

MACROINVERTEBRATE COMMUNITIES INHABITING SURFACE MINE
WETLANDS OF SOUTHWESTERN VIRGINIA

by

David Holton Jones

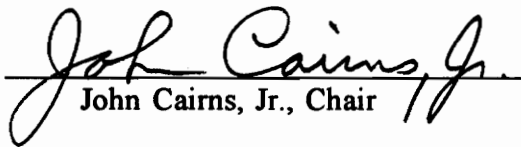
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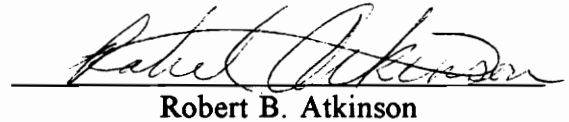
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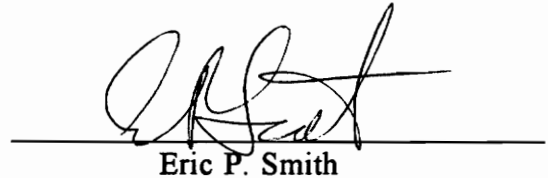
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ABSTRACT

Wetland acreage in Southwest Virginia has increased because of formation of wetlands on relic surface mine benches. Prior to the Surface Mining Control and Reclamation Act of 1977 (PL 95-87) once mining operations were completed the sites were abandoned. These areas presented novel landscapes in the rugged Allegheny Plateau physiographic region. Specifically, flat, compacted areas were created. In microdepression of these sites wetlands have formed. This study investigates the macroinvertebrate community associated wetlands of relic surface mining operations.

Surveys were conducted to identify what macroinvertebrates utilize these wetlands, to determine how this community was influenced by the physio-chemical characteristics of surface mine wetlands, and to develop design specifications for creating wetlands for current restoration efforts. Nine wetlands were sampled four times between July 1993 and May 1994. Seventy genera of macroinvertebrates were identified. The wetlands represented a range of physical and chemical parameters. The taxa richness appears to be related to these differences. Canonical Correspondence Analysis suggests that for the nine wetlands, the macroinvertebrate

taxa distribution is best explained by physical features of depth and duration of flooding, chemical parameters of iron, manganese, and sulfate concentration, and a biotic measure of plant litter biomass.

The findings of this study can be applied to ecological restoration. Wetland creation can be incorporated into current surface mine reclamation projects. To maximize the macroinvertebrate community of reclamation wetlands, sites must be positioned to avoid water quality problems, excavated to have a deeper portion which will remain inundated during all or most of the year, and have a gradually sloping substrate from the deepest area to the land surface. The final criteria being indirectly related to the macroinvertebrate community by influencing the macrophyte production, richness, and litter.

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GENERAL INTRODUCTION

Wetlands provide ecosystem services which benefit society. Services include water quality enhancement, flood peak regulation, and wildlife habitat (Mitsch and Gosselink 1993). With the recognition of these services, wetland destruction has been brought to the forefront of conservation issues. Over 50% of the natural wetlands in the United States have been destroyed (Tiner 1984, Dahl 1991). As society strives to protect existing wetlands, there has been a move to restore drained wetlands and create wetlands where none have existed before. However, not all wetland creation is planned. Wetlands unintentionally formed as a result of human activity have gained the interest of wetland ecologists. Research has been conducted on such wetlands as ball clay ponds and gravel pits (Barnes 1983, Friday 1987, Learner et. al. 1989, and Street and Titmus 1970). The coal producing region of the Appalachian Mountains is another area of unintentional wetland development (Klimstra and Nawrot 1985). Wetlands have formed on abandoned surface mine lands of the Allegheny Plateau physiographic region.

Wetlands on surface mines formed as a result of poor reclamation techniques. Prior to passage of the Surface Mining Control and Reclamation Act of 1977 (PL 95-87), sites were abandoned after the coal was removed. Here new landforms replace the natural topography and include vertical highwalls, benches, and outcrops. Highwalls are the cliffs of earth where excavation ceased. Outcrops are the areas where excavated material was pushed downhill. Benches are the flat, compacted areas

from which earth and coal were removed. In small depressions on the benches water is retained due to severe compaction. With time these areas are colonized by hydrophytes, and hydric soils develop. As such these sites meet the federal definition of wetlands.

As the hydrophyte community develops detritus accumulates. Accumulation of detritus influences wetland functions. Specifically, part of the litter biomass provides energy resources for primary and secondary macroinvertebrate consumers. Macroinvertebrates are a critical component of food chain support. Murkin and Wrubleski (1988) state that freshwater wetland foodwebs are detritus based. The trophic structure proceeds from plant detritus to micro-organisms, to invertebrate consumers and on to vertebrate consumers. As consumers, macroinvertebrates contribute to habitat functions by providing a link between primary production/detritus resource and higher trophic levels. In a review of the relationship between vertebrates and wetland invertebrates, Murkin and Batt (1987) state that all five classes of vertebrates have species which are dependent on this resource.

Macroinvertebrates are involved with nutrient cycling through the consumption of detritus and its exchange between trophic levels (Merritt et. al. 1984, and Murkin and Wrubleski 1988). Though the nutrient content of the macroinvertebrate standing crop is relatively insignificant, they play a distinct role in nutrient movement (Murkin and Wrubleski 1988). Macroinvertebrates affect the nutrient pool through transformation and translocation of organic material (Merritt et al 1984). The

collector-gatherer and scraper functional food groups consume detritus, algae, and microbes. As this material passes through the macroinvertebrate gut it is qualitatively and quantitatively altered. The fecal material provides particulate and dissolved organic matter which is utilized by primary producers and secondary consumers. Macroinvertebrates also enhance nutrient cycling through translocation or physical mixing (Merritt et al 1984). A large pool of nutrients is stored in the sediments. By burrowing and feeding in these sediments fauna, create bioturbations by circulation of water through burrows for respiration or excrement removal. This action mixes nutrients from the sediments into the water column, making them accessible for uptake.

Although macroinvertebrates are a major component of wetland faunas, research on the details of their abundance, distribution, and functioning in wetland habitats has only recently been initiated. Most macroinvertebrate studies have focused on lotic (stream) ecosystems. Krieger (1992) and Rosenberg and Danks (1987) suggest several reasons for the lack of research on macroinvertebrates in lentic systems: (1) the attention to control and/or eradication of a few pest insect species, (2) a perception that macroinvertebrates were economically trivial, and (3) the difficulties encountered in identification and sampling. Research has begun to increase as the role of aquatic insects in the food web and nutrient cycle are identified (Murkin and Wrubleski 1988).

This study investigates the macroinvertebrate community of surface mine

wetlands. These wetlands are an increase of wetland resources, however the ecological services provided by these wetlands is yet unknown. Information gained from this study contributes to society's knowledge of surface mine wetland ecology, wetland macroinvertebrate communities, and restoration ecology. In chapter one macroinvertebrate community structure is addressed. Chapter two focuses on the relations between the macroinvertebrate community and the environmental parameters of accidental wetlands.

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SURFACE MINE WETLAND MACROINVERTEBRATE ASSEMBLAGES

Chapter 1

INTRODUCTION

Although macroinvertebrates are a major component of wetland faunas, research on the details of their abundance, distribution, and functioning in wetland habitats has only recently been initiated. Most macroinvertebrate studies have focused on lotic (stream) ecosystems. Krieger (1992) and Rosenberg and Danks (1987) suggest several reasons for the lack of research on macroinvertebrates in lentic systems: (1) the attention to control and/or eradication of a few pest insect species, (2) a perception that macroinvertebrates were economically trivial, and (3) the difficulties encountered in identification and sampling. Research has begun to increase as the role of aquatic insects in the food web and nutrient cycle are identified (Murkin and Wrubleski 1988).

Wetlands on surface mines have been identified as novel wetland resources. Prior to passage of the Surface Mining Control and Reclamation Act of 1977 (PL 95-87), mines were abandoned after the coal was removed. Here new landforms replace the natural topography. Water is retained in small depressions due to severe compaction and sediment which limits infiltration. With time these areas are colonized by hydrophytes, and detritus accumulates. Accumulation of detritus can influence wetland functions. Part of the litter biomass provides energy resources for macroinvertebrates.

human disturbance (Barnes 1983, Fowler et. al. 1985, and Street and Titmus 1979). However, wetlands of contour mines in the Allegheny Plateau region have not been studied. These sites are unique because they are located along long narrow surface mine benches, occur at elevations higher than typical riverine wetlands, and are individually small, but exhibit a high density. The question to be answered by this project is what macroinvertebrates utilize these unusual ecosystems. The goals of the current study were to: (1) determine if macroinvertebrates are a component of accidental wetlands on surface mine benches of Southwest Virginia, (2) identify the structure of this macroinvertebrate community, (3) characterize the macroinvertebrate community response to disturbance, e.g., drawdown, and (4) estimate the potential of macroinvertebrates to colonize wetlands constructed for reclamation of surface mines.

METHODS

Macroinvertebrates were collected from nine wetlands located on three surface mine benches. The study sites were part of a larger project investigating the ecological services provided by surface mine wetlands of contour mine benches (Atkinson 19xx). The sites represented a variety of physical parameters (Table 1.1). These sites were small with an average size of 0.06 ha, ranging from 0.01 ha to 0.17 ha. Mean age of the wetlands was 17 years with the youngest being 9 years and the oldest 26. The depth of the wetlands was recorded for each site. With variation in depths of the wetlands some of the sites were seasonal or dry during one or more sampling periods.

Table 1.1: Chemical and physical parameters of nine surface mine wetlands, Wise County, VA, July 1993 to May 1994. Length to H2O is the distance to a colonizing source, H2O depth is the maximum depth, H2O fluct. is the water level fluctuation in standard deviations, and H2O permanence refers to seasonality of flooding.

SITE	pH	COND. uMHO	Fe mg/L	Mn mg/L	SO4 mg/L	Mg mg/L	HARD mg/L	ALKALIN. mg/l	AREA Hectare	LENGTH TO H2O meters	AGE years	H2O DEPTH meter	H2O FLUCT. Stan.Dev	H2O PERM. PERM.
B1	6.58	42	0.5	1.7	229	24.4	180	55	0.0438	*		0.66	1.9	Y
B2	6.27	143	5.7	4.7	927	84.8	618	59	0.1742	3		0.7	0.82	Y
B3	4.99	121	0.1	3.2	723	88.1	533	56	0.0789	37		0.45	1.77	N
L1	6.15	13	2.0	2.0	3	4.1	31	32	0.0129	40	19			N
L2	6.34	57	0.2	0.5	329	36.4	243	34	0.0645	71	18	0.52	0.37	Y
L3	6.23	9	0.8	0.5	9	5.7	37	42	0.0154	*	16	0.36	2.77	Y
T1	6.15	12	2.3	0.7	2	4.2	36	33	0.0485	61	12	0.07	2.96	N
T2	6.72	16	0.5	0.3	6	5.9	59	66	0.0715	49	9	0.35	2.96	Y
T3	6.84	16	2.5	0.9	35	5.2	49	52	0.0241	*	26			N
Powell River	7.54	59	0.1	0.1	158	17.4	171							

* No aquatic ecosystem apparent

Water chemistry parameters varied between sites (Table 1.1). High conductivity, metals concentrations, and sulfate concentration indicated stressed sites. Four species of macrophytes dominated different wetlands (Table 1.2). Typha was the dominate in four sites, Scirpus in three sites, Sparganium in one site, and the aquatic in the final site.

Table 1.2: Vegetation parameters of nine surface mine wetlands, Wise County, VA, July 1993 to May 1994.

Site	Plant Richness (Species)	Plant Biomass (g/m ²)	Dominate Species
B1	27	266	Spa Ame
B2	9	881	Typ spp
B3	29	328	Typ spp
L1	14	439	Sci Cyp
L2	15	368	Typ spp
L3	14	624	Typ spp
T1	19	981	Sci Cyp
T2	23	319	Myr spp
T3	31	493	Typ spp

Typ spp--Typha spp.

Sci Cyp--Scirpus Cyperanus

Myr spp--Myriophyllum spp

Spa Ame--Sparganium Americanum

Macroinvertebrate samples were taken on four dates: July 1993, September 1993, March 1994, and May 1994. Three samples were taken on each date using a D-frame net for sweep samples. This technique was selected because it (1) allowed sampling of both the water column and the top layer of sediment, (2) allowed sampling thick vegetation, (3) adequately sampled microhabitats, (4) was nondestructive, and (5) compared to various other types of sampling methods, offers the best results for taxa richness and relative abundance in wetlands (Cheal et. al 1993). A 1.0 mm mesh opening was selected to reduce clogging of the net. The samples were collected using a catch per unit effort (CPUE) technique. To standardize the sample effort a sample area of one quarter meter squared was swept. The quarter meter was maintained by placing a frame on top of the water and moving the net throughout the area. Samples were preserved in a 10% formaldehyde solution. In the lab samples were rinsed through a 1.0 mm mesh sieve. The macroinvertebrates were then picked from the remaining material. Identification was taken to genus using Merritt and Cummins (1984).

Macroinvertebrate richness was analyzed to identify differences between wetlands. Sites L1 and T3 were excluded from this analysis because they were flooded during the March 1994 sample. Hurlbert (1984) suggests that replicates within treatments are pseudo replication. As such the ANOVA test is performed with an inappropriate error term. To correct this problem the interaction term (sample date*site) was substituted for sample error. Duncan's multiple comparison test was

used to identify which sites are significantly different.

RESULTS

All nine wetlands contained macroinvertebrates (Table 1.3). A total of 14 orders, 40 families and 70 genera were identified in the samples. Insects were the most common invertebrates, and few other macroinvertebrates were represented. The most common orders were the Coleoptera (6 families, 26 genera), Diptera (8 families, most not identified to genera), Odonata (4 families, 13 genera), and Hemiptera (6 families, 7 genera). Annelidae was the only non-insect taxon which had great abundance. Several taxa were found in all sites. These were the Ceratopogonidae, Chironomidae, Tabanidae, and Annelidae. Additional species were found in all sites except the highest stressed sites (B2, L1, and T3 discussed below). These taxa are given in Table 1.4. Four sites had taxa limited to the one wetland T2, B1, B3, L2, and L3. Most functional feeding groups were represented. The predators dominated with 63% of the taxa, followed by the collector/gathers with 20%, shredders 14%, and scrapers 3%.

The sites differed in the number of taxa inhabiting them. For individual sites the richness ranged from 10 genera to 52. The lowest richness was found in two seasonal wetlands which were dry for the majority of the year. The taxa found in these sites were dominated by the diptera. Site B2 had the third lowest richness, 17 taxa. These three sites are referred to above as the highly stressed sites. The greatest

Table 1.3: Macroinvertebrate distribution of nine surface mine wetlands, Wise County, VA, July 1993 to May 1994.

	Black Creek			Low Splint Bench			Tyggart Bench		
	B1	B2	B3	L1	L2	L3	T1	T2	T3
Order Coleoptera									
Family:									
Chrysomelidae	12	1							
Dytiscidae Acilius			1						
Dytiscidae Agabates			1						
Dytiscidae Agabus			1	1		7		7	
Dytiscidae Anodocheilus							1		
Dytiscidae Celina	16	1	6		4	15	6	5	
Dytiscidae Coptotomus							3		
Dytiscidae Cybister							3	1	
Dytiscidae Hydaticus						1			
Dytiscidae Hydrovatus								1	
Dytiscidae Ilybius			1			1		5	
Dytiscidae Laccophilus	3		2				4	9	
Dytiscidae Laccodytes			7	1		1	14	2	
Dytiscidae Uvarus			4			2	5	2	
Dytiscidae spp			1		1	1			41
Gyrinidae Dineutus	7		1					2	
Haliplidae Haliplus								2	
Haliplidae Peltodytes	1		1		5	14	75	5	1
Hydrophilidae Berosus		1						1	
Hydrophilidae Enochrus			2				8	3	
Hydrophilidae Paracymus			4				2		
Hydrophilidae Tropisternus			2			2	11	8	
Noteridae Hydrocanthus			1				24	23	
Noteridae Notomicrus			1				19	12	
Noteridae Suphisellus							39	11	
Scirtidae Cyphon			2				1		
Scirtidae Scirtes			21		2	3	33	2	
Order Diptera									
Family:									
Ceratopogonidae	150	248	22	125	915	168	24	110	18
Chaoboridae Chaoborus	21					23		85	
Chironomidae	1802	208	36	11	1098	2337	46	231	36
Culicidae	4				21		1	10	
Stratiomyidae Odontomyia	10	1	6		71	13	8	161	1
Syrphidae					2				
Tabanidae	6	3	3	5	6	8	14	28	1
Tipulidae Helius			2	11	6	19	9	7	5

Table 3: Continued

	Black Creek			Low Splint Bench			Tyggart Bench		
	B1	B2	B3	L1	L2	L3	T1	T2	T3
Order Ephemeroptera									
Family:									
Baetidae Baetis	23	1			90	15		53	
Caebudae Caenis								7	
Order Hemiptera									
Family:									
Belostomatidae Belostoma									11
Corixidae Corisella	2					2	108	51	
Gerridae Gerris		1					2		
Nepidae Ranatra	4								3
Notonectidae Buenoa	18		1		6	23	3	3	
Notonectidae Notonecta	2				4	4			11
Veliidae Paravelia									1
Order Lepidoptera									
Family:									
Pyralidae							1	7	
Order Megaloptera									
Family:									
Corydalidae Chauliodes	1	1	3	12	12	5	17		14
Sialidae Sialis	1	31	4						6
Order Odonata									
Family:									
Aeshnidae Aeshna	14	4	5	3	11	3	4	12	
Aeshnidae Anax	17	9	1		18	19	1	18	
Coenagrionidae Argia	7								
Coenagrionidae Chromagrion	73	17	2		29				10
COenagrionidae Enallagma	145	17	10		53	15			310
Coenagrionidae Telebasis	2								
Corduliidae Neurocordula	1								
Lestidae Lestes	53				13	117	1		104
Libellulidae Lepthemis					2				1
Libellulidae Libellula						1			1
Libellulidae Macrodiplax						1			
Libellulidae Miathyria					4		127		
Libellulidae Pachydiplax	10		1		124	5	11		21

Table 3: Continued

	Black Creek			Low Splint Bench			Tyggart Bench		
	B1	B2	B3	L1	L2	L3	T1	T2	T3
Order Trichoptera									
Family:									
Leptoceridae							3	7	
Limnephilidae	9				8		2	7	
Phryganeidae	13		1	2	4	31	10	9	3
Polycentropodidae	1		1						
Order Annelidae	5	1	2	2	10	33	10	26	3
Order Collembola	1					1			
Order Gastropoda	16				5	5	12	3	
Order Amphipoda							2	1	
Order Homoptera		2	1		13	2		28	
Order Ararina			2					5	
TOTAL ABUNDANCE	2450	547	162	173	2537	2897	665	1563	88
TOTAL RICHNESS	33	17	36	10	28	33	38	52	10
MEAN RICHNESS	12.8	5.3	7.6	10	12.6	12.8	13	18.3	10
STANDARD DEVIATION	3.7	2.2	4.7	0	3.7	2.3	4.3	5.1	0

richness was found in site T2. The richness in this site was 52 taxa. The means and standard deviations by sample date are given in Figure 1.1. The results of ANOVA indicate that the means are significantly different. Duncan's multiple comparison show that at the $P < 0.05$ level the mean richness of site T2 is significantly higher than the other sites and that sites B2 and B3 are significantly lower than all other sites.

Table 1.4: Distribution of select taxa of nine surface mine wetlands, Wise County, VA, July 1993 to May 1994.

All Sites:

Diptera

Ceratopogonidae

Chironomidae

Tabanidae

Annelidae

All Sites except B2, L1, and T3:

Coleoptera

Dytiscidae Celina

Haliplidae

Peltodytes

Diptera

Stratiomyidae Odontomyia

Hemiptera

Notonectidae Buena

Odonata

Aeshnidae Aeshna

Aeshnidae Anax

Trichoptera

Phryganidae

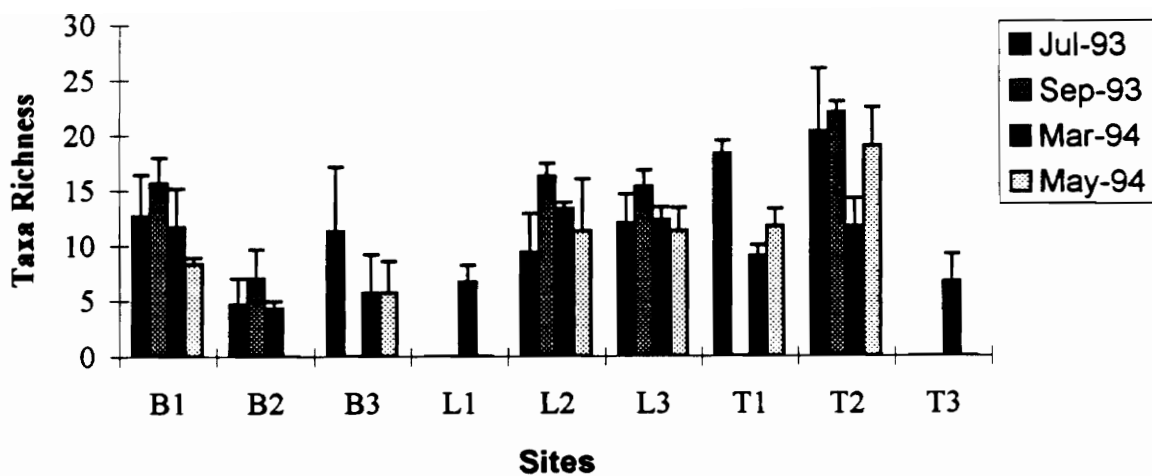


Figure 1.1: Macroinvertebrate richness of nine surface mine wetlands, Wise County, VA, July 1993 to May 1993. Error bars represent 1 standard deviation.

DISCUSSION

Macroinvertebrate Community

Macroinvertebrates inhabit wetlands on surface mine benches. This information is relevant when considering the evolution of these wetlands. Surface mine wetlands are opportunistic ecosystems. They form as a result of extreme landscape disturbance associated with surface mining. Environmental problems such as erosion, sedimentation, and high metals concentrations are found associated with former mining sites, including some sites in this study (Table 1.1). Additionally these wetlands are relatively young, with ages ranging from 10 to 30 years. Despite these problems,

macroinvertebrates have become established at all the study sites. Comparison of the macroinvertebrate communities of the surface mine wetlands to reference sites was impossible. No reference sites exist because natural wetlands which may have existed along floodplains have been destroyed or are impacted by sedimentation, and lentic wetlands located on mountain slopes do not exist. However, reference to wetlands literature provides studies which offer information on macroinvertebrates of various wetland types.

Bataille and Baldassare (1993) investigated the macroinvertebrates of prairie potholes in Manitoba, Canada. These wetlands are probably similar to surface mine wetlands in that they are shallow, contain dense hydrophytes, and the hydrology input is via precipitation or groundwater. In this study three wetlands were studied. Using two sampling methods they found 26 families of nektonic invertebrates and 50 families of emergent insects. In the current study, 40 families were identified. Of these, 34 families were aquatic insects. Magee et al (1993) studied the macroinvertebrates of two forested floodplain wetlands located along the Mississippi River that are inundated by seasonal flooding. The sites were sampled with traps for two years 55 taxa were collected. The list of taxa was dominated by families other than aquatic insects. The only insects in the top 80% of frequency of occurrence were the chironomidae. The taxa richness of this study was lower than the pothole study (Bataille and Baldassare 1993), but still higher than the current study. Rader et.al. (1994) investigated the macroinvertebrates along a nutrient gradient in the everglades of Florida. Using a D-

frame sweep net, 54 families of invertebrates were collected and overall richness was similar to the floodplain sites (Magee et. al. 1993), but higher than the current study. Aquatic insects in the everglades were represented by 27 families, compared to 36 families in the current study.

These studies (Bataille and Baldassare 1993, Magee et. al. 1993, and Rader et.al. 1994) suggest that the wetlands in the current study do not exhibit the richness or taxonomic richness of natural wetlands. Water chemistry, age, and size of surface mine wetlands could explain these differences. Barnes (1983) investigated succession in 10 ball clay ponds of England. Similar to the wetlands in this study, these ponds were formed after mining operations had altered the landscape. In the five pH neutral ponds 45 families were identified, whereas in the current study 40 families were collected. These values suggest that for similar wetland creation history, surface mine wetlands are not lacking in richness. Ails et. al. (1991) investigated agricultural rainwater evaporation ponds to determine their suitability for waterfowl habitat. These sites had high salt contents and the wetland plant and macroinvertebrates assemblages were species poor with only 15 macroinvertebrate families. In the current study site B2 had poor water and its macroinvertebrate community had 15 macroinvertebrate families.

Several studies investigated wetlands associated with surface mining. Bosserman, and Hill (1985) investigated the impact of acid mine drainage on wetlands in Western Kentucky. Sites with acid mine drainage had fewer taxa and higher

abundances than reference sites. This difference is related to water chemistry parameters. Sites with low pH, high sulfate concentration and high metals concentration exhibited lower richness. Usis and Foote (1991) investigated the effects of surface mining on a wetland Trichoptera, *Limnephilus indivisus*. This species' abundance declined in a wetland after impacts from surface mining. The results of the study suggest that this decline is related to high conductivity and suspended solids. Fowler et al (1985) studied the colonization of shallow ponds on a reclaimed surface mine. Two year old ponds had 36 families present, and the authors suggest that this richness will increase as the macrophyte community develops. These studies suggest that mining operations negatively impact aquatic systems. However systems designed and constructed for reclamation are ecologically beneficial.

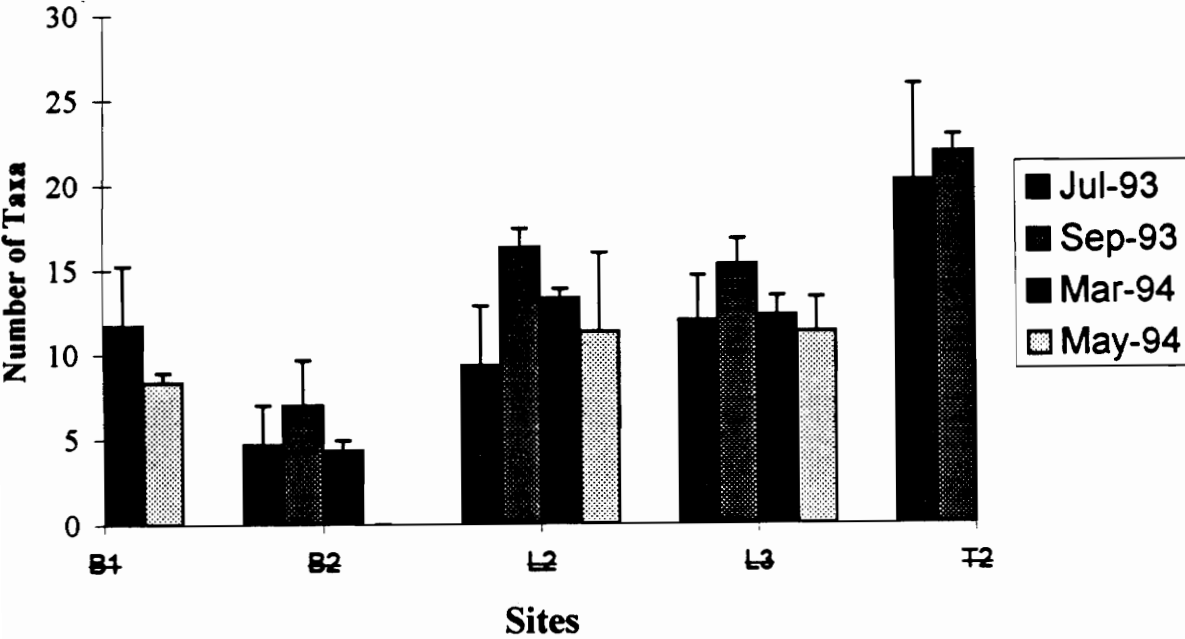


Figure 1.2: Macroinvertebrate richness of five permanently flooded surface mine wetlands, Wise County, VA, July 1993 to May 1994. Error bars represent 1 SD.

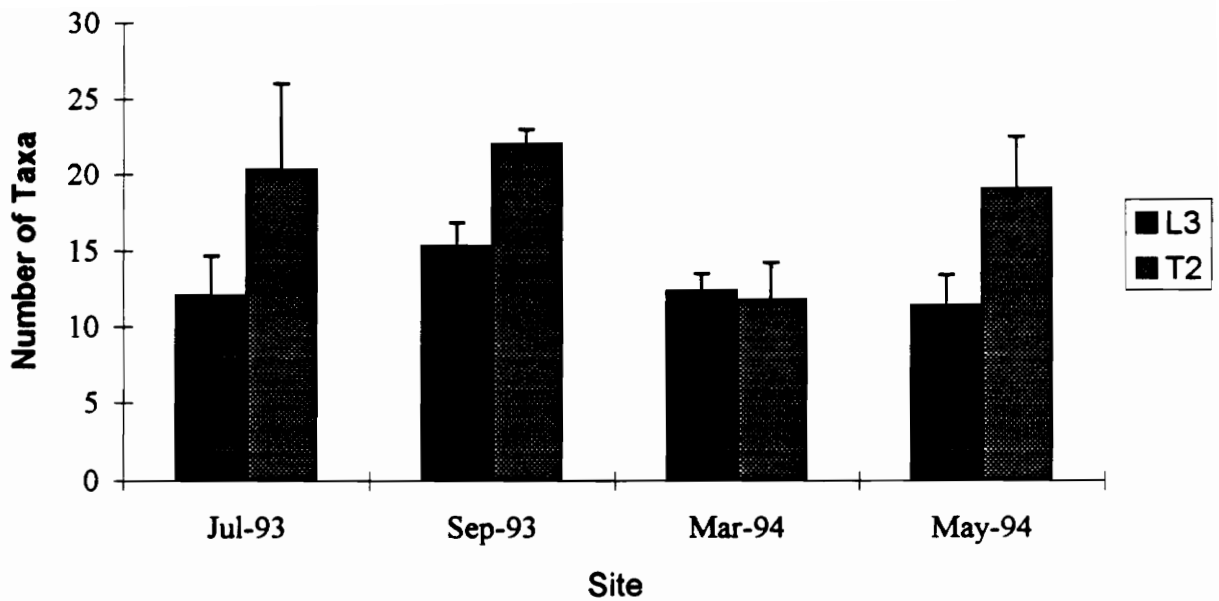


Figure 1.3: Macroinvertebrate richness of two surface mine wetlands, Wise County, VA, July 1993 to May 1994.

The data suggest that macroinvertebrate richness is influenced by water quality. Figure 1.2 shows the richness values for the permanent sites. The seasonal sites were omitted to eliminate any hydrological effects. Site T2 has significantly higher richness than the other sites, and it has the lowest values for conductivity and metals and sulfate concentration. Conversely, site B2 has significantly lower richness, and the highest water chemistry values. The other sites are intermediate between these two sites. However, the chemical parameters of site L3 appear closer to T2 than any of the other sites, but the richness is lower. Figure 1.3 shows this relationship. The difference in richness may be due to the difference in the dominate vegetation. Site T2 is dominated by an aquatic, *Myriophyllum* spp., which has high underwater surface

area. Whereas site L3 is dominated by an emergent, *Typha* spp., with low underwater surface area. Voits (1976) found that the macroinvertebrate community is related to the dominate vegetation in an aquatic ecosystem. As the plant surface area increases habitat diversity increases thus enabling more macroinvertebrates to occupy the system.

Macroinvertebrate Community Response to Disturbance

Wetlands are characterized by dynamic hydrologic regimes. The water fluctuation can be the driving force of macroinvertebrates of temporary waters (Neckles et al 1990, Batzer et. al. 1993, Bataille and Baldassarre 1993). Thus in seasonal wetlands, the macroinvertebrate community is determined by the length and frequency of flooding (Ebert and Balko 1987). Macroinvertebrates of temporary waters have developed strategies to withstand drought. Wiggins et al (1980) provide four methods for surviving dry periods: (1) permanent residents which have a resistant resting stage or diapause, (2) motile species which must oviposit in water, (3) motile species in which oviposition is independent of water, and (4) motile species which migrate to permanent water then return to oviposit in the newly flooded basin.

Most of the macroinvertebrate sampling in this study was conducted during the extreme drought conditions of 1993. While seasonal drawdowns are common for this type of system (Williams 1987), the 1993 drought provided an opportunity to quantify

macroinvertebrate response to severe hydrologic disturbance. In this study four of nine wetlands were dry during part of the year. All these sites contained macroinvertebrates after flooding. Two of the wetlands (T3 and L1) were inundated for only the March 1994 sample. The richness in these short season wetlands was the lowest of all the sites sampled if the poor water quality sites B2 and B3 (Table 1.1) are not considered. This community structure is characteristic of seasonal wetlands-- those which only have water present during the wet season. Seasonal wetlands typically have low diversity and high abundance (Neckles et. al. 1990). Figure 1.4 shows the richness of the five permanent sites (left side of x axis) and the four seasonal sites (right side of x axis) during the March 1994 sample when all sites were flooded.

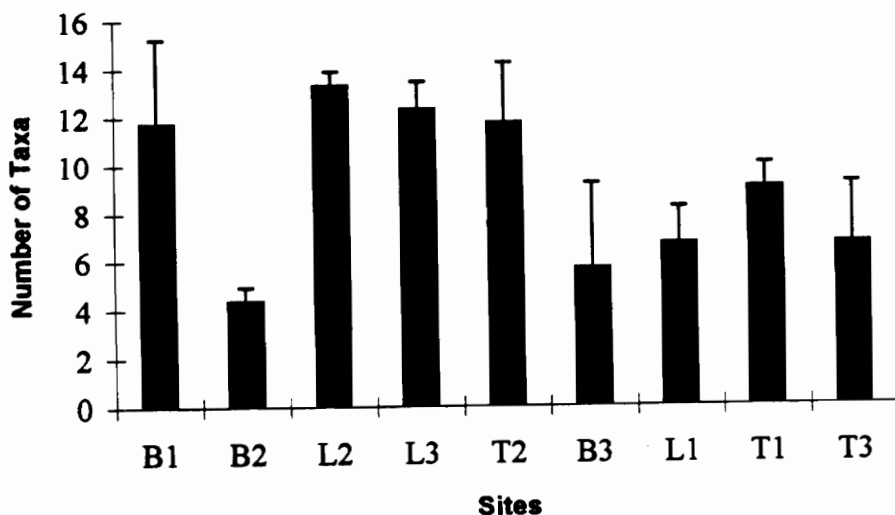


Figure 1.4: Macroinvertebrate richness of nine surface mine wetlands, Wise County, VA, March 1994. Sites are arranged on the X axis such that the five permanent sites are on the left side and the seasonal sites are graphed to the right.

Sites B2 and T1 were dry for only one sample period, September 1993. The overall richness for these two wetlands were second and third highest for this study. For the sites which went dry the richness range from highest to lowest. The seasonal wetlands have characteristic low diversity, but the sites which dried for shorter periods had higher richness. Ebert and Balko (1987) suggest that for temporary pools richness increases with increasing frequency and duration of flooding. These wetlands may typically exhibit stable water levels, and only become dry during extreme years (1993 was the second driest year recorded).

Resilience is the capacity of natural systems to absorb change without dramatically altering (Holling 1973). Surface mine wetlands in this study exhibited a range of hydrologic conditions during each sampling date. In spite of the variety of hydrologic responses to the 1993 drought, macroinvertebrate communities reestablished upon the return of flooded conditions. Thus, surface mine wetland macroinvertebrate communities demonstrated considerable resilience to severe drought (Figure 1.5).

Macroinvertebrate Colonization of Constructed Wetlands

Surface mine wetlands provide a template for the design of created wetlands as a component of current surface mine reclamation (Atkinson 1994). Created wetlands will provide ecological services to the post mining landscape. Several studies have investigated macroinvertebrate colonization of constructed wetlands. Fowler et. al. (1985) studied the colonization of surface mine sediment ponds in the Eastern

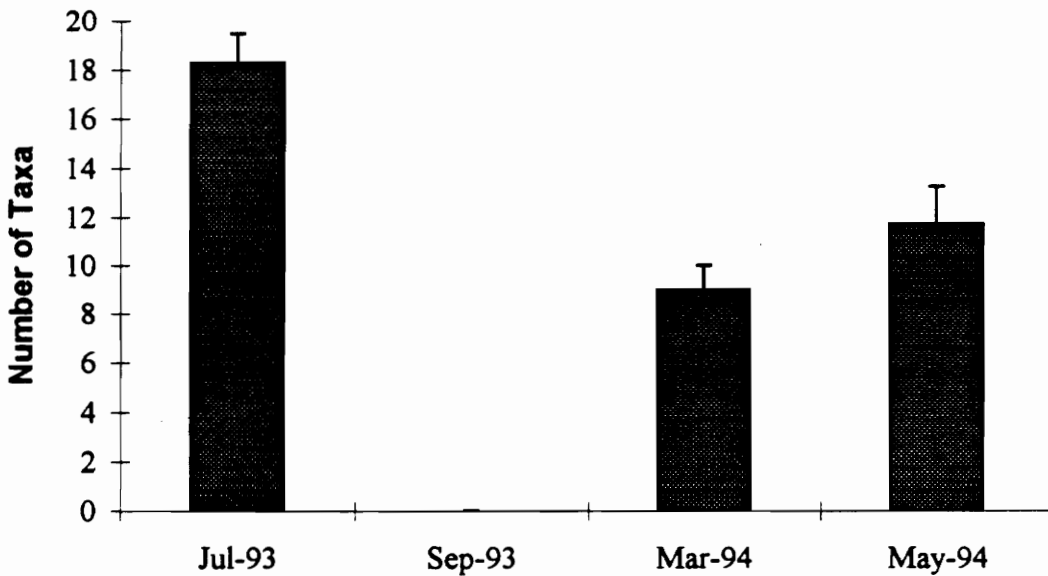


Figure 1.5: Macroinvertebrate richness of one surface mine wetland, Wise County, VA, July 1993 to May 1994.

Tennessee. After two years 36 families of macroinvertebrates were collected. With time it is assumed that this richness will increase. Barnes (1983) studied the colonization of ball clay ponds. It was determined that initial colonization of neutral ponds was rapid. Short dispersal distances was suggested as the mechanism for quick colonization. The Orders Coleoptera and Hemiptera were the earliest to colonize. As hydrophytes colonized and habitat complexity increased the macroinvertebrate community shifted from algivores and predators towards epiphyton grazers and detritivores. Street and Titmus (1989) investigated colonization of one and two year old gravel pits. These ecosystems had rapid colonization the first year. During the second year as hydrophytes developed the populations of sites decreased in similarity.

This differentiation was attributed to increased habitat complexity.

In the current study one constructed wetland was sampled in May 1995. The site was approximately one year old, had no hydrophytes growing, had been dry for several periods during the year, and had no epicenter for colonization for over 100 meters. However, 4 families were collected (Table 1.1). Similar to Barnes (1983) Coleoptera and Hemiptera were represented in first taxa to colonize these sites. The other two taxa were Odonata and Diptera. These taxa have been found in new isolated sites and both are known to disperse widely (Sheldon 1984). The colonization of this experimental site suggests that constructed wetlands will be inhabited quickly, and that hydrophytes are not a prerequisite for colonization. The results of Barnes (1983) suggest that as hydrophytes colonize and habitat complexity increases macroinvertebrate richness will increase. By constructing wetlands in areas without water quality problems it is expected that they will have macroinvertebrate communities similar to site T2 (Table 1.2). This site exhibited the greatest richness of all the wetlands sampled, and all the functional feeding groups were represented. It had permanent hydrology, moderate hydrology fluctuation, circumneutral pH and low concentrations of metals. By constructing wetlands similar to site T2, macroinvertebrates may become established and contribute to wetland ecological services that will improve reclamation such as nutrient cycling, trophic support, and energy transfer.

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ORDINATION OF MACROINVERTEBRATE COMMUNITIES IN SURFACE MINE WETLANDS OF VIRGINIA

Chapter 2

INTRODUCTION

Over 50% of the natural wetlands in the United States have been destroyed (Tiner 1984, Dahl 1991). As society strives to protect existing wetlands, there has been a move to restore drained wetlands and create wetlands where none have existed before. However, not all wetland creation is planned. Wetlands unintentionally formed as a result of human activity have gained the interest of wetland ecologists. Research has been conducted on such wetlands as ball clay ponds and gravel pits (Barnes 1983, Friday 1987, Learner et. al. 1989, and Street and Titmus 1970). The coal producing region of the Appalachian Mountains is another area of unintentional wetland development. This increase is a direct result of strip mining (Klimstra and Nawrot 1985).

Wetlands on surface mines formed as a result of mass movement of earth. Previous to current reclamation law (PL 95-87) after the coal was removed sites were abandoned. Here new landforms replace the natural topography. The typical post-mine landscape consists of vertical highwalls from the excavation of the mountainside; benches which are generally flat open areas proceeding away from the highwall; and the outslope, excavated material pushed over the hillside. Wetlands have become a

feature of most benches. Water is retained because the lack of slope and severe compaction reduces hydraulic conductivity. With time these areas are colonized by hydrophytes, plants with morphological, physiological and reproductive adaptations for living in saturated conditions (Mitsch and Gosselink 1993). As hydric soils develop, these sites meet the three criteria of the federal wetlands definition: hydrology, hydrophytes, and hydric soils.

Accidental wetlands are more than simple retention ponds. Along the surface mine bench these wetlands provide wildlife habitat. The diversity and structure of the wetland ecosystem offers patches of unique resources unlike typical dry depauperate sites recovering from an impact. Water is retained on site, hydrophytes colonize, and detritus accumulates. This detritus accumulation is significant and provides water quality enhancement functions (Mitsch and Gosselink 1993), but it also serves as an energy source for primary and secondary consumers. As consumers, macroinvertebrates contribute to habitat functions by providing a critical link between primary production/detritus resources and higher trophic levels (Murkin and Wrubleski 1988). Additionally they function in nutrient cycling aiding in the breakdown of detritus so nutrients become available for primary production (Murkin and Wrubleski 1988). These two functions of macroinvertebrates are a necessary process in wetland ecology.

Although macroinvertebrates are a major component of wetland faunas, research on the details of their abundance, distribution, and functioning in wetland

habitats has only recently been initiated. Most macroinvertebrate studies have focused on lotic (stream) ecosystems. Krieger (1992) and Rosenberg and Danks (1987) suggest several reasons for the lack of research of macroinvertebrates in lentic systems: (1) the attention to control and/or eradication of a few pest insect species, (2) a belief that macroinvertebrates were economically trivial, and (3) the difficulty in identification and sampling this community.

In this study canonical correspondence analysis (CCA) (terBraak 1986) was used to examine relationships between macroinvertebrate community structure and the environmental parameters of accidental wetlands. The direct gradient analytical method of CCA simplifies complex data such that relationships are revealed. CCA also provides an effective method for analyzing patterns in biotic communities and assessing the relative importance of multiple environmental variables on macroinvertebrate assemblages. CCA has been applied to several studies of macroinvertebrates in lentic and lotic systems (Gower et al 1994, Grantham and Hann 1994, Jackson and Harvey 1993, Johnson and Wiederholm 1989).

METHODS

Macroinvertebrates were collected from nine accidental wetlands located on three surface mine benches in Wise County, VA. The study sites were part of a larger project investigating the ecological services provided by surface mine wetlands of contour mine benches (Atkinson 1995). The sites represented a range of physical

Table 2.1: Chemical and physical parameters of nine surface mine wetlands, Wise County, VA, July 1993 to May 1994. Length to H2O is the distance to a colonizing source, H2O depth is the maximum depth, H2O fluct. is the water level fluctuation in standard deviations, and H2O permanence refers to seasonality of flooding.

SITE	pH	COND. uMHO	Fe mg/L	Mn mg/L	SO4 mg/L	Mg mg/L	HARD mg/L	ALKALIN. mg/l	AREA Hectare	LENGTH TO H2O meters	AGE years	H2O DEPTH meter	H2O FLUCT. Stan.Dev	H2O PERM.
B1	6.58	42	0.5	1.7	229	24.4	180	55	0.0438	*		0.66	1.9	Y
B2	6.27	143	5.7	4.7	927	84.8	618	59	0.1742	3		0.7	0.82	Y
B3	4.99	121	0.1	3.2	723	88.1	533	56	0.0789	37		0.45	1.77	N
L1	6.15	13	2.0	2.0	3	4.1	31	32	0.0129	40	19			N
L2	6.34	57	0.2	0.5	329	36.4	243	34	0.0645	71	18	0.52	0.37	Y
L3	6.23	9	0.8	0.5	9	5.7	37	42	0.0154	*	16	0.36	2.77	Y
T1	6.15	12	2.3	0.7	2	4.2	36	33	0.0485	61	12	0.07	2.96	N
T2	6.72	16	0.5	0.3	6	5.9	59	66	0.0715	49	9	0.35	2.96	Y
T3	6.84	16	2.5	0.9	35	5.2	49	52	0.0241	*	26			N
Powell River	7.54	59	0.1	0.1	158	17.4	171							

* No aquatic ecosystem apparent

parameters (Table 2.1).

Water chemistry and physical characteristics were determined at each wetland. Chemical parameters measured were pH, conductivity, alkalinity, hardness, sulfate, iron, magnesium and manganese concentrations. Physical parameters recorded included temperature, surface area, distance to next aquatic ecosystem, age, water depth, water level fluctuation, and the duration of flooding.

Vegetation surveys were conducted in the accidental wetlands in another study (Atkinson 1995). In that study, vegetation richness, biomass, and dominant plant species were determined. This data was incorporated into the macroinvertebrate community analyses. The values are given in Table 2.2.

Macroinvertebrate samples were taken on four dates: July 1993, September 1993, March 1994, and May 1994. Three samples were taken on each date using a D-frame net for sweep samples. This technique was selected because it (1) allowed sampling of both the water column and the top layer of sediment, (2) allowed sampling thick vegetation, (3) adequately sampled microhabitats, (4) was nondestructive, and (5) compared to various other types of sampling methods, offers the best results for taxa richness and relative abundance in wetlands (Cheal et. al 1993). A 1.0 mm mesh opening was selected to reduce clogging of the net. The samples were collected using a catch per unit effort (CPUE) technique. To standardize the sample effort an area of one quarter meter squared was swept. The quarter meter was maintained by placing a frame on top of the water and moving the net throughout

the area. Samples were preserved in a 10% formaldehyde solution. In the lab samples were rinsed through a 1.0 mm mesh sieve. The macroinvertebrates were then picked from the remaining material. Identification was taken to genus using Merritt and Cummins (1984).

Table 2.2: Vegetation parameters of nine surface mine wetlands, Wise County, VA, July 1993 to May 1994.

Site	Plant Richness (Species)	Plant Biomass (g/m ²)	Dominate Species
B1	27	266	Spa Ame
B2	9	881	Typ spp
B3	29	328	Typ spp
L1	14	439	Sci Cyp
L2	15	368	Typ spp
L3	14	624	Typ spp
T1	19	981	Sci Cyp
T2	23	319	Myr spp
T3	31	493	Typ spp

Typ spp--Typha spp.

Sci Cyp--Scirpus Cyperinus

Myr spp--Myriophyllum spp

Spa Ame--Sparganium Americanum

The invertebrate data set was analyzed using conical correspondence analysis (CCA) (ter Braak 1986). CCA examines variation in community composition by constraining ordination axes to be linear combinations of environmental variables. In the ordination diagram, arrows represent environmental variables and points the weighted averages of species and site scores. The direction of the arrows indicate maximum change in the species ordination, and its length is proportional to the magnitude of change in that direction. Species-environmental correlation coefficients provide a measure of how well the environmental data account for the extracted variation in the biological data. Weighted inter-set correlation coefficients were used to identify the relative importance of environmental factors in determining the site scores derived from ordination of the species data.

Transformations were performed on the data. Species data and environmental variables were evaluated for skewed distributions. The $\ln(x+1)$ transformation was performed on the species abundance, conductivity, and concentrations of metals and sulfate.

RESULTS

Macroinvertebrates were present at all sites. A total of 14 orders, 40 families and 70 genera were collected. Insects were the most common invertebrates with few other macroinvertebrates represented. The most common orders were the Coleoptera (6 families, 26 genera), Diptera (8 families, most not identified to genera), Odonata (4

families, 13 genera), and Hemiptera (6 families, 7 genera). Annelidae was the only non-insect taxon which had great abundance. Several taxa were found in all sites (Table 2.3). These were the Ceratopogonidae, Chironomidae, Tabanidae, and Annelidae. Additional species were found in all sites except the highest stressed sites (B2, L1, and T3 discussed below). Four sites had taxa limited to the one wetland T2, B1, B3, L2, and L3.

Table 2.3: Distribution of select taxa of nine surface mine wetlands, Wise County, VA July 1993 to May 1994.

All Sites:

Diptera

Ceratopogonidae

Chironomidae

Tabanidae

Annelidae

All Sites except B2, L1, and T3:

Coleoptera

Dytiscidae Celina

Haliplidae

Peltodytes

Diptera

Stratiomyidae Odontomyia

Hemiptera

Notonectidae Buena

Odonata

Aeshnidae Aeshna

Aeshnidae Anax

Trichoptera

Phryganiedae

The sites differed in the number of taxa inhabiting them. For individual sites the richness ranged from 10 genera to 52. The lowest richness was found in two seasonal wetlands which were dry for the majority of the year (L1 and T3). The taxa found in these sites were dominated by the diptera. Site B2 had the third lowest richness, 17 taxa and it had the most extreme water quality values (Table 2.1). These are the three highly stressed sites mentioned above. The greatest richness was found in site T2 with 52 taxa.

The nine wetlands exhibited a range of physical and chemical variables (Table 2.1). The sites were small with an average size of 0.06 ha, ranging from 0.01 ha to 0.17 ha. Mean age of the wetlands was 17 years with the youngest being 9 years and the oldest 26. Depths ranged from 0.02 m to 0.89 m with a mean of 0.47 m. Following from variable depths, 5 of the sites were flooded for the entire study, and 4 were seasonal or dry during one or more sampling periods. Water chemistry parameters varied between sites. All sites had pH measures above 6.0 except site B2 with a mean pH of 5.0. In general the sites located at Black Creek (site names beginning with 'B') had higher values for conductivity, iron, manganese, and sulfate concentrations. One other site not located at Black Creek, L2, also exhibited high conductivity and sulfate concentration. High conductivity, metals concentrations, and sulfate concentration indicated stressed sites.

Canonical correspondence analysis was used to identify relationships between the wetland macroinvertebrate community and site physical and chemical parameters.

Data on 18 environmental variables were collected. Of these four were initially discarded because they were too variable. The remaining variables were analyzed by CCA. The Canoco program's forward selection option was used to evaluate the contribution of the 18 environmental variables. Six were selected for the final analysis: iron, manganese, and sulfate concentration, maximum depth, permanence of inundation, and litter biomass. Variables were omitted if they had low correlation with the canonical axes, and thus offered little explanation for the distribution of macroinvertebrates. The selected environmental variables, twenty-seven samples and 70 species were used in the ordination procedure. Figure 2.1 shows the results of the ordination of sites and macroinvertebrate taxa with respect to environmental variables. Sites B1, L2, and L3 were placed near the depth arrow and the point for permanence (points were used for nominal variables). These sites were flooded for the entire study, whereas sites L1 and T1 were seasonal, and thus, they were placed opposite the permanent sites. Not all the permanent sites were located in the proximity of the permanence variable point. Instead sites B2 and T2 were placed on opposite ends of the water quality vectors. These two sites have very different measures of conductivity, iron, manganese, and sulfate concentrations (Table 2.1). B3 and T3 were also aligned with poor water quality arrows.

Table 2.4 shows the eigenvalues, the percent variance of the species environment relation and the intersite correlation of environmental variables with the ordination axes. The eigenvalues are small indicating that the variability is found in

the species distribution. However, the species site matrix of ranked CCA scores shows the structure of the species data is arranged in a diagonal band representing axis one (Table 2.5). Though the axis is short it does describe the structure of the species distribution. Those species falling outside this band represent random noise unexplained by the taxa structure. The cumulative percent variance indicates that the environmental variables describe 85% of the spread of the CCA species ordination. The intersite correlations of environmental variables indicate the strength of the relations between the individual environmental variables and the site scores. Ranking these values for axis one showed that permanence, depth and sulfate are negatively correlated to axis one, while litter, iron and manganese were positively correlated with this axis. Ranked intersite correlations of axis 2 showed iron and manganese as being the foremost variables influencing this axis.

DISCUSSION

Although accidental wetlands have increased the net wetland acreage in southwest Virginia these sites differ from natural wetlands in that they are impacted by surface mining prior to SMCRA (PL95-87). Water quality problems exist even though mining had ceased nearly twenty years earlier. However, macroinvertebrate communities have colonized all the sites. In this investigation, macroinvertebrate communities have been shown by CCA ordination to be related to physical properties of depth and length of inundation, influenced by biological input as litter and related

to three water chemistry variables (iron, manganese and sulfate concentration) .

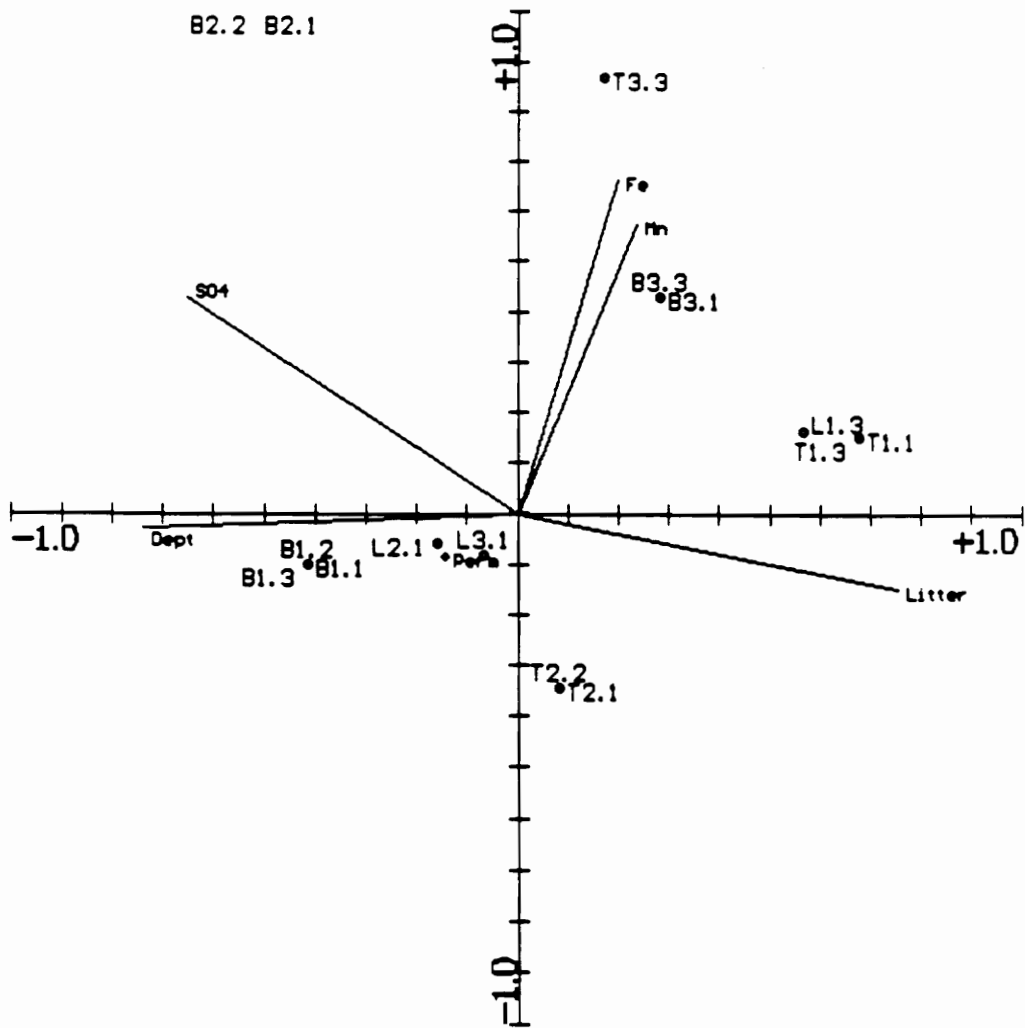


Figure 2.1: Site and environmental variable biplot based on CCA of nine surface mine wetlands Wise County, VA, July 1993 to May 1994. Environmental variables are represented as vectors, except hydrology permanence which is a point because it is a nominal variable. The symbols for environmental vectors are: iron (Fe), manganese (Mn), and sulfate concentration (SO₄), water depth (Dept), seasonality/permanence (Perm), and litter biomass (Litter). Sites are shown as points. The letter and first number indicate site and the second number indicates sample date (1 July 1993, 2 September 1993, 3 March 1994, and 4 May 1994). Some of the sample points are hidden by labels of others. Table 2.5 gives an indication of the location of hidden points along the x-axis.

Table 2.4: Results of canonical correspondence analysis for 9 surface mine wetlands, Wise County, VA, July 1993 to May 1994.

	1	Axis 2	3	4
Eigenvalues	0.23	0.17	0.09	0.07
Cumulative % variance of species-environment relation	33.4	58.9	72.6	83.4
Interset correlation of environmental variables with axes:				
Fe	0.19	0.63	0.08	0.24
Mn	0.22	0.55	0.04	0.29
SO ₄	-0.62	0.41	-0.02	-0.43
Depth	-0.70	-0.03	-0.02	-0.46
Permanence	-0.75	-0.45	0.09	0.19
Litter biomass	0.71	-0.14	-0.36	0.04

Hydrology and litter explain the spread of CCA axis 1. Permanence and depth are related in that deeper sites will be flooded for longer periods. Wiggling et. al. (1980) suggest that macroinvertebrates of temporary waters have developed strategies to withstand drought. These strategies are: (1) permanent residents which have a resistant resting stage or diapause, (2) motile species which must oviposit in water, (3) motile species in which oviposition is independent of water, and (4) motile species which migrate to permanent water then return to oviposit in the newly flooded basin. This theory suggests that different species are able to colonize temporary aquatic

ecosystems. Table 2.5 gives the taxa list for all wetlands arranged by score rank of CCA axis 1. The species at the top are associated with shallow, seasonal wetlands and those at the bottom are found in deeper, permanent sites. Looking at the distribution, those at the top are predominately coleoptera suggesting that other taxa which can not readily migrate or diapause cannot colonize these sites. Taxa such as Baetis, Culicidae, Chaoborus, and Nepidae are located toward the bottom of the table indicating they are associated with permanent hydrology.

Litter is also strongly correlated to axis 1. There is no difference between the functional feeding groups of the macroinvertebrates at the two ends of axis 1 suggesting that litter is not influential as a food source. Instead it may be related to plant dynamics and habitat. Atkinson (1995) found that plant biomass production was highest in seasonal wetlands. It follows that the biomass of herbaceous plants supports the litter biomass, so seasonal wetlands will have greater amounts of litter. This attribute of seasonal wetlands provides habitat for macroinvertebrates. Due to the high organic material in the substrate, during dry periods sections of the wetland will remain moist for longer periods. Elbert et. al. (1987) found that the species composition of seasonal ponds is related to the frequency and length of duration. Litter affects the length of duration, so seasonal sites with high litter will have a different macroinvertebrate assemblage than a similar site with small amounts of litter.

Iron and manganese concentration have the most influence on axis 2 (Table 2.4). Figure 2.1 shows this relationship. The poorest water quality site, B2, is placed

at the top of the graph and the best water quality site, T2, is placed at the bottom. Poor water quality's negative influence on macroinvertebrates is well known, so this relationship is expected. Gower et. al. (1994) investigated the influence of high metal concentration in streams on macroinvertebrate communities. The high metal concentration was due to historical mining in the region of study. They used CCA and found that the macroinvertebrate communities were partitioned along a metals gradient with the most tolerant species separated from less tolerant species. Other studies have investigated the effects of mining on wetland macroinvertebrates (Bosserman and Hill 1985, Usis and Foote 1991). In these studies high levels of metals, sulfate and conductivity had a negative impact on the macroinvertebrate communities. However, Bosserman and Hill (1985) used richness and abundance in multiple regression, and Usis and Foote (1991) only investigated effects on abundance of one species. In this study the entire taxa list of the wetlands is analyzed to identify relationships. The results support the findings of the prior two studies.

Canonical correspondence analysis suggests that part of the variability of surface mine macroinvertebrate community structure is explained by environmental variables. The variables with the highest relationship to the macroinvertebrate community are depth, permanence, litter biomass, and iron, manganese, and sulfate concentrations. These findings support prior studies on the influence of mining on aquatic fauna. This information can be used in current restoration efforts. Accidental wetlands provide ecological services to the post mining landscape. Since current

reclamation regulation requires the removal of the structures which allow accidental wetland formation, no wetland development occurs on post- SMCRA mine sites. It is proposed that wetlands be constructed on reclaimed surface mines for wildlife habitat. Macroinvertebrates are an important component of wetland ecosystems. As such the information from this study can be utilized to encourage maximum development of wetland functions and values. Macroinvertebrate design considerations are derived from the results of this study. Specifically, wetlands should be constructed such that they have a deeper portion up to one meter deep. They should be located to avoid water quality problems. And lastly, the substrate should gradually slope from the deepest point to the landscape surface.

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GENERAL CONCLUSIONS

Macroinvertebrates inhabit wetlands which have formed on relic surface mine benches. In this study all nine sites contained macroinvertebrates. Seventy genera of macroinvertebrates were present. For individual wetlands the maximum taxa richness was 53 and the minimum richness was 17. These values are lower than the richness of various natural wetlands. However, macroinvertebrate studies have been conducted in wetlands with similar origins as those of the current study. In ball clay extraction wetlands and agricultural drainwater ponds the range of taxa richness (15-40 taxa) was comparable to those of this study.

The structure of the macroinvertebrate community appears related to the physio-chemical parameters. Macroinvertebrate richness between sites was significantly different. Canonical Correspondence Analysis (CCA) was used to identify correlations between taxa distribution and environmental variables. The results may account for the differences between wetlands. The CCA analysis indicates that the macroinvertebrate distribution is related to the physical characteristics of depth and duration of flooding, the chemical parameters of iron, manganese, and sulfate concentration, and a biotic parameter of litter biomass.

The wetlands of this study can serve as models for designing wetlands on current surface mine reclamation. The results of the CCA analysis suggest specific criteria for optimal macroinvertebrate community development. Wetlands should have adequate depth such that they are flooded for all or most of the year. The site of the

wetland construction should be located in an area without water quality problems. The morphology of the wetland basin should have an extremely gradual slope. This slope will maximize the diversity of the vegetation community which provides resources and habitat for macroinvertebrates.

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4/94 Inducted Phi Kappa Phi Honor Society

5/84 Forest Products Research Society Outstanding Member

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5/81 Key Club Man of the Year

12/79 Boy Scouts Eagle Award

4/79 Boy Scouts God and Country

10/79 Appalachian Council Scout of the Year

GRANTS

7/1/93-6/30/94 Powell River Land Use Project. \$3,100. Macroinvertebrate richness and abundance in surface mine wetlands. Support: partial salary, travel, and supplies.

Matching funds from the Department of Biology, Virginia Tech \$500.

7/8/93 Graduate Research Development Project. Applied for \$300. Macroinvertebrate richness and abundance of accidental wetlands on surface mines in Southwest Virginia. Support: supplies.

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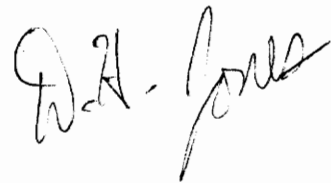
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A handwritten signature in black ink, appearing to read "D.A. Jones". The signature is written in a cursive, flowing style.