastolic pressure shows irregular variations (Rabatin et al., 1977)

EYE-HAND COORDINATION: Again, the peak and trough difference in the data analyzed for mean eye-hand coordination for the female students (Fig. 3) was significant ($0.0005 < p \leq 0.005$) as was the

fit to a cosine curve ($p = 0.002$). Peak efficiency (the trough of the rhythm, in this case, since the least value is the fastest) occurred at 1930 and corresponded to the time of peak body temperature. The peak and trough in the data for the male students (Fig. 3) was not significantly different, However, their peak efficiency occurred at 1630, the same as their peak for body temperature. In general, female students showed better eye-hand coordination than the male students. The slowest mean time for the female students to perform the test was 44.66 ± 1.89 seconds as compared to the fastest mean time of 42.14 ± 2.59 for the male students. The least amount of eye-hand coordination occurred at 0430 for both male students and female students. This corresponds to the findings of Glascow et al. (1982) and should be of considerable interest to those whose jobs and/or lives may depend on efficient eye-hand coordination in the early morning hours. It should be pointed out that several man-made disasters in the recent past happened during these early hours. Included among these disasters are the Three-Mile Island Nuclear Plant incident, the Chernobyl nuclear disaster, the Union Carbide chemical disaster in Bopal, India, and the Exxon Valdez oil spill.

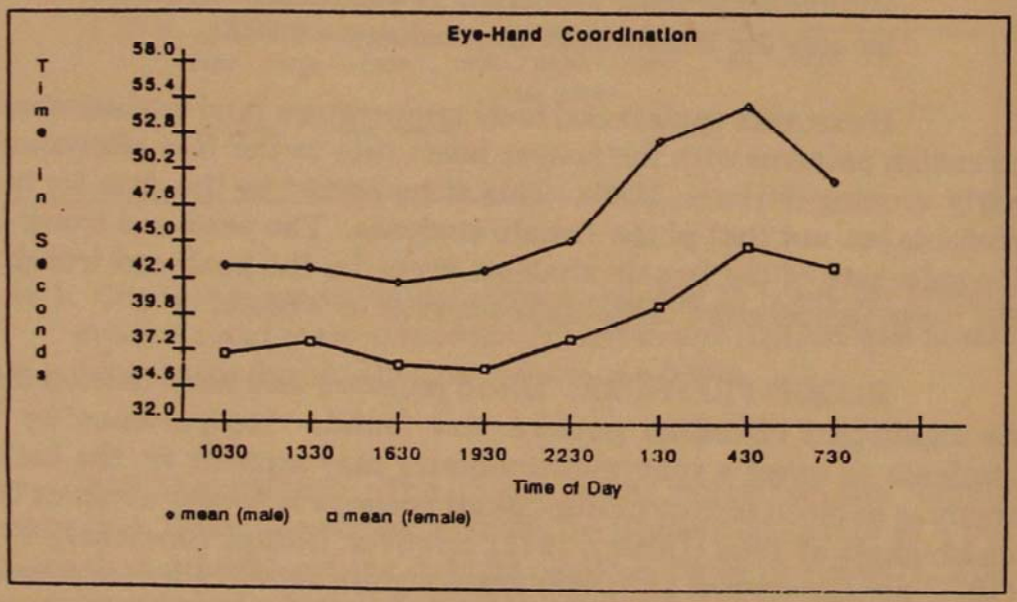

Figure 3. Changes in mean eye-hand coordination during the 24 hours for female and male students. A significant rhythm was found for only the female students, $p = 0.002$.

NUT-THREADING RING TEST: Although both mean peak and trough times for male students and female students were significantly different according to Student's t-test (both: $0.01 < p \leq$

0.025) neither fit a cosine curve. Both groups decreased the time needed to thread 3 nuts over the threaded portion of the ring significantly, probably due to a learning curve. The female students were still a little faster than the male students, and again there was a slowing of performance at 0430.

PEAK EXPIRATORY FLOW: Peak expiratory flow varied dramatically from person to person but the data, although peak and trough were significantly different for the female students according to Student's t-test ($0.025 < p \leq 0.05$), but did not show a circadian rhythm for either female students or male students.

GRIP STRENGTH: Although 3 of the 4 sets of data for grip strength had significant rhythms with the mean cosinor (right hand data for female students were not significant), neither right nor left hand mean grip strength for boys had significantly different peaks or troughs using Student's t-test. However, as expected the right hand had more grip strength than the left (all subjects were right handed) and the male students had greater grip strength than the female students. Also as expected, the trough (least amount of grip strength) occurred at 0430.

Conclusions

Although circadian rhythms for male students were not significant for mean body temperature, mean radial pulse, or mean eye-hand coordination in this study, individual variation in each of the above was perceived. A larger sample is necessary before concluding that there is a lack of circadian rhythms among male students. A correlation in time of peak body temperature and peak eye-hand coordination, but not for pulse, was found for the female students. The data from eye-hand coordination tests, using both the "bean jar coordinometer" and the "ring apparatus", as well as the data from the grip strength test, suggest that the best time of day for the average teenage student to do critical work is not in the early hours of morning (i.e., 0430). In general, it is the opinion of the authors and the students involved that the knowledge of the existence of rhythms in one's own body is an important part of one's education.

Two things are evident from this study: the definition of homeostasis should be modified to include the fluctuations and the dynamic nature of physiology that occur in the biological processes of the human body during a 24 hour period; and that the teenage

population does have circadian rhythms in some physiological parameters.

Acknowledgment

This work was supported by a National Science Foundation Young Scholars Grant (RCD-8550176) to Bethany College

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Minutes of the Annual Business Meeting
West Virginia Academy of Science
66th Annual Meeting
West Virginia Institute of Technology
Montgomery, West Virginia 25136

April 6, 1991

1. 10:24 AM General Meeting called to order by Dr. Don Tarter.

2. Welcome by Dr. Tarter.

3. 10:25 AM: Welcome by Dr. Robert Gillespie, President WVIT.
   a. "The Academy has much to offer education in West Virginia."
   b. Introduction of A. J. Fields and Michael Demchek, West Virginia Outstanding Science Teachers of the Year.
   c. Dr, Gillespie recounted the April 5, 1991 speaker from the National Geographic Society, Barry Bishop.

4. 10:31 AM: Don Tarter introduced Speaker of the House Chuck Chambers. Speaker Chamber's theme was the environment in West Virginia. He recounted many items that were being accomplished and many more that needed attention. "West Virginians have to seize control of their own destiny."

5. 12:26 PM: General Business Meeting

   a. President Tarter thanked John Parks for being planning chairman.

   b. Roy Clarkson gave the Treasurer's Report. Elizabeth Swiger said she had audited the books and found all in order.

   c. President Tarter presented Roy Clarkson a plaque for his service over the years as treasurer.

   d. Tom Pauley appointed chairman of the nominating committee.

   e. Andy Cook accepted as new treasurer.

   f. The Junior Academy was under the direction of Amy Kokesh.

g. Andy Cook welcomed the Academy to hold its 1992 meeting at West Liberty,.

h. It was moved, seconded and passed that the 1993 meeting be held at Davis and Elkins College.

i. It was moved by John Parks and seconded by Rathan Mitra that the names of 3 reviewers be forwarded with papers to be published in the *Academy Proceedings*. This should help Editor Keller with the *Proceedings.*

j. It was moved, seconded, and passed that occasional papers would not be a part of the regular *Proceedings*. Steve Stephenson discussed this idea for considerable time.

k. President Tarter thanked John Chisler for holding the past several science fairs at Glenville State College.

l. The Outstanding High School Science Teacher Awards were presented to:
   1. A. J. Fields, South Charleston High School
   2. Michael Demchek, Jefferson High School

m. President Tarter gave a memorial tribute to Joe Glencoe of West Virginia Wesleyan who recently died.

n. It was moved, seconded, and passed that we dismiss the meeting.

Phillip Cottrill
Secretary

WEST VIRGINIA ACADEMY OF SCIENCE
ANNUAL TREASURER'S REPORT

1990

April 6, 1991
WVAS Annual Meeting
West Virginia Institute of Technology
Montgomery, West Virginia

January 1, 1990 to December 31, 1990

CASH BALANCE JANUARY 1, 1990		$4,812.03
CASH RECEIPTS	$1,595.00	
Institutional Membership	1,400.00	
Libraries	750.00	
Contributions (Talent Search)	110.00	
Annual Meeting*	549.00	
Interest (Checking Account)	176.36	
Advertisements	225.00	
Abstract Charges	100.00	
Contributions: Du Pont	400.00	
WVU Library (Exchange Copies)	325.00	
TOTAL CASH RECEIPTS FOR YEAR	5,630.35	
TOTAL CASH RECEIPTS PLUS BALANCE JANUARY 1. 1989		$10,442.39
CASH DISBURSEMENTS		
Printing (McClain)	4,218.30	
Annual Meeting**	620.76	
Postage	365.85	
Editorial Assistance	108.00	
Talent Search	175.00	
Glenville College (Science Teachers Directory	325.00	
Best Student Paper	100.00	
Plaques	159.85	
Copying	262.31	
Misc.***	59.03	
TOTAL DISBURSMENTS		$6,394.10
BANK BALANCE DECEMBER 31, 1990		$4,048.29

IN ADDITION TO ABOVE: CD NO. 001-D158-99 is held by The First National Bank of Morgantown:P Value on anniversary date April 8, 1990 was $6.664.46.

Respectively submitted,

Roy B. Clarkson Treasurer, WVAS

* Annual Meeting (Receipts)

Roy Clarkson (Till Money)	$100.00
Registration	204.00
Lunch	245.00
TOTAL	$549.00

** Annual Meeting (Disbursements)

Ed Keller (Copying)	$98.16
Roy Clarkson (Till Money)	100.00
Lunch	245.00
Speaker (Honorarium)	100.00
Kinkos (Copying)	63.60
TOTAL	$620.76

*** Misac. (Disbursements)

Ed. Resource Dist (Refund)	$2.00
Nat. Assn. Acad. Sci. (Dues)	27.00
Princeton Univ. (Refund)	3.00
Kinkos)Copying Member. App.)	27.03
TOTAL	$59.03