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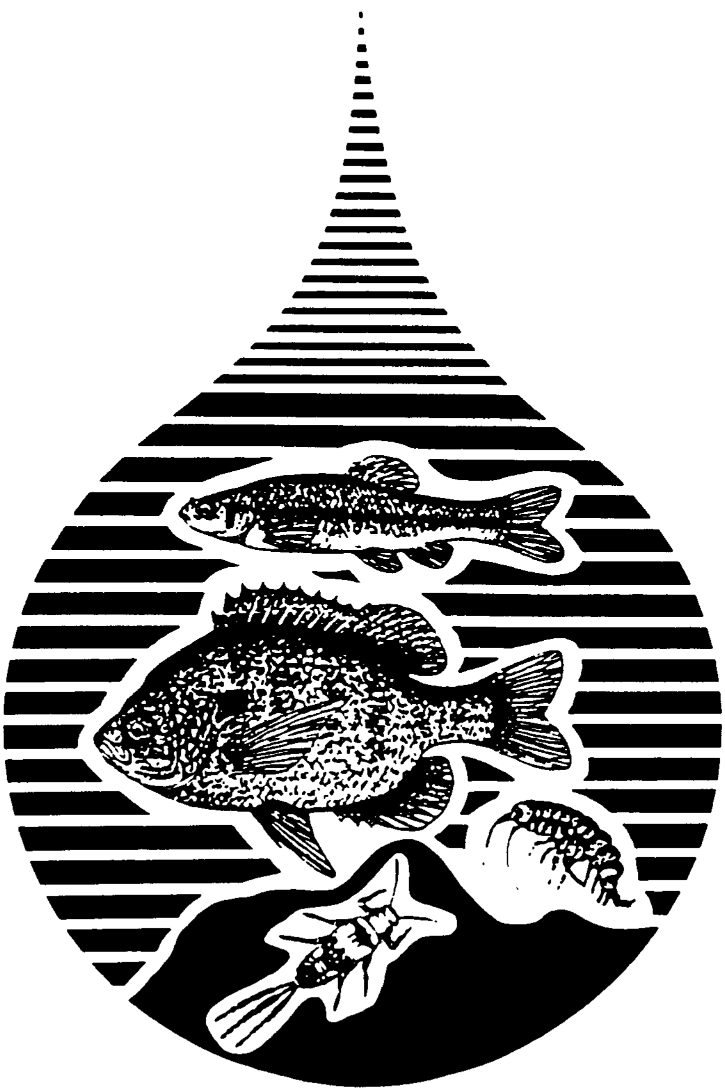
1991 Midwest Pollution Control Biologists Meeting

Environmental Indicators: Measurement and Assessment Endpoints

Lincolnwood, Illinois

March 19-22, 1991



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PROCEEDINGS OF THE 1991 MIDWEST

POLLUTION CONTROL BIOLOGISTS MEETING

ENVIRONMENTAL INDICATORS: MEASUREMENT AND ASSESSMENT ENDPOINTS

Held in

LINCOLNWOOD, ILLINOIS

MARCH 19-22, 1991

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Cover: Cover design and illustration by Blaine D. Synder of EA Engineering, Science and Technology, Inc. Depicted is a fathead minnow, a bluegill, a gammarid amphipod, and an emphemerellid mayfly superimposed on a drop of water. This design was developed for USEPA's Rapid Bioassessment Program, Assessment and Watershed Protection Division, Office of Water, Washington, D.C.

ACKNOWLEDGEMENTS

After several years of hosting the Biocriteria and Ecological Assessment Conferences in Region V, it has become a regular event our Agency can be proud of. The theme of this years meeting "Environmental Indicators: Measurement and Assessment Endpoints" is another example that Region V pollution control biologists are in the forefront of their field bringing together innovative approaches for addressing national issues relating to water quality. In addition to the keynote paper by Dr. James Karr, a total of 22 papers were presented at this years meeting. Of significance was the two workshops including the Oligoechaete taxonomy Workshop (Dr. Donald Klemm) and Sediment Toxicity Testing Workshop (Marsha Nelson and Jim Coyle). The first meeting of the Regional-State Biocriteria Workgroup (chaired by Thomas Simon and Chris Yoder) met and discussed issues pertaining to lake and large river biocriteria development.

The editors of this years meeting wish to thank the Region V, Instream Biocriteria and Ecological Assessment Committee and Region V management for supporting this meeting.

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Components of Biological Integrity: Their Definition and Use In Development of An Invertebrate IBI

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Abstract
Protection of quality water resources is critical to the maintenance of our way of life. Recent threats, such as the drought in California, fish consumption advisories, and contamination of beaches, are illustrative of the extent of abuse of water resources. These widespread declines in the quality of water resources have altered societal perceptions of and goals for the management of those resources. Growing interest in biological assessment in the last decade is in sharp contrast to the status quo of earlier decades. In this paper, we briefly review the evolution of water law and outline the conceptual foundations of ambient biological monitoring. We illustrate the use of those foundations as we outline our efforts to develop a methodology for use of invertebrates in assessing biological integrity.

Water Law

Early in this century, streams and lakes were viewed as sources of water or as locations for the discharge of societal wastes. Eventually, concern was expressed for the role of water pollution in the spread of human health problems; microbial contamination and oxygen-demanding wastes were early concerns and the threat of toxic contamination continues to expand.

As problems with and perceptions of water resources have changed, water law has changed as well. The first major water legislation in the United States was passed in 1889 to control oil pollution and protect navigation. Throughout the early part of this century and into the 1940's, legislative actions dealing with water resources tended to be relatively weak and provided little or no money. By the 1950's and 1960's legislation was tougher, but it concentrated on the development of construction grant programs to treat domestic effluent.

Passage of the Water Quality Act Amendments of 1972 (Public Law 92-500) brought a new approach with incorporation of the stated goal of protecting the fishable and swimmable status of these resources through control of point and non-point sources of pollution. Timetables, deadlines by which society had to respond to and protect water resources, were developed. For the first time, the phrase "biotic integrity" came into clean water legislation with the charge "to restore and maintain physical, chemical, and biological integrity of the nation's waters." But even that innovation did not stimulate a very broad perspective. The dominant approach continued to be control of chemical contaminants. Over the past decade the failure of that approach has become obvious. By the 1980's, new phrases such as "anti-degradation" and "ambient biotic assessment" were added to the lexicon of water resource professionals. Calls for adoption of biological criteria became common in the late 1980's (Karr 1991).

Overall, early legislative trends can be characterized by several common themes: an inordinate concentration on chemical contamination; funding for technology development and construction of wastewater treatment

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facilities; and increased enforcement efforts directed towards control of point-source pollution. Non-point sources of pollution were not seriously considered in water quality legislation until 1972. Even then, efforts to control non-point sources were relatively weak, primarily because they utilized point-source approaches and conceptual frameworks that are inappropriate for treatment of non-point source contamination (Karr 1990a).

Pollution Defined

The narrow contaminant perspective misconstrues the intent of the Clean Water Act. Pollution is defined in the 1987 Act as human "alteration of the chemical, physical, biological, or radiological integrity of water." That definition clearly goes beyond treatment of chemical contamination to a broader conception of factors responsible for degradation of water resources. An integral component of any effort to use that broad conception of pollution is the evaluation of the resource's ability to sustain a balanced biological community. If a water resource is degraded to the point that it does not support a healthy biological community, it very likely will not support one or more beneficial uses.

Assessing Water Quality

Toxicity testing and chemical evaluations of water samples have long been the mainstay of water resource evaluation. Each provides valuable information but, when conducted in the absence of ambient biological monitoring, they do not provide sufficient information to protect water resources. In a recent study, water chemistry data failed to detect 50% of the impairment in Ohio surface waters that was detected with integrated biological and chemical monitoring (Rankin et al. 1990). Thus, increased use of ambient biological monitoring is essential for the protection of water resources. Why has use of direct biological evaluations been so limited?

First, early efforts to maintain the quality of water resources were narrowly conceived and planned (Karr 1991). Water pollution control engineers dominated the agenda because chemical contamination was viewed as the problem. Water resource leadership was not familiar with, nor did it understand, the ecological dynamics that are important in influencing the effects of toxic compounds or other chemical contaminants. Further, they did not appreciate that degradation of water resources may be caused by factors other than chemical contamination. Ecologists and biologists must share the blame for inadequate incorporation of biological insights into water resource management. Most ecologists and biologists were either unable or unwilling to translate the foundations of their ecological knowledge into useable methodologies to evaluate the quality of water resources. The lack of a defensible conceptual definition of biological integrity also limited progress and use of biological monitoring. The phrase biological integrity was used in the Clean Water Act (PL 92-500), but development of a conceptual foundation was not vigorously pursued. The lack of standardized field methods to sample the biological community, to analyze the results of sampling, and the lack of procedures to synthesize that information into assessments of the conditions of a water resource also limited the utility and, thus, use of biological monitoring. Finally, misconceptions about the costs of biological monitoring perpetuated the idea that biological monitoring was too expensive.

The development of a broader perspective to ambient biological monitoring is critical to protection of water resources. Two concepts are important in the development of this broader perspective:

> **biological integrity** - "the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition and functional organization comparable to that of natural habitat in the region" (Frey 1975, Karr and Dudley 1981).

ecological health - "... a biological system...can be considered healthy when its inherent potential is realized, its condition is stable, its capacity for self repair when perturbed is preserved, and minimal external support for management is needed " (Karr et al. 1986).

Evidence that biological integrity and ecological health are seriously threatened is widespread. Forty percent of the molluscs of the Ohio River drainage were listed as rare, endangered, or extinct by 1970 (Stansbery 1970). Two-thirds of the fishes of the Illinois River and 43% of Maumee River fish species have declined in abundance substantially or have disappeared in the last century (Karr et al. 1985). For the fishes of California, sixty-four per cent are in a list that ranges from extinct (6%) to declining populations (22% - Moyle and Williams 1989); only 36% of species have stable populations. In North America, 364 fishes warrant protection because of their rarity (Williams et al. 1989). Despite massive expenditures to improve water quality, none of 251 fishes listed as rare in 1979 could be removed from the list in 1989 because of successful recovery efforts (Williams et al. 1989).

As these examples demonstrate, water resources are still not adequately protected. Inadequately treated problems include toxics, non-point sources, habitat destruction, and altered stream flows. The limited use of biological factors in evaluating the quality of water resources perpetuates these problems and results in continuing declines in the health, the biological integrity, of water resource systems.

The Constituents of Biological Integrity

Weaknesses of most past approaches of biological monitoring include 1) a narrow conception of the factors responsible for degradation and 2) a limited perspective on the components of biological integrity. Over the past decade, my colleagues and I have identified five primary classes of variables that humans impact that result in the degradation of water resources:

1. Water quality - temperature, turbidity, dissolved oxygen, organic and inorganic chemicals, heavy metals, toxic substances, etc.

2. Habitat structure - substrate type, water depth and current velocity, spatial and temporal complexity of physical habitat.

3. Flow regime - water volume, temporal distribution of flows.

4. Energy source - type, amount, and particle size of organic material entering stream, seasonal pattern of energy availability.

5. Biotic interactions - competition, predation, disease, parasitism.

Karr et al. (1986, see also Karr 1991) provide a more detailed analysis of these factors and how human actions impact the quality of water resources. Among the five, water quality has been the primary subject of efforts by USEPA and equivalent state agencies. The U. S. Fish and Wildlife Service and state fish and game agencies have treated physical habitat degradation. In recent years, those same agencies evaluated altered flow regimes with the instream-flow methodology. Few have dealt with alteration of energy sources that drive stream biology, and most impacts on biotic interactions have come from efforts to introduce exotics and/or through harvesting of top predators. Overall, the determinants of water resource quality from a biological perspective are complex, and the simplistic EPA approach of making water cleaner is inadequate. We must evaluate all water resource degradation to identify the factors responsible for degradation and then treat the problem in the most cost-effective and efficient manner. Ambient biological monitoring offers unique opportunities to detect, analyze, and plan treatment of degraded resources.

Table 1. Components of biological integrity.

Elements	
	Genes within Populations
	Populations within Species
	Species within Communities/Ecosystems
	C/E within Landscapes
	Landscapes within Biosphere
Process	
	Nutrient Cycling
	Photosynthesis
	Water cycling
	Evolution/Speciation
	Competition/Predation
	Mutualisms

The components of biological integrity are also narrowly conceived by most individuals and agencies charged with protecting water resources (Karr 1990, in press). Two major aspects of biological systems - elements and processes - must be protected (Table 1). The most commonly cited aspects on the elements side are the species of plants and animals in aquatic communities. Additional critical components of the elements include the genetic diversity within those species and the assemblages (communities, ecosystems, and landscapes) upon which those species depend. At the level of processes, a myriad of interactions ranging from energy flow and nutrient dynamics to evolution and speciation are critical to the maintenance of biotic integrity. Given sufficient technology, we could maintain species in zoos and genetic diversity in gene banks but in the absence of complex species assemblages and the processes that keep them in existence, we are not protecting biotic integrity. The advantages of this approach to protecting the quality of water resources are diverse (Table 2).

Table 2. Advantages of ambient biological monitoring.

1. Broadly based ecologically
2. Provides biologically meaningful evaluation
3. Flexible for special needs
4. Sensitive to a broad range of degradation
5. Integrates cumulative impacts from point source, non-point source, flow alteration, and other diverse impacts of human society
6. Integrates and evaluates the full range of classes of impacts (water quality, habitat structure, etc.) on biotic systems
7. Direct evaluation of resource condition
8. Easy to relate to general public
9. Overcomes many weaknesses of individual parameter by parameter approaches
10. Can assess incremental degrees and types of degradation, not just above or below some threshold
11. Can be used to assess resource trends in space or time

Assessing Biotic Integrity

Critical components of a comprehensive approach to the protection of biotic integrity include evaluation of ecological attributes from the individual to the assemblage level. Further, an evaluation must be made with respect to the expectation for a relatively undisturbed natural habitat for that region, "regional reference site(s)." Within this framework, an efficient, accurate assessment of the status of the water resource is possible using biological monitoring. Further, an assessment is likely to detect degradation, regardless of the factor responsible for that degradation. Biological monitoring is at a threshold in the ways that it can be used and in the potential for development of methodologies and indexes that can provide useful answers to water resource problems. One of the most important contributions of the recent growth in interest in biological monitoring has been recognition of the need to set standards as a function of local and regional expectations. Indeed, that should have been done for chemical and physical criteria as well. For example, total phosphorus standards should vary regionally and according to primary use among Minnesota lakes with values ranging from less than 15 to 90 ug/l (Hieskary et al. 1987).

Many examples of use of ambient biological monitoring have been documented in the past decade (Karr et al. 1986, Ohio EPA 1988, Steedman 1988, Simon et al. 1988, Davis and Simon 1989, Davis 1990). Ohio EPA has been the most innovative and comprehensive in their development and use of biological monitoring (Ohio EPA 1988, 1990, Rankin et al. 1990, Ohio EPA 1991, Thoma 1991) but many other states are rapidly developing sophisticated approaches as well (e.g. Michigan, Wisconsin, Nebraska, Illinois, etc. - these proceedings). For example, in the Scioto River near Columbus, Ohio, a complex of water resource problems representative of many areas in the U. S. can be seen. Monitoring of the biota of the river over the last decade has shown substantial improvement in biological integrity in association with improvements in wastewater treatment plants (Fig. 1). However, because of the widespread degradation due to untreated factors (habitat degradation, non-point source pollution, input from combined sewer overflow), the biotic communities of the Scioto River adjacent to Columbus remain well below what might be expected in that region.

Successful efforts to protect water resources using biological monitoring have incorporated the following characteristics of biological systems: 1) their dynamics at a variety of relevant spatial and temporal scales and 2) appropriate metrics at three levels: a) ecosystem (productivity, decomposition, nutrient cycling, atmosphere/biosphere/geosphere interactions); b) population/community (community structure, species richness, species interactions, functional groupings, population structure); and c) health of individual organisms.

We must be innovative in incorporating these into water resource evaluations. Some can be incorporated directly and easily (e.g., population size, species richness) while others are more difficult or expensive to measure directly (Karr 1991). For example, the total productivity of an ecosystem is very difficult to measure. We should seek ways to measure productivity, or a surrogate of productivity that is indirect but reliable. Alternatively, we might develop more cost effective ways to measure productivity by improvements in technology.

Since early in this century, beginning with the work of Kolkwitz and Marsson (1907) and Forbes and Richardson (1928), Forbes (1919) and continuing to the present, biologists have noted a number of biological patterns associated with increased human influence within a watershed: the number of species declines, a small group of intolerant species disappear quickly, trophic specialists decline while trophic generalists increase. Effective biological monitoring can structure these general observations to define hypotheses (e.g, Table 3 for hypotheses implicit in IBI) and predictions about expected pattern in aquatic biota under varying levels of human influence. If after evaluating each hypothesis, we find general broad correlations, relationships between human disturbances and these attributes of the community, these then become assumptions. That is, we assume that, on average, these relationships accurately reflect the influence of human activities on natural communities. Thus, we have a fairly robust inference about the extent of degradation in biological integrity at a site.

Indexes of biotic integrity, such as IBI, are, thus, a quantitative expression of a number of known relationships between human disturbance and the characteristics of the resident biota. These indexes have four important properties. First, the accumulated information

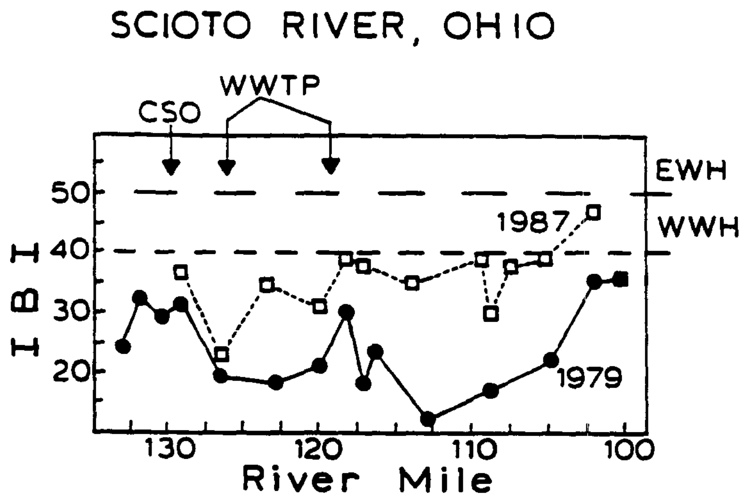

Figure 1. Longitudinal trend in IBI for the Scioto River, Ohio in and downstream from Columbus Ohio, 1979 and 1987. CSO = Combined sewer overflow; WWTP = Wastewater treatment plant inflow; WWH = Warmwater habitat; EWH = Excellent warmwater habitat. Stream flow is from left to right. (From Yoder 1989).

Table 3. Hypotheses/assumptions about biological patterns associated with increasing human effects on stream biota (modified from Fausch et al., 1990).

1. Number of native species and those of specific taxa on habitat guilds declines
2. Number of intolerant species declines
3. Proportion of individuals that are members of tolerant species increases
4. Proportion of trophic specialists such as insectivores or top carnivores declines
5. Proportion of trophic generalists, especially omnivores, increases
6. Fish abundance generally declines
7. Proportion of individuals in reproductive guilds requiring silt-free coarse spawning substrate declines
8. Incidence of hybrids increases
9. Incidence of externally evident disease, parasites, and morphological anomalies increases
10. Proportion of individuals that are members of introduced species increases

provides greater resolving power for the overall index than for each metric. The many components of biotic integrity (elements and processes) and the complexity of ecological systems, limits the likelihood that any single metric can be used to assess all forms of degradation and be sensitive across the full range of degradation. The magnitude of variation involved in assessments using only a single metric derives from natural variation and sampling error. As a result, no single metric is absolutely reliable in its ability to predict (with narrow precision) the state of biological integrity. A suite of metrics is better to insure more or less independent evaluations of site quality. Although site status is only generally known based on each individual metric (Fig. 2 upper), the addition of other metrics improves the resolving power of the approach; strong inferences can be made when multiple metrics are used (Fig. 2 lower). That is, each metric has a level of precision below 100% (perhaps 70-80%), but combining many metrics with that level of accuracy and across a variety of attributes of the biota, narrows the range and improves the precision of the estimate of biological integrity at a site.

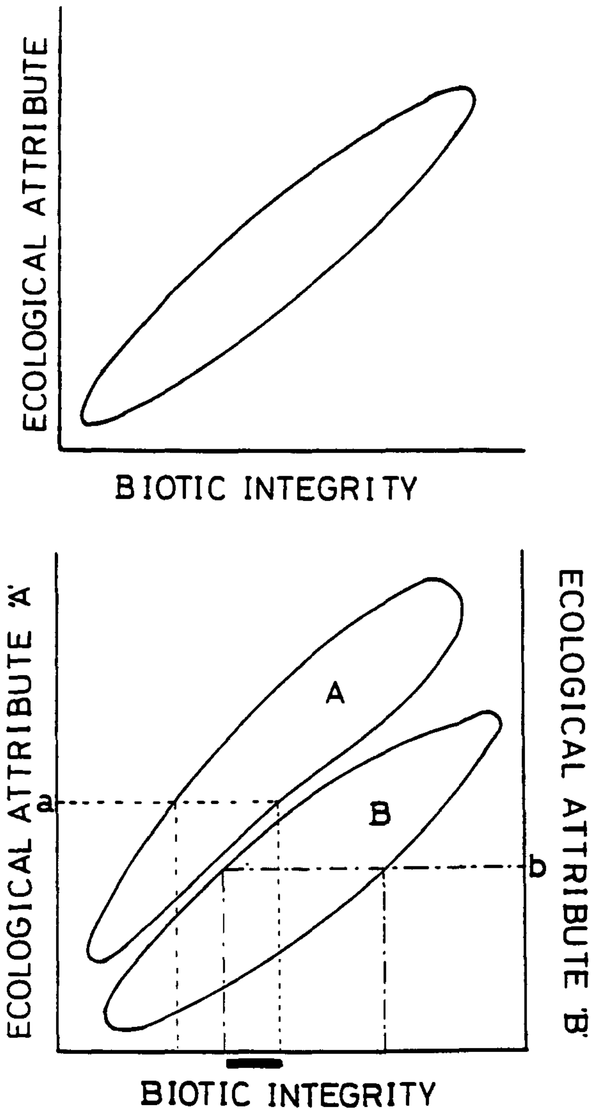

Figure 2. Conceptual depiction of the relationship between a single ecological attribute (IBI metric-upper panel) and two ecological attributes (lower panel)and biotic integrity. Note that simultaneous use of two metrics narrows the identified biotic integrity level (dark horizontal bar) relative to use of a single attribute (metric) (from Karr in press).

Second, the sensitivity of each metric varies with position along a gradient from undisturbed sites (high biotic integrity) to disturbed sites (low biotic integrity). Metrics such as total number of species seem to decline monotonically across the full range of degradation (Fig. 3). Intolerant species disappear before degradation has proceeded very far while the number of anomalies changes little until the area is severely degraded. In contrast, proportion of carnivores declines slowly with mild human impacts and declines rapidly at intermediate stages (Fig. 3). Carnivores disappear from a range of heavily degraded sites. Redundancies exist among metrics and relative sensitivities vary across the range of biotic integrity.

Third, biological monitoring as used in IBI acknowledges and accounts for natural geographic variation. Historically, that has not been done with physical/chemical parameters despite the reality of natural variation in those attributes. Accurate assessment of biological integrity requires fine tuning as one moves regionally. In fact, that is a major problem with historical chemical monitoring where expectations were not adjusted regionally. For many chemical attributes, failure to account for regional natural variation in contaminant levels is a serious error.

Fourth, evaluations attempted using the multimetric approach yield either narrative or numerical results (or both) to satisfy regulatory requirements.

Developing an Invertebrate IBI

The use of invertebrates to assess specific anthropogenic impacts on stream biota has a long history (e.g., Chutter 1972, Hilsenhoff 1977, Winner et al. 1980, Rosenberg et al. 1986). However, comprehensive attempts to evaluate stream biotic integrity using invertebrates have been attempted only recently (e.g., Hilsenhoff 1982, 1987, 1988, Ohio EPA 1988, Lenat 1988, Lang et al. 1989, Plafkin et al. 1989). Early in the 1980's Ohio EPA began to adopt the concepts involved in IBI for evaluations using benthic invertebrate communities. They developed a ten metric index (Invertebrate Community Index, ICI) that parallels the original IBI (Ohio EPA 1988). We applaud these approaches but feel that none combines metrics that evaluate both elements and processes of biotic integrity. Further, multimetric indexes have not involved evaluation of the robustness of the individual metrics. Thus, we outline our ongoing effort to develop a comprehensive invertebrate index for streams of the Tennessee Valley. We discuss 1) formulation of invertebrate metrics and 2) evaluation of the ability of individual metrics to determine biological integrity.

Our first task was to develop, a priori, metrics that characterize important elements and processes occurring in streams. We also intend to represent the full biological hierarchy from individual to community levels. Taxa richness (e.g., number of Plecoptera taxa) and community composition (e.g., proportion of tolerant organisms) metrics are the most widely used and highly developed metrics in existing invertebrate indexes (e.g., Ohio EPA 1988, Plafkin et al. 1989). Metrics designed to measure ecological processes and community function (e.g., proportion of grazers as a "surrogate" measure of periphyton production) have not been widely used nor fully investigated. Our goal is to investigate both types of metrics using stream benthic invertebrate databases provided by the Tennessee Valley Authority.

To develop metrics associated with ecological processes and community function we placed organisms (usually genera) into biotic categories describing trophic status, functional group classification, feeding mechanism, and habit (Table 4). Inclusion of trophic category metrics is usually not done, because most benthic biologists prefer categorization of stream organisms by functional-feeding group (Cummins 1973, Merritt and Cummins 1984). Our approach allows us to investigate how the dominance of grazer-scraper or omnivore guilds, for example, changes across sites. Inclusion of the habit biotic category allows us to examine patterns of loss of taxa in particular habitats. For example, sprawlers are usually associated with mineral substrate, while climbers are often associated with submerged or emergent vegetation.

Using the philosophy of the IBI, we developed 28 metrics in three distinct categories; taxa richness and community composition, trophic and functional group composition, and abundance (Table 5). Taxa richness and community composition metrics are often used in biological monitoring (Ohio EPA 1988, Plafkin et al. 1989). These include metrics like total taxa richness (Metric 1, Table 5), richness of intolerant insect orders (5, 6, 8), and the percent contribution of individuals in tolerant groups (15, 16) to the total community. As in IBI, taxa richness of intolerant groups often reflects levels of degradation (e.g., Lenat 1988).

Several taxa richness and community composition metrics relate to molluscs, an especially rich and sensitive group in the Tennessee Valley. Three involve native snail and long-lived mussel taxa (2, 3, 4). The mussel fauna of the Valley was thought to be the most diverse in the country and is declining (Isom 1969, Ahlstedt 1983, Starnes and Bogan 1988). Consequently, mussel taxa richness should reflect levels of biotic integrity. We also included two metrics that measure the proportion of Corbicula fluminea in the community (13, 14). The exotic Corbicula invaded the Tennessee River and its tributaries. Although there is some question as to the tolerance level of Corbicula, it certainly appears to be an "opportunistic" species (Prezant and Chalermwat 1984), and we hypothesize that it might be able to invade communities where other groups, especially native mussels, have declined.

Some metrics in the taxa richness and community composition category involve the number of taxa in specific habit categories. Skaters, planktonic organisms, divers, and swimmers spend most of their time in the water-column (9). Sediment-surface taxa (10)

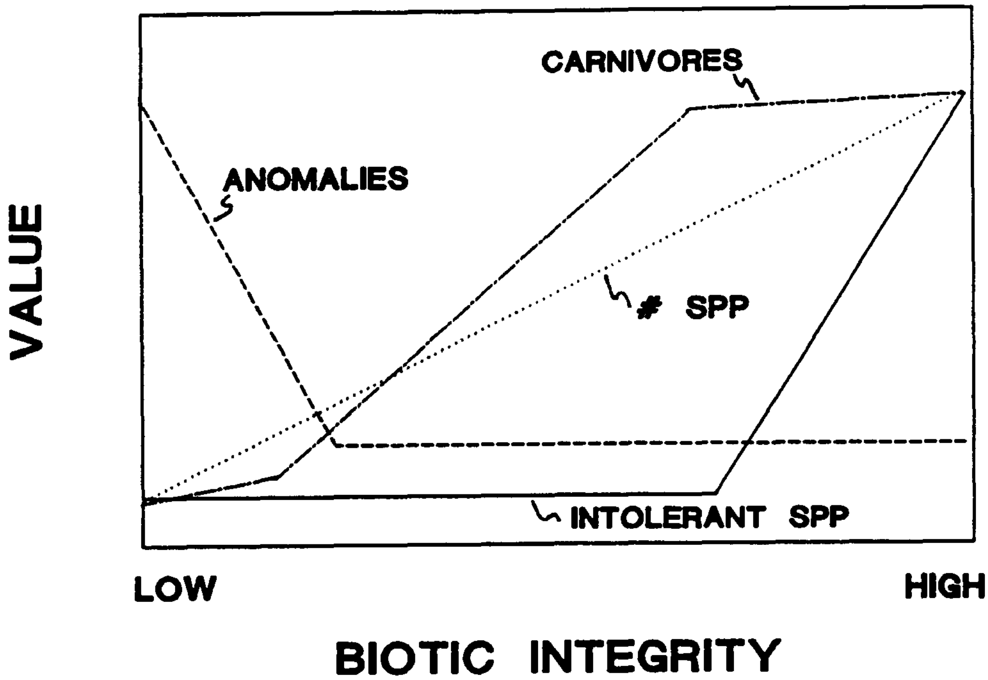

Figure 3. Conceptual depiction of the range of sensitivity of four IBI metrics across the gradient from low to high biotic integrity.

include clingers and sprawlers, whose lifestyles place them primarily on benthic substrates. Climbing taxa (11) spend much of their time on submerged or emergent vegetation or debris, while burrowers (12) live within the substrate. We hypothesize that declining numbers of taxa in the sediment-surface, water-column, and climbing guilds should reflect degradation of specific habitat types, while increasing taxa richness in the burrowing guild should indicate degradation. For instance, declining taxa richness in the group comprising the sediment-surface taxa should reflect degradation of the mineral substrate perhaps due to sedimentation.

Our second set of metrics includes two broad groups, trophic and functional-feeding group categories (Table 5). Although trophic categories are rarely used in benthic invertebrate studies, we include both trophic and functional group categories to increase the possibility of detecting change in the resource base of the community. Using trophic status, we hypothesize that we can determine how the detritus food base of the community, for example, changes by monitoring organisms in the detritus-feeding guild (18). We also can determine how collector-filterers (23) or grazer-scrapers (24; and their underlying resource base) change across sites. Finally, we included a metric, percent of individuals in the sample that are strictly predatory (26, consume only other animals in final developmental stages), to monitor the top trophic (and functional-feeding) levels in the community.

The third group, the abundance metrics, includes the total numbers of individuals (27)

Table 4. Biotic categories used in classification of invertebrates.

1. TROPHIC CATEGORY
   A. Herbivore
   B. Carnivore
   C. Detritivore
   D. Scavenger (Detritivore,Herbivore)
   E. Omnivore (Detritivore, Herbivore, Carnivore)
2. FUNCTIONAL GROUP*
   A. Shredder
   B. Collector
   C. Grazer
   D. Parasite
   E. Predator
3. FEEDING MECHANISM*
   A. Chewers
   B. Filterers
   C. Gatherers
   D. Scrapers
   E. Engulfers
   F. Piercers
4. HABIT*
   A. Skaters
   B. Planktonic
   C. Divers
   D. Swimmers
   E. Clingers
   F. Sprawlers
   G. Climbers
   H. Burrowers
   I. Attachers

* From Merritt and Cummins 1984

and the extent to which a single taxon or a few taxa dominate the community (28). These metrics have been used in other explorations of community assessment; however, their properties as individual metrics have been inadequately investigated. We explored a number of cutoff points (1-5 species) for the dominance metric, and at present are using the percent of individuals in the two most abundant taxa.

Our second objective is to determine how well each individual metric distinguishes biological integrity. To begin this process we examined individual metrics to determine if they vary predictably across rivers and streams monitored by the Tennessee Valley Authority. We use data from the Fixed Water Quality Monitoring Sites, an assessment program begun in 1986. Currently, there are sites on 12 tributaries of the Tennessee River; however, initially invertebrate data were collected for only six sites -- Clinch, Powell, Sequatchie, Elk, and Duck Rivers and Bear Creek. TVA used the fish IBI at four of these sites (Clinch, Powell, Sequatchie, Bear) and we have used IBI scores to make preliminary rankings (Clinch = Powell > Sequatchie > Bear) (Saylor et al. 1988, Saylor and Ahlstedt 1990). Our intuition is that the Elk and Duck Rivers probably fall between Sequatchie and Powell. We examined patterns of metrics across the sites (rivers). If a metric exhibits no discernable pattern or extreme variability across rivers (the four with "known" impact) then it is thought not to be able to distinguish sites. Using this approach, we deleted 10 metrics from further consideration (Table 5).

Although we looked at patterns for all our metrics, we discuss only a few. Total number of invertebrate taxa increases almost linearly from most degraded to least degraded sites (Fig. 4A). Surface-taxa richness shows the same pattern, probably indicating that the lost taxa occupy hard surfaces (Fig. 4B). The proportion of Corbicula (Fig. 5A) and proportion of collector-filterers (Fig. 5B) show reverse relationships, increasing from least degraded to most degraded sites. Corbicula increases dramatically only in the two most degraded systems, while collector-filterers increase almost linearly. These two metrics seem to provide differing areas of sensitivity to degradation, much as percent omnivores and percent anomalies do in the IBI (Karr, in press).

After we determine the relationships between our metrics and the levels of degradation at the

Table 5. Metrics proposed and being tested for inclusion in an invertebrate IBI.

POTENTIAL METRICS	HYPOTHESIZED TREND WITH DEGRADATION
I. Taxa Richness and Community Composition	
1. Total taxa richness	decline
2. Native snail and mussel taxa*	decline
3. Unionid taxa*	decline
4. Intolerant snail & mussel taxa	decline
5. Ephemeropteran taxa	decline
6. Trichopteran taxa	decline
7. Dipteran taxa*	increase
8. Plecopteran taxa	decline
9. Water-column taxa*	decline
10. Sediment-surface taxa	decline
11. Climbing taxa*	decline
12. Burrowing taxa*	increase
13. % individuals as _Corbicula_	increase
14. % bivalves as _Corbicula_*	increase
15. % individuals as oligochaetes	increase
16. % individuals as chironomids	increase
II. Trophic and Functional-Feeding Group	
17. % individuals as omnivores and scavengers	increase
18. % individuals as detritivores	increase
19. % individuals as herbivores*	decline??
20. % individuals as carnivores*	decline
21. % individuals as shredders	???
22. % individuals as collector-gatherers	increase
23. % individuals as collector-filterers	increase
24. % individuals as grazer-scrapers	decline??
25. % individuals as parasites*	increase
26. % individuals as strict predators	decline
III. Abundance	
27. Abundance	decline
28. % individuals in the two most abundant taxa	increase

*Metrics that have been dropped from further analyses.

Fixed Station Sites, we will test our metrics in one of two ways. First, water quality measurements have been taken on the Fixed Station Sites during the period of the invertebrate studies (Parr 1991). We will combine specific water quality data with information on land use practices occurring in each of the drainages and correlate these factors with our invertebrate metrics. This will strengthen our inferences concerning the ability of the metrics to distinguish degraded conditions. Second, we will independently evaluate individual metrics by applying them to data collected on other streams with known impacts.

Concurrently, we are exploring a number of properties associated with benthic sampling and level of taxonomic identification. For instance, at the Fixed Station Sites replicate samples are collected using different methodologies at each site; typically, several Hess samples (taken in pools and runs, the data presented previously), several Surber samples (taken in riffles), and qualitative samples (taken across all habitats) are available. We are evaluating the extent to which any one sampling method provides reliable evaluations of the biotic integrity. Our early conclusions suggest that Hess samples better differentiate sites than either Surber or qualitative samples. We also are interested in whether metric behaviors are consistent across stream size (headwater streams to large rivers), or if we have to score metrics differently as a function of stream size.

Our goal with respect to the development of an invertebrate community index is to investigate and test a number of hypotheses regarding individual metrics (as discussed above), score those metrics that seem to successfully reflect biological conditions, and develop an index from that based on a number from as few as 8 to as many as 14 useful metrics. After putting together an index that we feel is relatively reliable, we will seek data from areas of known impact to determine the ability of our invertebrate index to evaluate conditions resulting from varying human impacts.

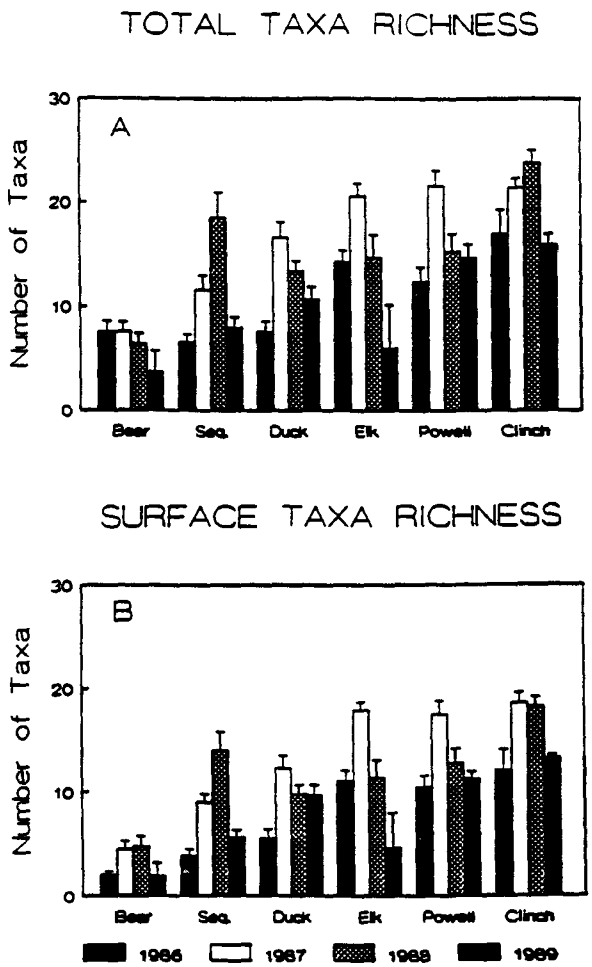

Figure 4. Mean and standard error bars for two invertebrate IBI metrics for six Tennessee River tributaries during four consecutive years (1986-1989). Note that the rivers are plotted from low quality (Bear Creek) to highest quality (Clinch River). A. Total Taxa Richness. B. Surface Taxa Richness. Both decrease with increasing human influence.

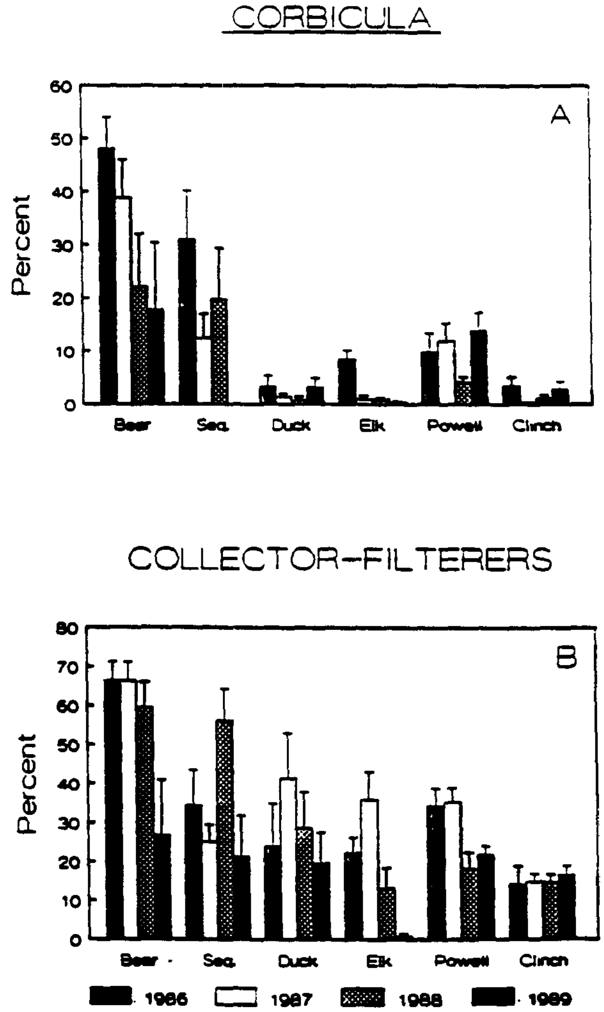

Figure 5. Mean and standard error bars for two invertebrate IBI metrics for six Tennessee River tributaries during four consecutive years (1986-1989). Note that Corbicula (A) increase, especially in the most degraded sites, while the collector-filterers (B) increase gradually along the gradient toward Bear Creek.

Summary

In closing, we want to emphasize three points. Historically, water chemistry and fish tissue sampling and toxicity testing have been the principal approaches used in the evaluation of water resources. Unfortunately, a number of forms of degradation imposed on aquatic resource systems by human society are not fundamentally chemical. As long as we depend solely on chemical analyses we are not likely to detect and treat the degradation caused by those factors. We view the array of water quality or water resource sampling programs as a tripod (some have inappropriately compared it to a three-legged stool). With a tripod, the relative length of the legs (monitoring approaches) can be altered to suit the landscape of local water resource problems.

Second, a number of major transitions during the past decade have stimulated rapid changes in societal approaches to water resource protection. Assessment of water resources today is broader than the assessment of the chemical quality of the water, a development that was precipitated by the widespread recognition that the quality of water resources continues to decline. The broader societal goals for the protection of water resources requires the use of the broader range of disciplines to inform water resource decisions. Furthermore, decisions based on a narrow disciplinary approach often must be questioned when placed in the larger disciplinary context. As a result, the dogma of many disciplines is under more careful scrutiny. The dogma of the past cannot be accepted uncritically if we are to properly protect water resources. The planning perspective in protection of water resources is expanding in both space and time. Planning should be done for periods of decades, not for next year. Planning can and should be done over entire watersheds, not over short river reaches. The emergence of a landscape ecology view of water and other natural resources is instrumental in making society aware of the need to plan in a wider geographic context.

The role of biology will continue to expand. The most important challenge is for biologists and ecologists to be more effective at translating the knowledge about biological systems into the tools that can be used by society in protecting the biological and non-biological components of those water resources (Karr 1991).

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Effects of Extremely Low Frequency (ELF) Electromagnetic Fields on the Diatom Community of the Ford River, Michigan

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Abstract
The effects of 76 Hz extremely low frequency (ELF) electromagnetic radiation produced by the U.S. Navy's ELF andtenna on riverine diatom communities have been intensively studied since 1983 at two sites in the fourth order Ford River in northern Michigan. Data from a control site were compared to data from an experimental site under the antenna. Background data on the diatom community were collected from June 1983 through April 1986; transitional data were collected on ELF effects during a variable power testing period from May 1986 until October, 1989; and data from a fully operational system were collected from October 1989 through September 1990. Diatoms were monitored at a site near the antenna and at a control site where they received 5.5 times less exposure to longitudinal electric fields and 330 times less exposure to magnetic flux than they received at the antenna site. Paired t-test analyses between sites showed that chlorophyll a and organic matter accrual rates were different between the sites in 1990, the first year of fully operational ELF exposure, whereas they were not different when data from 1983 through 1990 were compared. Before and after, control and impact analyses (BACI) and randomized intervention analyses (RIA) suggested that the relationship between sites had changed for chlorophyll a biomass, diatom cell density, total diatom biovolume, and species diversity and evenness after ELF testing began as compared to data collected prior to such testing indicating a possible ELF effect. Chlorophyll *a* gave the strongest evidence for an ELF effect of any of the 9 community based algal parameters examinded. Analysis of covariance (ANCOVA) with longitudinal electric field included as a covariant suggested that this type of electric field was not responsible for the changes observed through 1989 (1990 exposure data were not available at the time of this analysis). Ongoing analyses are being conducted to determine if observed changes are related to the greater exposures to longitudinal fields experienced by the algae in 1990 or to changes in magnetic flux or to weather related variables such as water temperature. Stepwise regressions indicated that water temperature was the most important of the weather related variables in explaining variance in diatom community data. We also examined ELF effects on 3 of the most common species of diatoms present in the summer and 4 of the most common species present in the winter. The abundance of none of these species changed between the before and after data sets providing no evidence for ELF effects at the individual population level.

Key Words: Diatoms, ELF electromagnetic radiation, rivers, diversity, density, species composition, chlorophyll *a*.

Introduction

In 1982, a study to determine the effects of extremely low frequency (ELF) electromagnetic radiation on diatom (Division Bacillariophyta: Class Bacillariophyceae) (classification after Patrick and Reimer 1966) communities in the Ford River, Michigan was initiated. The ELF electromagnetic radiation was to be produced by a 56 mile long antenna (one 28 mile NS leg and two 14 mile long EW legs that crossed the NS leg) to be built by the U.S. Navy for communication with deeply submerged submarines. The initial subcontract with IIT Research Institute (funded as part of their contract with the U.S. Navy) called for three years of before and three years of after data for determining ELF effects at paired sites with one located under the site where the antenna was to be built (experimental site) and the other far enough away so that ELF electromagnetic radiation would be at least an order of magnitude less than the radiation received by organisms under the antenna (control or reference site). After 10 months of selecting preliminary sites and collecting data on the biota, chemistry, and physical characteristics of the sites, and on the ambient ELF fields (collected by IITRI) at the sites to make sure that the final sites selected were as comparable as possible, background data collection began at the two selected sites. Background or before data collection continued from June, 1983 to May, 1986; transitional data were collected from May, 1986 to October, 1989; and fully operational data have been collected since October, 1989. The transitional data were collected during a period of intermittent antenna testing that consisted of only 35 days of testing for the NS leg that crossed the aquatic sites in 1986 (75 days for all legs) at 4-6 amps; limited exposure at 15 amps in 1987; limited exposure at 75 amps in 1988; intermittent exposure at 150 amps in most of 1989 before going operational in October, 1989 at 150 amps and 76 Hz. This paper includes data on the effects of ELF fields on the benthic algal community through early September, 1990. The objective of this paper is to summarize our results on ELF effects on the diatom community in the Ford River. To our knowledge, no other studies of ELF effects on natural, in situ algal communities have been conducted.

Methods and Materials

The two sites chosen for the study of ELF effects were in riffle zones of the fourth order Ford River in northern Dickinson County in Michigan's upper peninsula. The Ford River is part of the Lake Michigan drainage. The Ford River catchment lies between the Escanaba and Menominee River catchments, and the river empties into upper Green Bay just south of Escanaba, Michigan. At the two study sites, the stream varies from 9-12 m wide during low flow, and depth varies from less than 0.3 m during low flow to more than 2 m during floods. Chemical and physical data for the river have been extensively monitored, and preliminary data are available from Burton et al. (1991 a, b) and Oemke and Burton (1986). The river can be characterized as a hardwater, high alkalinity (about 120 - 180 mg $CaCO_3$/L), low nutrient (less than 10 μg soluble reactive P/L and less than 60 μg inorganic N/L), brown water trout stream with a bottom that varies from sand and silt in the pools to gravel to cobble in the riffles. Most of the land in the catchment is in successional forest with *Populus tremuloides* dominating the upland areas and *P. balsamifera* and/or *Alnus rugosa* dominating riparian zones. Extensive *Alnus rugosa* and some *Thuja occidentalis* swamps in the drainage basin along with forest humus and litter are likely sources of the organic matter that imparts the brown color to the river. The pH in the river varies between 7.5 and 8.2 most of the time, and dissolved oxygen remains near saturation throughout the year.

The experimental site was located within 50 m of the point where the ELF antenna crossed the Ford River (T43N:R29W:Sec. 14), while the control or reference site was about 8 km downstream (T43N:R28W:Sec. 21). These two sites were closely matched in exposure to ambient ELF electromagnetic levels prior to operation of the antenna. At full power in 1989, 5.5->10 times more ELF radiation was received by the biota at the experimental site than at the control site (e.g. the experimental site received 5.5 times more exposure to longitudinal electric fields; 540 times more exposure to transverse electric fields, and 330 times more exposure to magnetic flux than did the control site at 76 Hz according to measurements made by IITRI in 1989). At full power of 150 amps, biota at the experimental site received ELF exposure at a rate of 61 mv/m^2. Placement of samplers in 1990 was adjusted to insure that biota at the experimental site received at least 10 times more exposure for all components of ELF electromagnetic radiation measured at 76 Hz during full power operation than did the biota at the control sites.

Standard (25X76 mm), glass microscope slides were held vertically into the current in plexiglass diatom racks using a modification of the diatometer illustrated in Patrick and Reimer (1966) except that no deflector shield was placed in front of the slide holder, and the racks were fastened directly to standard construction style clay bricks that were placed directly on the stream bottom. The slide racks were placed in riffle areas, and current velocity through the slide holders was carefully matched using a current meter. Positions of the racks were checked and adjusted weekly to insure that current velocity differed little between sites. Care was also taken to place the racks in areas of the stream that were open to sunlight during most of the day. Some early morning and late afternoon shading by riparian vegetation and/or banks of the streams was unavoidable but shading differences between sites was minimal.

Colonization times of 28 days for the mature community and 14 days for chlorophyll a accrual rates during the ice-free seasons were selected based on the findings of Oemke and Burton (1986) for this river during start up studies in 1982. Winter sampling occurred at 28 day intervals from 1983-84 through 1986-87, at 56-60 day intervals in 1987-88, and has remained at 42 day intervals since that time. Processing of diatoms for counting, calculations, and other procedures followed the methods discussed by Oemke and Burton (1986) as did procedures for determining chlorophyll a and organic matter accrual rates and biomass standing crops. We use accrual rates as crude estimates of primary productivity as recommended in Standard Methods (A.P.H.A. 1985). We prefer to refer to these measures as accrual rates, since productivity is underestimated or affected by an unknown amount due to sloughing losses, leakage of cellular contents, death and senescence of cells, etc.

All statistical procedures except for before and after, control and impact (BACI) analyses and random intervention analyses (RIA) were performed using StatView 512+ (copyright 1986 by Abacus Concepts, Inc.), a software program for the Apple Macintosh plus from Brainpower, Inc. of Calabasas, CA. Procedures for BACI and RIA analyses are summarized in Eggert et al. (1991), a companion paper in this volume. Differences between treatments were accepted as significant at the $p>0.05$ level unless otherwise specified.

Results

There were no significant differences for any of the nine community level benthic algal parameters monitored at the antenna and control sites from 1983 through 1990 according to paired t-tests analyses (Table 1). Values at the control site and the antenna site were significantly correlated for each of these parameters (Table 1). Minimum detectable differences were in the 25-30 % range for most parameters with Shannon-Wiener diversity (H') and evenness (J') being the most sensitive indicators for detection of differences between the sites (5-7 % - Table 1). Diatom cell density and total diatom biovolume (calculated from individual cell volumes and density) were the least sensitive parameters for detection of differences between the sites (48 -53 % minimum detectable differences - Table 1).

Table 1. Paired t-test results (DF = 83 - 85), correlation coefficients*, and percent minimum detectable differences ($p < 0.05$) between the antenna and control sites for benthic algal parameters for 1983 - 1990.

Parameter	t-test	correlation coefficient	minimum detectable difference
Chlorophyll a Biomass	NS	0.85	29 %
Chlorophyll a Accrual	NS	0.83	32 %
Organic Matter Biomass	NS	0.70	23 %
Organic Matter Accrual	NS	0.61	27 %
Diatom Cell Density	NS	0.90	48 %
Diatom Cell Volume	NS	0.96	25 %
Total Diatom Biovolume	NS	0.70	53 %
Species Diversity	NS	0.70	7 %
Species Evenness	NS	0.79	5 %

* all are significant at the $p < 0.01$ level.

We also compared the antennna to the control site for each year of the study using the paired t-tests. Few differences were detected on a year by year basis. The first fully operational year for ELF exposure was 1990. Comparisons between the antenna and control sites for 1990 using paired t-test analyses suggested that two of the parameters listed in Table 1, chlorophyll *a* biomass and daily accrual rates of organic matter, had been affected by ELF exposure. Chlorophyll *a* biomass was slightly but significantly higher under the antenna than it was at the control site. However, chlorophyll *a* daily accrual rates were not significantly different between the sites in 1990. Conversely, organic matter biomass was not signifcantly diffferent between the two sites in 1990 even though organic matter daily accrual rates were. Since algal and bacterial biomass should dominate production of organic matter on slides oriented vertically to the current (little settling of suspended organic matter should occur in this orientation), it is surprising that results from the two parameters are not more closely correlated. No significant differences for any of the other seven community based parameters listed in Table 1 were detected in 1990. No significant differences had occurred between sites overall (Table 1) or even for 1989, the year of intermittent exposure to 150 amp operation of the antenna. Significant results should occur by chance alone 5 % of the time. With two of the nine community based parameters (22 %) being significantly different between the sites in 1990, differences appear to be real. We feel that it is too early to put much emphasis on these analyses after only a single year of fully operational data and will

Table 2. Summary BACI and RIA statistics. Before statistics are from June 1983 to April 1986. After statistics are from May 1986 to September 1990. N in parentheses for BACI and RIA respectively. NS = $p > 0.05$. N = 83 - 85 overall, 48 - 53 for summer, 33 - 35 for winter samples.

Parameter	BACI	RIA
Chlorophyll *a* Biomass	$p < 0.01$	$p < 0.05$
Summer Data Only	$p = 0.06$	$p < 0.01$
Winter Data Only	NS	NS
Organic Matter Biomass	NS	NS
Summer Data Only	$p < 0.01$	$p < 0.01$
Winter Data Only	NS	NS
Diatom Cell Density	$p < 0.01$	$p = 0.06$
Summer Data Only	$p < 0.01$	$p = 0.18$
Winter Data Only	NS	NS
Diatom Cell Volume	NS	NS
Summer Data Only	NS	NS
Winter Data Only	NS	NS
Total Diatom Biovolume	$p < 0.05$	$p = 0.09$
Summer Data Only	NS	NS
Winter Data Only	NS	NS
Species Diversity	$p < 0.05$	$p < 0.05$
Summer Data Only	NS	NS
Winter Data Only	NS	NS
Species Evenness	$p < 0.01$	$p < 0.01$
Summer Data Only	$p < 0.05$	$p < 0.05$
Winter Data Only	$p < 0.05$	$p < 0.05$

await the 1991 data before drawing firm conclusions.

Studies that use comparisons between a single control and reference site have a potential problem with pseudoreplication (Stewart-Oaten et al. 1986, Carpenter et al. 1989), and there is some question as to whether or not paired t-tests are appropriate ways to analyze the data. The two statistical procedures suggested as alternatives by Stewart-Oaten et al. (1986) and Carpenter et al. (1989) to the more traditional paired t-test approach have been used on our data as an additional way to detect potential differences between the two sites that may be related to ELF electromagnetic fields (Eggert et al. 1991). Since RIA analyses require a minimum number of 40 observations, analyses are only possible at this time using all the transitional and operational data (May 1986 - September 1990). We ultimately plan to stratify results into before data (June 1983 - April 1986), transitional data with the ELF antenna undergoing testing (May 1986 -

September 1989), and operational data (October 1989 - September 1992). Both the RIA and BACI analyses test for differences in the relationship of the data between the two sites before and after a potential impact occurs. The means do not have to be the same to show no significant difference. Suppose, for example, that density had consistently been higher at the control site than at the impact site prior to the onset of the potential environmental stress (e.g ELF exposure in this study). No impact would be indicated by either analysis if this difference remained consistent after onset of the potential stress (e.g. exposure to ELF fields). If, however, the relationship changed such that mean density decreased at the impact site but not at the control site, BACI and RIA analyses would indicate a significant change even though the means might now be comparable. This significant change could signal a potential significant change in density at the impacted site related to ELF exposure (Eggert et al. 1991).

Results of the preliminary BACI analyses for 7 of the 9 community based parameters reported in Table 1 indicated that significant differences have occurred between the before and transitional/operational data for 5 of the 7 parameters examined using the overall data set (line one for each parameter in Table 2). RIA analyses confirmed these differences as being significant for 3 of the 5 parameters. Both RIA and BACI analyses showed that before data were different from transitional/operational data for chlorophyll *a* biomass and species diversity and evenness indicating a potential ELF effect on these parameters (Table 2). BACI analyses showed that before diatom cell density and total diatom biovolume data differed significantly from after data, but RIA analyses failed to confirm these results at the $p > 0.05$ level (differences were significant at the $p > 0.10$ level - Table 2). No differences were detected for any of these 5 parameters using the paired t-tests on the combined data (Table 1).

Winter data are more variable than summer data, are collected at less frequent intervals, and could mask differences in the data sets. Therefore, we stratified the data into data collected from mid-April through October (summer data) and data collected from November to April (winter) to see if we could improve results (Table 2). Of course, smaller sample size results in less power to detect differences. Even so, differences occurred in the organic matter biomass summer before and after data, that were not evident in the overall data set even though significant differences in some of other parameters disappeared (Table 2). Species evenness was the only parameter that showed a consistent difference in the winter as well as in the summer and overall (Table 2).

BACI analyses were used to compare year to year differences, since it is less sensitive to sample size than is RIA analyses. While some of the other parameters showed differences in at least one of the before years compared to at least one of the transistional/operational years, only chlorophyll *a* biomass showed a significant difference between data collected in 1990, the only fully operational year so far, and each of the before ELF years (83, 84, and 85). Since paired t-test analyses had also indicated that differences between the control and antenna site were siginifcant in 1990, chlorophyll *a* offers the best evidence that ELF electromagnetic radiation may have caused a difference in benthic algae between the two sites. Chlorophyll *a* was slightly but significantly greater at the antenna site than at the reference site in 1990. If ELF has caused an effect, it has resulted in slightly increased biomass of chlorophyll *a* at the antenna site.

Since a variety of differences were detected between the before and transitional/operational data, we analyzed the data using analysis of covariance (ANCOVA) with cumulative exposure to ELF longitudinal fields over the 28 day colonization period as the covariant for the 1986 through 1989 after data (Table 3). The 1990 ELF exposure data are not yet available. The only difference between sites detected in

Table 3. Analysis of covariance (ANCOVA) statistics for 1986 - 1990. Before and after t-test comparisons are for before period from June 1983 to April 1986 and May 1986 to September 1990 respectively. NS = $p > 0.05$.

	Between Site t-Tests		
Parameter	Before	After	ANCOVA
Chlorophyll a Biomass	$p < 0.05$	NS	NS
Organic Matter Biomass	NS	NS	NS
Diatom Cell Density	NS	NS	NS
Diatom Cell Volume	$p < 0.05$	NS	NS
Total Diatom Biovolume	NS	NS	NS
Species Diversity	NS	$p < 0.01$	$p < 0.05$
Species Evenness	NS	$p < 0.01$	$p < 0.05$

the after data using between site t-tests were for species diversity and evenness (Table 3). ANCOVA did not change any of these relationships (Table 3) indicating that diffences between sites was not related to exposure to longitudinal electric fields from 1986 through 1989. Therefore, differences detected between sites as summarized in Tables 1 and 2 and above must be related to some factor other than ELF longitudinal electric fields or to the greater differences in longitudinal electric fields in 1990, the first year of fully operational data. Exposure to increased magnetic flux could be the explanation as could between year differential responses to weather related factors between the sites. Stepwise regression analyses strongly implicated water temperature as the most important weather related factor in explaining variance in the benthic algal community. Future analyses will explore water temperature and ELF generated magnetic flux exposure as covariants that may explain differences in the before and after data between sites. As soon as 1990 data on longitudinal electric fields are available to us, we will incorporate these data in our ANCOVA analyses.

Since BACI and RIA anayses had indicated differences in the before and transitional/ operational data in species diversity and evenness, we selected three of the more common species of the diatom flora from the summer period and four from the winter period for further analyses (Table 4). There were no significant differences for any of these species between the before and transitional/operational data (Table 4). Differences in diatom species diversity and evenness must, therefore, be related to differences in rarer species rather than these more common forms.

In summary, data collected to date on response of the benthic algal community to ELF electromagnetic fields suggest that changes may be occurring in the algal community that may be related to exposure to ELF electromagnetic fields. These changes include

Table 4. Summary BACI and RIA statistics for selected diatom species. Before statistics are from June 1983 to April 1986; N = 44-45 for summer and 31 for winter. After statistics are from May 1986 to September 1990; N = 45-46 for summer and 33 for winter. NS = $p > 0.05$.

	BACI	RIA
Summer		
Achnanthes minutissima	NS	NS
Cocconeis placentula	NS	NS
Cymbella minuta	NS	NS
Winter		
Achnanthes minutissima	NS	NS
Fragilaria vaucheriae	NS	NS
Gomphonema olivaceum	NS	NS
Synedra ulna	NS	NS

changes in chlorophyll *a* biomass, organic matter accrual rates, diatom density, and diatom species diversity and evenness. The strongest evidence for such changes comes from the chlorophyll *a* biomass data. There is no evidence to suggest that any significant changes have occurred in numbers or volume of the more common algal species in the community. Potential effects on the community cannot be completely confirmed at present due to only a single year of exposure to the fully operational ELF antenna. Studies on the benthic algal community over the next one or two years should clarify the preliminary results reported in this paper.

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A Comparison of RIA and BACI Analysis for Detecting Pollution Effects on Stream Benthic Algal Communities

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Abstract
Before and After, Control and Impact analysis (BACI) and Randomized Intervention Analysis (RIA) are used to overcome pseudoreplication in sampling designs that emphasize data collection at paired control and impacted sites. Both methods were applied to data collected on the potential effects of 76 Hz extremely low frequency (ELF) electromagnetic radiation on benthic diatom communities at two sites in the Ford River, Michigan. Data on the diatoms were collected as part of the U.S. Navy funded studies (through IIT Research Institute) on ELF effects from an ELF communication antenna. Data on chlorophyll a, diatom species diversity, and diatom abundance were collected at an impact site under the antenna and at a control site receiving 7-10 times less ELF radiation than the impact site. Data were divided into a 3 year before period and a 6 year after period. Both procedures detected differences ($p < 0.05$) in the relationship between the two sites in chlorophyll a and species diversity before antenna operation as compared to the period after antenna operation began, but detected no difference in diatom abundance. The RIA procedure was limited by sample sizes less than 40, limiting our ability to detect monthly differences with it. The small sample size problem emphasizes the need for long term monitoring. The parametric (BACI) and randomization (RIA) procedures offer powerful, complimentary tools for the detection of pollution effects using control and impact sites.

Key Words: Statistical applications, BACI analysis, RIA analysis, diatoms, rivers, ELF electromagnetic radiation.

Introduction
The experimental design of large-scale monitoring efforts used to determine environmental effects of a pollutant has commonly been plagued by the problem of psuedoreplication as defined by Hulbert (1984). A typical sampling design, set up to determine whether a known pollutant will adversely affect a biological parameter, consists of replicated sampling over time at a control and impact site. However, funding and logistic constraints associated with environmental monitoring often makes the replication of treatments (sites) impossible. This lack of replication, therefore, invalidates the use of inferential statistics such as analysis of variance in interpreting the data (Stewart-Oaten 1986).

The problem of statistically detecting non-random changes between a single control and impacted site has received some attention in the literature (Stewart-Oaten et al. 1986, Carpenter et al. 1989, Carpenter 1990, Jassby and Powell 1990, Reckhow 1990).

Stewart-Oaten et al. (1986) have introduced the Before and After, Control and Impact analysis (BACI), which requires paired sampling at control and impact sites, both before and after perturbation. The mean of the "before" differences between sites is compared to the mean of the "after" differences between sites by a t-test. If the magnitude of the difference between sites changes significantly following addition of the pollutant, there may be a perturbation effect. The procedure assumes that the following criteria are met: (1) the measures of the parameters at any time are independent of the measures at any other time, and (2) the differences between control and impact sites of the "before" period are additive.

Randomized Intervention Analysis (RIA) represents a non-parametric alternative to the BACI analysis (Carpenter et al. 1989). RIA is based on replicated sampling over time, before and after an impact, at control and experimental sites. A mean difference between sites is calculated from both "before" and "after" data sets. The absolute value of the difference between these means represents the test statistic. Random permutations of the time series of inter-site differences provide an estimate of the distribution of the test statistic. The proportion of randomly created differences between means that are greater than the observed difference between means, determines whether a significant change has occurred between sites. By using a randomly created error distribution, the RIA design does not require transformations for non-additive data. RIA does require the assumption that errors are independent from one another through time. However, Carpenter et al. (1989) indicated that serial correlation did not lead to ambiguous results in 97 percent of the autocorrelated cases that they examined.

The objective of this paper is to demonstrate the potential use of RIA and BACI as statistical tools for the detection of pollution effects in environmental monitoring. Here we compare and contrast both methods using diatom abundance, species diversity and chlorophyll a standing stock data sets collected as part of an on-going study to monitor potential effects of 76 Hz extremely low frequency (ELF) electromagnetic radiation on stream benthic diatom communities at a control and impact site in the Ford River, Michigan.

Methods and Materials

The data were obtained from an ongoing study of the effects of ELF electromagnetic radiation (generated by the U.S. Navy's ELF antenna) on the Ford River ecosystem in Michigan's upper peninsula (see Burton et. al. in this volume for more details on this study). Glass microscope slides held in plexiglass carriers and incubated in the river for 28 days (42 days during the winters of the last three years of the study) were used to sample the periphyton community at two sites in the river. The impact site lies directly beneath the antenna and receives about 7 times the exposure of the control site, which is about 8 km downstream from the antenna. Pre-operational data were collected every 28 days between June 1983 and May 1986. Operational data were collected every 28 days during the spring, summer and fall and every 48 days during the winter between June 1986 and September 1990. Testing on the antenna began in May 1986 at 4 amps (impact site exposure rate = 1.6 mv/min). On April 28, 1987 the power was increased to 15 amps (6.1 mv/min at the impact site) and on November 15, 1987 the power was increased to 75 amps (31.0 mv/min at the impact site). Testing at full power (150 amps, 61.0 mv/min at the impact site) began on May 1, 1989 and full operations at 150 amps began on October 7, 1989. The antenna was operated in a fairly irregular pattern during the testing period from May 1986 to October 1989.

BACI analysis and RIA were conducted on chlorophyll *a* standing crop (10 replicates per site per sampling date), species diversity (3 replicates per site per sampling date), and individual diatom species abundances (3 replicates per site per sampling date). Each data set was split up into "before" and "after" periods with all sampling dates from June 1983 to April 1986 as the "before" period and all dates from May 1986 to September 1990 as the "after" period. The data for the biological parameters and diatom abundances were also divided into summer and winter seasons to statistically examine seasonal variations for these parameters. Seasons consisted of a Summer (May to October) and a Winter (November to April) period. Those seasons prior to Summer 1986 represented the "before" period, while the "after" period consisted of all seasons after May 1986. Using the BACI technique, we also compared individual seasons of the "before" period to other "before" seasons to determine whether any differences occurred prior to impact. Each of the "before" seasons was then compared to each of the "after" seasons to see whether significant differences between sites had occurred. Finally, peak diatom abundances were compared between sites using BACI for those months of the year when species such as Achnanthes minutissima become dominant.

The BACI analyses were run as specified by Stewart-Oaten (1986). Data points from each sampling date were entered into files using Statview 512+ on a Macintosh Plus microcomputer. The set of "before" data were tested for additivity using Tukey's one degree of freedom test for non-additivity. If the slope of the regression of differences between sites against the means of both sites varied significantly from zero ($p < 0.05$), then data were transformed. The log (x + 1) transformation was used for non-additive biological data, while the arcsin square root of the mean transformation suggested by Steel and Torrie (1960) was used for proportional data. According to Stewart-Oaten et al. (1986) the independence of error assumption required by the BACI analysis may be considered to be "plausible if large, local, long-lasting random effects are unlikely". While our initial analysis of the data indicated that this assumption was indeed plausible, any significant or questionable results were closely examined for possible serial correlation problems. The Durbin-Watson test (1951) was used to test for independence of errors. If the "before" data sets met the additivity and independence of error criteria, differences between sites for each time period were compared with an unpaired two-tailed t-test. Data sets which failed to meet the stringent requirements of BACI analysis were analyzed using the non-parametric RIA test.

Data for RIA were entered into data files using the Supercalc software program and transferred to separate ASCII files for each parameter for each site. By using a randomly created error distribution, RIA eliminates the criterion for additive data. RIA calculations were performed on an IBM microcomputer using the RIAPUB program obtained from Dr. Stephen R. Carpenter. The program is interactive in nature and is applicable for most studies of this type. A probability distribution created by RIAPUB through random permutations of the time-series of inter-site differences determined whether a significant change between sites occurred after antenna operation.

Results and Discussion

Pooled and seasonal chlorophyll a standing crop data for the "before" period were found to be non-additive using Tukey's test. A plot of mean standing crop against differences between sites using raw data showed the slope to be significantly greater than zero (Figure 1a and 1c). The log (x + 1) transformed data set was retested for additivity and the slope of the regression line was found to be not significantly different from zero (Figure 1b and 1d). Results of a BACI unpaired t-test on pooled chlorophyll a data indicated a significant difference ($p < 0.01$) between "before" and "after" period mean differences (Table 1). When the data

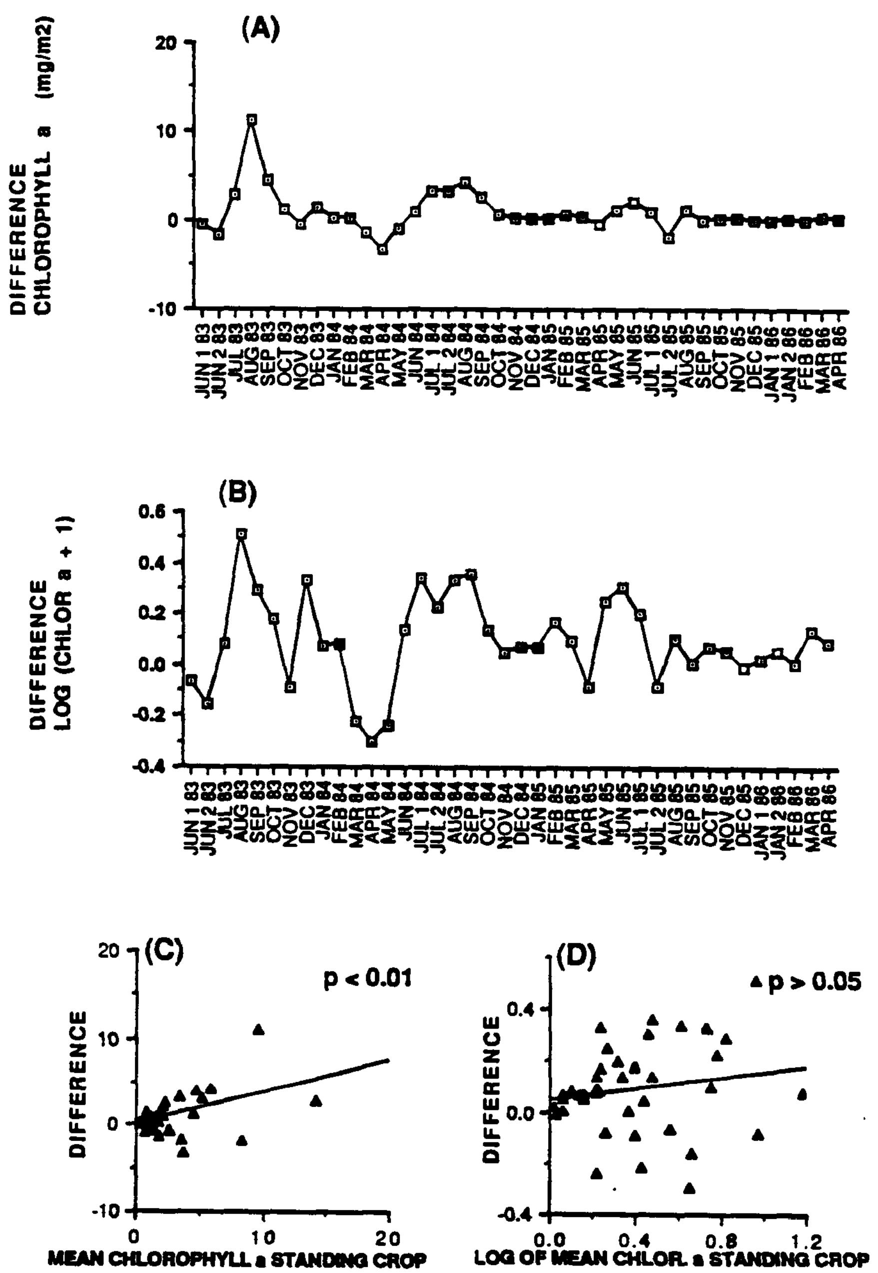

Figure 1. Non-additive and additive chlorophyll a data. (A) Difference between sites of raw chlorophyll a data for "before" period. (B) Difference between sites of log(x+1) transformed chlorophyll a data for "before" period. (C) Tukey's test for non-additivity on raw chlorophyll a data (significant at $p < 0.05$). (D) Tukey's test for non-additivity on log transformed chlorophyll a data (non significant).

Table 1. Summary of BACI and RIA comparisons for chlorophyll a, species diversity and diatom abundance between control (FCD) and experimental (FEX) sites for 1983-1990. N in parentheses for BACI and RAI, respectively.

Parameter	Comparison	BACI Signif. ($p < 0.05$)	RIA Signif. ($p < 0.05$)
Chlorophyll a	6/83-4/86 vs. 5/86-9/90 (84) (84)	$p < 0.01$	$p < 0.05$
	Summer 83-85 vs. 86-90 (49) (51)	$p = 0.06$	$p < 0.01$
	S 83/88 (9)	$p < 0.05$	
	S 83/90 (9)	$p < 0.05$	
	S 84/87 (11)	$p < 0.05$	
	S 84/88 (10)	$p < 0.05$	
	S 84/90 (10)	$p < 0.01$	
	S 85/87 (11)	$p < 0.05$	
	S 85/88 (10)	$p < 0.01$	
	S 85/90 (10)	$p < 0.01$	
	Winter 83-85 vs. 86-89 (33) (33)	NS	NS
Species Diversity	6/83-4/86 vs. 5/86-9/90 (83) (85)	$p < 0.05$	$p < 0.05$
	Summer 83-85 vs. 86-90 (48) (51)	NS	NS
	Winter 83-85 vs. 86-89 (33) (34)	NS	NS
Diatom Abundance A. minutissima			
	Summer 83-85 vs. 86-90 (45) (46)	NS	NS
	Winter 83-85 vs. 86-89 (31) (33)	NS	NS
	May & June 83-85 vs. May & June 86-90 (12)	NS	--

were broken down on a seasonal basis, significant differences between the summers of 83, 84 and 85, and the summers of 87, 88 and 90 were found. A closer inspection of the chlorophyll a "before" data set revealed that the independence assumption was not completely satisfied. Results of a Durbin-Watson test on "before" period data indicated that a significant ($d = 1.14$, $p < 0.05$) serial correlation problem existed.

Chlorophyll a standing crop data were then analyzed using the non-parametric randomized intervention analysis. The inter-site relationship changed over time for both the entire data set and the summer seasonal data (Table 1). Due to the lack of sensitivity of RIA at observation numbers less than 40, year-to-year comparisons could not be run (Carpenter et al. 1989).

Diatom species diversity data were found to be additive using Tukey's test for pooled (p <

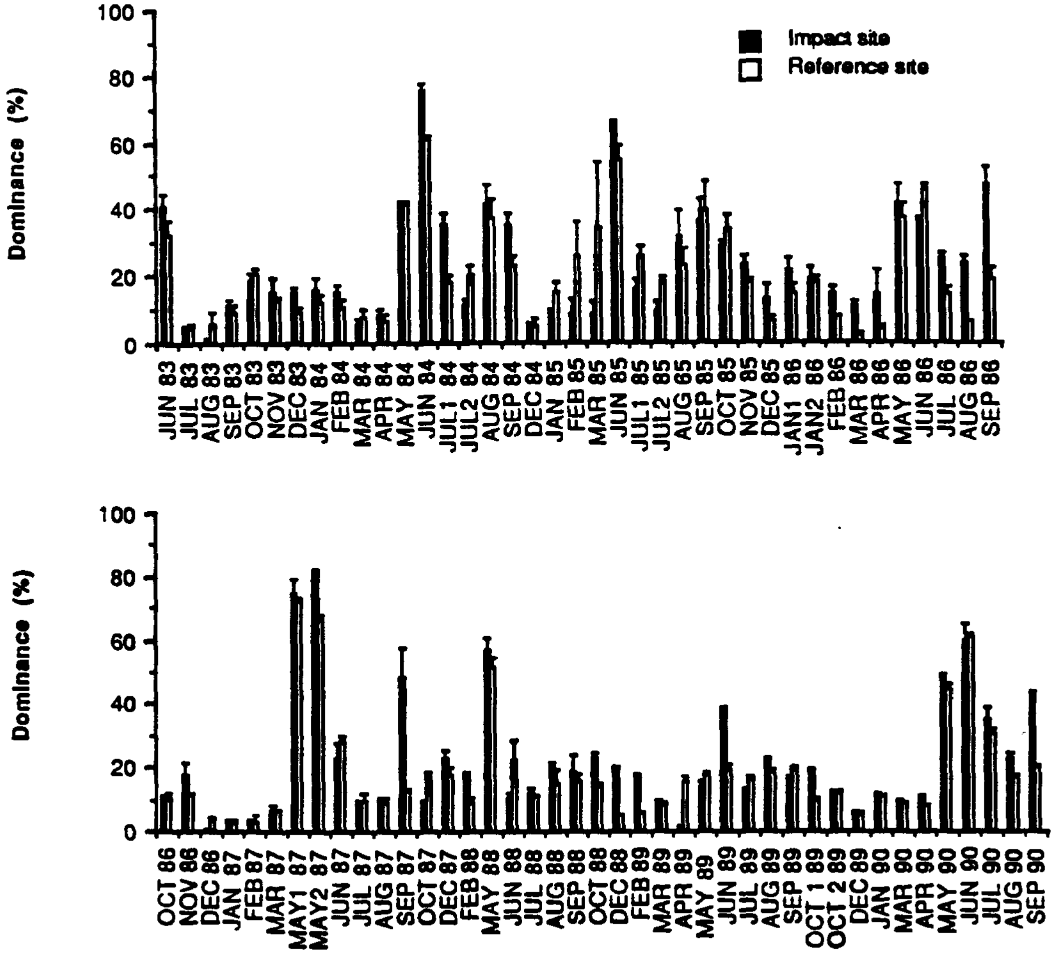

Figure 2. *Achnathes minutissima* percent dominance for the Ford River, 1983-1990.

0.84) data sets, and summer ($p < 0.90$) and winter ($p < 0.96$) data sets. Results of Durbin-Watson's test indicated no serial correlation problem ($d = 1.60$, non-significant at $p < 0.05$). Since all data sets for diversity passed BACI's additivity and independence tests, an unpaired t-test was run on untransformed data for pooled, seasonal and year-to-year comparisons. Results of BACI t-tests demonstrated significant changes ($p < 0.05$) in the inter-site relationship for the pooled"before" and "after" data (Table 1). Seasonal pooled comparisons were not significant. The only year-to-year comparison that was significant was the summer 1983 to summer 1987 comparison.

Species diversity pooled and seasonal data analyzed using RIA reflected the results of the BACI analysis (Table 1). In this instance, additional statistical analysis of diversity data using the non-parametric randomized intervention analysis was redundant. A check of all data sets indicated that all assumptions of BACI had been satisfied, thus making RIA unnecessary.

The abundance of a dominant diatom species Achnanthes minutissima has followed a predictable pattern of high dominance during summer periods and low dominance during the winter (Figure 2). All diatom abundance data were transformed using the arcsin square root of the mean transformation and tested for additivity. Seasonal transformed data for the before period were marginally additive (summer: $p < 0.06$ and winter: $p < 0.08$). Durbin-Watson independence tests of summer data found no significant autocorrelation problem ($d = 1.98$), while winter data were significantly autocorrelated ($d = 0.90$, $p < 0.05$). Results of unpaired t-tests for A. minutissima indicated that there were no significant inter-site changes in mean differences for either seasonal "before" and "after" periods, or year-to-year comparisons. Since the winter abundance data were found to be significantly autocorrelated, BACI results were verified using RIA. RIA reflected the results obtained in the BACI comparisons of both the summer and winter abundance data (Table 1).

In an attempt to detect even more subtle changes in diatom abundances, we ran BACI analyses on monthly data at peak A. minutissima abundances (Figure 2). All May and June data for the years 1983-1985 were pooled to represent the "before" period and all May and June data for 1986-1990 as the "after" period so that mean differences beween sites could be examined. The data appeared to be significantly negatively serial correlated ($d = 3.33$, $p < 0.05$). Since negative autocorrelations are conservative with regard to probability levels, an unpaired t-test was run on the monthly data. There was no significant change in the inter-site relationship after antenna operation according to the BACI analysis (Table 1). This comparison could not be verified with RIA due to the limited number of observations available.

For the chlorophyll a standing crop and species diversity parameters where significant differences were found using either BACI or RIA, the data were scrutinized further to determine whether ELF electromagnetic radiation or another factor had caused the observed differences. Significant differences found by BACI or RIA do not imply that a suspected perturbation has caused a change, nor do these tests reveal at what point in time the change occurred. Ecological and procedural considerations should be examined in all cases. Analysis of covariance (ANCOVA) of chlorophyll a standing crop with ELF exposure included as a covariant indicated that a variable other than ELF electromagnetic radiation caused the change in relationship between sites. Significant positive correlations between water temperature and chlorophyll a during drought periods from 1986 to 1990 suggest that the observed differences were related to weather variables. ANCOVA of species diversity data using ELF exposure as a covariate also indicated that a factor other than antenna exposure was responsible for the significant BACI and RIA results.

Along with the need for careful interpretations regarding the possible sources of variation, data set sample sizes should be considered when deciding on the appropriate statistical analysis. Generally, the statistical power of non-parametric tests such as RIA is smaller than that of a similar parametric test (Welkowitz et al. 1976). When populations are normally distributed, the number of observations required by RIA should be larger than the sample size required by the BACI analysis in order to obtain the same amount of power. Carpenter et al. (1989) reported that RIA could consistently detect manipulation effects with sample sizes of 40 or more. Stewert-Oaten et al. (1986) did not suggest a minimum sample size for the BACI analysis. Although the authors did present an example in which only 23 data points were used to detect a manipulation effect, Monte Carlo simulations are required to definitively determine the effective sample size required by the BACI method. Thus, year-to-year BACI comparisons presented in this study should be interpreted with some caution, since BACI's ability to detect perturbation effects with sample sizes of nine to eleven (Table 1) data points remains unknown. The sample size problem also emphasizes the need for long term monitoring of potential pollutant effects.

In summary, an environmental impact study should provide quantitative evidence to support regulatory decisions regarding potential environmental pollutants. Both the BACI analysis and RIA offer a means of quantitatively detecting whether perturbations such as toxic effluents, pipeline construction, or power plant discharges may be impacting an ecosystem. The BACI and RIA results should be interpreted with some care and caution however, as significant findings do not imply that the suspected pollutant has caused the observed differences. Our analysis of ELF effects on a riverine algal community using BACI and RIA suggests that the following statistical protocol will accurately and quantitatively allow the detection of environmental perturbations. First, the parametric BACI analysis should be used for data sets satisfying plausible assumptions of independence and additivity. If the relationship between control and impacted sites has changed significantly over time, or if the independence, normality or additivity assumptions appear to be questionable, then the non-parametric randomized intervention analysis may be used to examine the data. Finally, if the inter-site relationship is found to change over time using RIA, final conclusions of the perturbations effects should be based on ANCOVA results (using the magnitude of the perturbation as the covariate) and/or other ecological considerations. When used in this manner, BACI and RIA represent complimentary and practical tools with which to make sound ecological decisions regarding potential environmental impacts.

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The Freshwater Annelida (Polychaeta, Naidid and Tubificid Oligochaeta, and Hirudinea) of the Great Lakes Region--an Overview

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Abstract
The segmented worms are important components of benthic communities in nearly every freshwater biotope. They are widely distributed, and some groups are found in great abundance. Several of the annelid groups have been used for monitoring and detecting changes in water quality and physical habitats. The habitat and water quality requirements as well as the pollution tolerance of many species of freshwater annelids have been documented in the literature by a few investigators. Practical taxonomic keys are now available to species, but many benthic water quality assessment studies still do not treat the annelid groups adequately because the investigators lack the knowledge and experience in using these keys. Furthermore, most bioassessment monitoring studies do not use adequate sampling and processing (preservation) techniques for aquatic annelids. The inadequate treatment by some investigators represents a loss of valuable ecological information for use in biological assessment of the quality of water resources, water pollution, or other changes in aquatic ecosystems resulting from natural causes or anthropogenic activities. The current aspects of morphology, taxonomy, distribution, and organic pollution to polychaetes, naidid and tubificid oligochaetes, and leeches of the Great Lakes Region species are presented and discussed.

Key Words: macroinvertebrates, polychaetes, oligochaetes, leeches, pollution, water quality, organic enrichment.

Introduction
Benthic animals, including the segmented worms, are commonly used to demonstrate the effects of pollution on the biological integrity of surface waters and changes in the biotic community (species composition, presence or absence, and relative abundance of tolerant and intolerant species) resulting from natural causes and destructive activities by man (Aston 1984; Brinkhurst 1974a,b; Carr and Hiltunen 1965; Goodnight and Whitley 1960; Hiltunen 1967, 1969a-c, 1971; Hiltunen and Manny 1982; Howmiller and Scott 1977; Milbrink 1983; Sawyer 1974; Klemm 1991 and papers cited therein). This paper is a taxonomic overview of the freshwater polychaetes, naidid and tubificid oligochaetes, and leeches of the Great Lakes region with emphasis on their use to demonstrate pollution effects and changes in biotic community. A checklist of the species is found in Table 1.

Annelida is an important and major phylum in the animal kingdom. The body of annelids is divided into rings (somites) or segments with serially arranged organs. The phylum includes three major classes, Polychaeta, Oligochaeta, and Hirudinea. The distribution of aquatic annelids is usually determined by the physical, chemical, and biological characteristics of the environment. Published accounts are relatively sketchy for understanding the roles that some of these characteristics play in the distribution of annelids (gross chemical pollution not

Table 1. Checklist of Polychaetes, Naidid and Tubificid Oligochaetes, and Leeches in the Great Lakes Region.

Class Polychaeta: Order Sabellida

Family Sabellidae
Manayunkia speciosa

Class Oligochaeta: Order Tubificida

Family Naididae
Allonais pectinata

Amphicaeta america

Amphicaeta leydigii

Arcteonais lomondi

Bratislavia unidentata

Chaetogaster diaphanus
Chaetogaster diastrophus
Chaetogaster limnaei
Chaetogaster setosus

Dero digitata
Dero furcata
Dero nivea
Dero obtusa
Dero vaga

Haemonais waldvogeli

Nais alpina
Nais bretscheri
Nais barbata
Nais behningi
Nais communis
Nais elinguis
Nais pardalis
Nais pseudobtusa
Nais simplex
Nais variabilis

Ophidonais serpentina
Paranais frici

Piguetiella michiganensis
Piguetiella blanci

Pristina aequiseta
Pristina breviseta
Pristina leidyi
Pristina longiseta bidentata
Pristina longiseta longiseta
Pristina plumaseta
Pristina synclites

Pristinella acuminata
Pristinella jenkinae
Pristinella osborni

Ripistes parasita

Slavina appendiculata

Specaria josinae

Stephensoniana trivandrana

Uncinais uncinata

Vejdovskyella comata
Vejdovskyella intermedia

Table 1. Checklist of Polychaetes, Naidid and Tubificid Oligochaetes, and Leeches in the Great Lakes Region (continued).

Class Oligochaeta: Order Tubificida

Family Tubificidae

Aulodrilus americanus
Aulodrilus limnobius
Aulodrilus pigueti
Aulodrilus pluriseta

Bothrioneurum vejdovskyanum

Branchiura sowerbyi

Haber cf. speciosus

Ilyodrilus templetoni

Isochaetides freyi
Isochaetides curvisetosus

Limnodrilus cervix
Limnodrilus cervix (variant form)
Limnodrilus claparedianus
Limnodrilus hoffmeisteri
Limnodrilus hoffmeisteri (spiralis form)
Limnodrilus hoffmeisteri (variant form)
Limnodrilus maumeensis
Limnodrilus profundicola
Limnodrilus udekemianus

Phallodrilus hallae

Potamothrix bavaricus
Potamothrix bedoti
Potamothrix hammoniensis
Potamothrix moldaviensis
Potamothrix vejdovskyi

Psammoryctides californianus

Quistadrilus multisetosus

Rhyacodrilus coccineus
Rhyacodrilus montana
Rhyacodrilus punctatus
Rhyacodrilus sodalis

Spirosperma ferox
Spirosperma nikolskyi

Tasserkidrilus harmani
Tasserkidrilus kessleri
Tasserkidrilus superiorensis

Teneridrilus flexus

Tubifex ignotus
Tubifex tubifex

Varichaetadrilus augustipenis

Class Hirudinea: Order Arhynchobdellida

Family Haemopidae

Haemopis grandis
Haemopis lateromaculata
Haemopis marmorata
Haemopis plumbea
Haemopis terrestris

Family Hirudinidae

Macrobdella decora
Philobdella gracilis

Table 1. Checklist of Polychaetes, Naidid and Tubificid Oligochaetes, and Leeches in the Great Lakes Region (continued).

Family Erpobdellidae

Erpobdella dubia	Mooreobdella bucera
Erpobdella parva	Mooreobdella fervida
Erpobdella punctata	Mooreobdella microstoma
Nephelopsis obscura	

Class Hirudinea: Order Rhynchobdellida

Family Glossiphoniidae

Actinobdella annectens	Helobdella fusca
Actinobdella inequiannulata	Helobdella papillata
Actinobdella pediculata	Helobdella stagnalis
	Helobdella transversa
Alboglossiphonia heteroclita	Helobdella triserialis
Desserobdella michiganensis	Marvinmeyeria lucida
Desserobdella phalera	
Desserobdella picta	Placobdella hollensis
	Placobdella montifera
Gloiobdella elongata	Placobdella ornata
	Placobdella papillifera
Glossiphonia complanata	Placobdella parasitica
	Theromyzon biannulatum
	Theromyzon rude

Family Piscicolidae

Cystobranchus meyeri	Piscicola geometra
Cystobranchus verrilli	Piscicola milneri
	Piscicola punctata
Myzobdella lugubris	
	Piscicolaria reducta

withstanding). Despite the fact that annelids may occur in all aquatic habitats and in great numbers, especially certain oligochaete groups, it must be stressed that much work remains to be done on the ecology and pollution biology of the annelids. The improper or inadequate treatment of the segmented worms is attributable, in part, to investigators that lack appropriate experience or do not understand the morphological terms and characters used in practical keys. To interpret the quality of water resources, the water quality requirements and pollution tolerances, the animals should be identified to the species level (Resh and Unzicker 1975).

Class Polychaeta: Order Sabellida: Family Sabellidae

General Morphology and Taxonomy

Polychaetes are segmented annelids typically with parapodia associated with each body segment. A high degree of modification in the basic plan of many polychaetes has resulted in different modes of existence, ranging from sedentary (tubicolous) forms to highly free-moving (errant) forms. The group is very diverse, most forms are mainly found free living; some are commensal with other invertebrates, and only a few are parasitic. The class contains about 85 families and many species, most of which are marine forms. World-wide, only 10 families are represented in freshwater.

In North America only four families and 11 species are represented (Klemm 1985a,c): Nereididae with six species, Ampharetidae with one species, Sabellidae with one species, and Serpulidae with two species. In the Great Lakes Region only the family, Sabellidae is represented by one species, Manayunkia speciosa; it is sedentary and inhabits a tube built of mud or sand and mucus. The size of this species is usully 2 to 5 mm long. The body is divided into distinct regions, the thorax and abdomen, with reduced or vestigal parapodia, with simple capillary chaetae and hooks or uncini. The prostomium is small or indistinct, without appendages. In Sabellidae, the anterior end is modified to form a branchial (tentacular) plume or crown surrounding the mouth which is used for food getting (filter feeding) and respiration. For more information on the taxonomy of the North America freshwater forms, see Klemm (1985a,c).

General Distribution and Ecology

This species is widely distributed in the Neartic region, from Duluth Harbor in western lake Superior to St. Marys River, Lake St. Clair, the Ottawa River in Ontario to Western Lake Erie, Lake Ontario, and the upper St. Lawrence River; eastward to the Finger Lakes and Hudson River, New York, Schuylkill River and Delaware River, Pennsylvania, Egg Harbor River, New Jersey, and Lake Champlain, Vermont, south to North Carolina, South Carolina, and Georgia. It has also been reported from the Pacific northwest, California, and Oregon to Alaska. One reason for not detecting this species more often is its small size; the sieve used to process the sample may have mesh openings too large to retain the specimens. Most specimens may pass through a Standard No. 30 sieve, and a Standard No. 60 should be used.

Mackie and Qadri (1971) reported, during a limnological survey of the Ottawa River, that specimens of M. speciosa occurred only in substrates composed of silt and sand and in moderately moving waters. Hiltunen (1965) also found a large number of M. speciosa at the mouth of the Detroit River, suggesting some relationship between water movement and the frequency of occurrence. Specimens in the Mackie and Qadri study did not occur in polluted water where BOD value exceeded 4 ppm nor where the DO content was less than 5 ppm. M. speciosa has also been found in lentic habitats, many locations in Lake Erie (Hiltunen 1965, Krieger 1990), and in several lakes in Alaska (Holmquist 1973). Spencer (1976) found it in Cayuga Lake, New York at depths of 20 m or less where densities of more than 1000/sq. m were found occasionally. Poe and Stefan (1974) reported this polychaete from the Schuylkill River, Pennsylvania, near the type-locality, and they reported that it appears to have a wide range of tolerances for environmental parameters such as DO (1.8 to 14.0 ppm), depth (0.3 to 16.0 m), pH (6.8 to 8.8), and water temperature (2.8 to 28.3°C) (obviously as low as 0°C because it survives ice-cover seasons), and concluded that the only environmental factor which may limit its distribution is the requirement for fine particulate material in the substrate for the construction of the tube in which it lives (gross chemical pollution notwithstanding).

Class Oligochaeta

Introduction

Naidids and tubificids are predominantly found in freshwater but some are strictly marine forms. Most oligochaetes have chaetae, with a few exceptions, and have no parapodia as in the polychaetes. The body is segmented into somites or compartments separated by septum, and each segment by convention is indicated by a Roman numeral, progressing from anterior to posterior. Segment I (including mouth and prostomium) is devoid of chaetae, hence numerical orientation of segments is achieved by counting posteriad of the chaetophorous segments, beginning with II. Normally each segment bears four fascicles "bundles" of chaetae, two dorso-lateral and two ventro-lateral. There are two basic types of chaetae (crotchets and capilliforms) whose numbers and morphology in the various body regions are taxonomically important. A crotchet can be straight or curved (sigmoid), and usually possess a more or less median thickening (the node or nodulus), and may be simple-pointed or have a bifid (cleft) distal end. Crochets are found in all oligochaetes. Capilliform chaetae which are elongate and simple-pointed, may be smooth or finely serrated. Capilliform chaetae when present, are found only in the dorsum of the Naididae and Tubificidae. For more detailed information on aquatic oligochaete biology, see Brinkhurst and Jamieson (1971), Brinkhurst and Cook (1980), Bonomi and Erseus (1984), and Brinkhurst and Diaz (1987).

Order Tubificida: Family Naididae

General Morphology and Taxonomy

Naidids are relatively small, commonly I mm to IO mm and more or less transparent when alive. All or nearly all can be identified to species by the external morphology, particularly the shape and arrangement of the chaetae. There are 20 genera and more or less 48 species known or likely to occur in the Great Lakes region. In North America 2I genera and 75 species are reported. Keys work well for most species, but some species descriptions are incomplete for North American material. The kinds of chaetae are much like those in the Tubificidae, except the naidids have dorsal acicular (short needle-like) chaetae that accompany the long capilliform (hair) chaetae. Some species of naidids also bear pectinate chaetae like the tubificids. Dorsal chaetae can begin in segment II or posteriad to it; dorsal chaetae that accompany capilliform (hair) chaetae are often very different from ventral chaetae. Dorsal fascicles often contain 1-2 capilliform chaetae and I-2 acicular chaetae. In summary, some naidids have ventral chaetae only (Chaetogaster spp.); other species have dorsals and ventrals with bifids only, while still other species have ventrals bifid and dorsals with capilliform plus simple, bifid, pectinate, or palmate acicular chaetae. The dorsal chaetae may begin in II, III, or further back, usually V or VI, rarely beyond. Some species may have eyes; may be found budding (a form of asexual reproduction), and when sexually mature, may bear genital chaetae in segments V or VI; spermathecae in segments IV, V, or VII; male pores on segments V, VI, or VIII. However, these features are not used in species identification. For more information on taxonomy of the naidids, see Hiltunen and Klemm (1980, 1985), Brinkhurst (1986), and Brinkhurst and Kathman (1983).

General Distribution and Ecology

The naidids are an ecologically diverse group (Leaner 1979) and are found in both lotic and lentic waters. The naidids are widely distributed and commonly inhabit the littoral zones of lakes or other shallow waters in streams, ditches, and ponds. Some species are sediment dwellers (like tubificids) while other species are characteristically found among the aquatic plants. Naidid populations are usually reduced where siltation and mud occur. Plants with a thick growth habit and well-developed periphyton community can support sizeable naidid populations. Riffles and similar areas where the substrate is primarily sand and gravel often contain substantial naidid populations. Longitudinal zonation of naidids along rivers has been demonstrated. Learner et al. (1978) concluded that factors associated with changes in altitude and slope of a river (water velocity, substrate type, presence and type of vegetation, and the influence of municipal and industrial wastes) can be important in influencing the distribution of naidids. They are generally less significant in lakes where they are confined primarily to the littoral zone. The behavior of most naidids is unlike that of most other oligochaetes because some naidids can swim as well as crawl, and others are small enough to be passively carried by strong water movements.

Feeding habits of most species are unknown, but some have been observed to feed on detritus, grazing upon bacteria, protozoans, and algae. Probably most oligochaetes are herbivorous, but some Chaetogaster species are primarily or perhaps entirely predaceous. Reproduction occurs by paratomy (architomy, asexually budding), where the posterior segments of the naidid develop into daughter zooids that break free after development is complete, or by fragmentation. Sometimes the worms are found consisting of two or more individuals that have not yet separated from the parent (anterior) section. Sexual reproduction is considered uncommon in many species.

Order Tubificida: Family Tubificidae

General Morphology and Taxonomy

Tubificids are medium-sized to large worms, commonly more than 20 mm long, that never have eyes, never reproduce by asexual budding, but occasionally regeneration of the posterior section in some species suggests fragmentation. A variety of chaetae are found among the species. Crotchets are always present but capilliform (hair) chaetae may or may not be present depending on the species. Most species are red when alive and coil or loop when disturbed. In the Great Lakes region there are presently 17 genera and 37 species. There are 21 genera and 64 species reported to occur in North America. Tubificids are identified by the characteristic shape of the somatic chaetae and their genital chaetae (spermathecal or penial chaetae) if present, or by mature male genitalia. In some species penis sheaths in segment XI are especially helpful in species identification. Spermathecae are located in segment X, and males pores are in Segment XI. Dorsal chaetae always begin on segment II, dorsal chaetae are often broadly similar in form to ventral chaetae; dorsal fascicles often bear a complement of more than 2 capilliform (hair) chaetae and 2 or more crotchets. Therefore, some species of tubificids have dorsal and ventral chaetae bifid; other species have dorsal capilliform and pectinate chaetae and ventrals mostly bifid. Pectinate chaetae may be narrow and hairlike distally in appearance. Some species have dorsal capilliform and bifid chaetae and ventral bifid chaetae. For more information on the taxonomy of the tubificids, see Stimpson, Klemm, and Hiltunen (1982, 1985) and Brinkhurst (1986, 1989).

General Distribution and Ecology

Tubificids are most commonly found in soft sediments rich in organic matter; several tubificid species characteristically live in large numbers in habitats that receive organic pollution (Aston 1984, Brinkhurst 1974a,b, Carr and Hiltunen 1965, Goodnight and Whitley 1960. Hiltunen 1967, 1969a-c, 1971, Hiltunen and Manny 1982, Howmiller and Scott 1977, Krieger 1990, Milbrink 1983). Tubificids respire cutaneously, but some species can tolerate anoxic conditions and environmental stresses (e.g., Limnodrilus hoffmeisteri and Tubifex tubifex). A number of species in the family are very stress-sensitive (Hiltunen 1967, Howmiller and Scott 1977, Milbrink 1983). Tubificids burrow in soft sediments, often in tubes of mud and mucus secretions, as the classic name implies. A few species occur in fine gravel or sand. The quantity and quality of organic matter reaching the sediment may be more important in determining which tubificid species will occur in a locality (gross chemical pollution notwithstanding) than the physical-chemical variables of water or sediment (Brinkhurst and Cook 1974).

Tubificids are mostly deposit feeders living on organic detritus and its associated bacteria, microflora, and fauna. Tubificids typically feed with their heads buried below the sediment surface with their tails protruding above it. The feeding activities of tubificids play an important role in mixing the physical and chemical characteristics of sediments (Brinkhurst 1974). Most tubificids reproduce by sexual reproduction even though they are hermaphrodites. Tubificids enclose their eggs in cocoons and deposit them on sediments.

Class Hirudinea (Not Hirudinoidea)

Leeches are serially segmented worms and are considered closely related to the oligochaetes. They are also hermaphroditic, i.e., they contain both male and female organs in each individual. Muscles and a hydrostatic skeleton are used in locomotion. The nervous, excretory and vascular systems are segmentally arranged. Leeches have well-developed anterior and posterior sucker, 34 segments (indicated by Roman numerals I-XXXIV)) which are subdivided into annuli, a reduced coelom and intestinal caeca, and usually two separate male and female gonopores with male gonopore anterior to the female gonopore. Leeches are devoid of chaetae, except Acanthobdella peledina, a leech which has chaetae in the anterior segments of the cephalic region (Klemm 1985b,c). Although some leeches are well adapted to a sanguivorous existence, the group is also well-represented by species which are both predatory and can engulf small animals whole or parasitic fluid-feeders. Leeches are found on all the continents, in terrestrial, freshwater, estuarine, and marine environments.

General Taxonomy and Morphology

Five families in the orders Arhynchobdellida and Rhynchobdellida are represented in the Great Lakes region: Haemopidae, Hirudinidae, Erpobdellidae, Glossiphoniidae, and Piscicolidae. Nineteen genera and 43 species have been recorded from that region. Twenty-one genera and 66 nominal species presently are reported to occur in North America. For more information on the taxonomy of leeches, see Klemm (1985b,c, 1991) and Sawyer (1972, 1986).

Leeches are found in most freshwater habitats, but are often ignored by biologists because they are thought to be difficult to identify to genus and species. Also, investigators neglect them because they lack an understanding of the diagnostic (morphological) terms and characters used in keys to identify specimens to the lowest taxonomic level.

The external morphological characters are usually sufficient for the identification of most leeches. Internal characters used to identify certain species of Haemopis are discussed in Klemm (1985b,c). The general external diagnostic features that are important for identifying the leeches to species are: size of mouth, general shape of body, form of suckers, form of cephalic region, number and arrangement of eyes, jaws and teeth, eyespots (ocelli), papillae, pulsatile vesicles, digitate processes on rim of caudal sucker, caudal-sucker separation from body on narrow pedicle, copulatory gland pores, the number of annuli between gonopores, and pigmentation patterns. Typically, the mouth opening of the haemopids and hirudinids is medium to large, occupying the entire sucker cavity, and the body is large, linear, elongate and well-muscled, length 75-300 mm. They are good swimmers. Haemopids and hirudinids always have 5 pairs of eyes. The mouth opening of erpobdellids is medium, occupying the entire sucker cavity; the body of erpobdellids is moderate size, linear, elongate, length to 100 mm, and they are also good swimmers. They usually have 3, or 4 pairs of eyes (or eyes absent). The mouth of glossiphoniids is a small pore on the rim or within the oral sucker cavity; the body of this group is dorso-ventrally flattened with the posterior half usually much wider than the tapering cephalic end, length to 40 mm. They have l, 2, 3, or 4 pairs of eyes. The mouth of piscicolids is a small pore within the oral sucker cavity, and the body of the piscicolids is cylindrical, narrow, posterior half can be slightly flattened, length to 30 mm. The body may be divided into a narrow neck (trachelosome) and wider body (urosome) regions; caudal sucker with or without eyespots, and body with or without pulsatile vesicles. Piscicolids can have one or two pairs of eyes (or eyes absent).

General Distribution and Ecology

In North America, freshwater leeches reach their greatest species diversity in lakes, permanent and temporary ponds, woodland pools, bogs, wetlands, rivers, and streams (Klemm 1972, Sawyer 1972). Klemm (1977, 1991) summarized the distribution of leeches in the Great Lakes states (Illinois, Indiana, Michigan, Minnesota, New York, Ohio, and Wisconsin) bordering the Great Lakes, including Ontario, Canada. Additional collecting in all habitats, substrate types, and host organisms will undoubtedly extend the regional distribution of some taxa.

The leeches of the Great Lakes region are a significant part of the continental freshwater fauna. Leeches are biologically important in food webs, and at trophic levels they function mainly as ectoparasites, predators, or both. An important ecological factor in the distribution of leeches is the availability of prey. Other environmental factors, such as composition of substratum, lentic or lotic waters, depth, size and type of water body, hardness and pH, dissolved solids, water temperature, dissolved oxygen, siltation and turbidity, and salinity are characteristics of aquatic habitats that also influence leech distribution and abundance (Klemm 1972, 1991; Sawyer 1974), not withstanding toxic substances in the aquatic environment. We have a relatively sketchy understanding of the role that these and other environmental factors play in the distribution of leeches. It must be stressed that much work remains to be done before we can have a clear picture of the problem.

The little we know of the feeding habits of leeches indicates that they are far more diverse than most people realize; many are not sanguivorous (blood feeders). The Haemopidae have no teeth or have varied and poorly developed jaws that are armed with small numbers of blunt teeth for masticating food, and are able to swallow prey whole. They are mainly predators of macroinvertebrates, but the ones with teeth are perhaps capable of sucking blood. In the Hirudinidae, the jaws are well developed, armed with numerous small, sharp, saw-like teeth suitable for making cuts in the epidermis of prey, such as reptiles, amphibians, and mammals. These leeches are blood sucking ectoparasites. Surprisingly, studies on the North American haemopids and hirudinids indicate that these leeches are predominantly predatory and extremely opportunistic, and consume larvae and eggs of amphibians and small invertebrates. They dwell mostly in freshwater, but some species can travel overland, and a few species are terrestrial. In Erpobdellidae, the mouth is large and adapted to predation. It contains a muscular pharynx for crushing and swallowing macroinvertebrate prey whole. They are highly mobile and are good swimmers. They live exclusively in freshwater. The Glossiphoniidae are without teeth or jaws and have a very small oral opening (pore). This name refers to the mechanism by which these leeches feed. They insert a tube-like proboscis into their prey and suck out the body fluids. The glossiphoniids parasitize turtles, mollusks, waterfowl, fishes, amphibians, mammals, including man, and even other leeches. They are ectoparasites or predators. Most travel slowly with a looping movement, but a few species are active swimmers. Brooding behavior is well developed, and cocoons are brooded over substrate or directly on the venter of the parent. They are found exclusively in freshwater. The Piscicolidae are primarily ectoparasites on fish. Some are permanent parasites on specific hosts, but most are opportunistic and feed on a variety of host fishes. A few feed on invertebrate groups, such a Decapoda and Cephalopoda. Piscicolids generally have a large and well-developed anterior sucker surrounding the mouth. As in the glossiphoniids, the oral opening is small. To feed, piscicolids insert a protruding sucking proboscis into their host. Parent leeches lay hard-shelled cocoons on a substrate, but they do not brood their cocoons or young. Only a few piscicolids are active swimmers. Muscles are generally poorly developed and locomotion is usually by looping movements over a substrate or host. Most species are marine, but some are found in brackish or fresh waters.

Tolerance to Organic Pollution

The species of annelids in the Great Lakes region that are commonly or occasionally associated with organically enriched waters are indicated in Table 2A-C. The tolerance values in Table 2A-C can be used with the Trophic Condition Index (Howmiller and Scott 1977; Milbrink 1983) and modified Hilsenhoff Biotic Index (Klemm et al. 1990; Plafkin et al. 1989). However, more pollutional studies are needed for these annelid groups because little is known about their tolerances and the biological effects of various contaminants. For more information on the water quality requirements and pollution tolerance of freshwater naidids, tubificids, and leeches, see Brinkhurst (1974a,b), Carr and Hiltunen (1965), Hiltunen (1967, 1969a-c), Howmiller and Scott (1977), Klemm (1972, 1991), Klemm et al. (1990), Krieger (1990), Milbrink (1983), and Sawyer (1974).

Table 2A. Pollution Tolerance of Selected Freshwater Annelids

Taxa	Tolerance to Organic Wastes* T	F	I
ANNELIDA - POLYCHAETA			
SABELLIDAE			
Manayunkia speciosa		3	
ANNELIDA - OLIGOCHAETA			
NAIDIDAE			
Amphichaeta americana		2	
Chaetogaster diaphanus		2	
C. diastrophus		2	
Dero digitata		2	
D. nivea		3	
D. obtusa		3	
D. pectinata		2	
Nais barbata	4		
N. behningi		3	
N. bretscheri		3	
N. communis	4		
N. elinguis	5		
N. pardalis	4		
N. simplex		3	
N. variabilis	5		
Ophidonais serpentina	4		
Pristina aequiseta		3	
Slavina appendiculata		2	
Specaria josinae		2	
Stylaria fossularis		3	
S. lacustris		3	
Vejdovskyella comata			1
V. intermedia	4		

*Ranking from 0 to 5 with 0 being the least tolerant. T = tolerant; F = facultative; I = intolerant

General Collection and Preservation

Aquatic worms are usually collected using dredges, grabs, cores and other sampling devices that provide bulk collections of bottom subtrate. This material is then sieved or hand-picked so that the organisms are separated from the accompanying silt and debris. This must be done carefully, especially if a sieve is used. The abrasion of the soft-bodied worms against a sieve surface may break specimens or damage the specimens by breaking or displacing chaetae, particularly capilliform (hair) chaetae, for example. Although a US Standard No, 30 mesh sieve (28 meshes per inch, 0.595 mm openings) is usually used, it should be noted that many small individuals may be lost during the sieving process and that the use of a finer sieve (for example, No. 60 mesh, 0.25 mm opening) or no sieving at all may be required to ensure collection of all individuals. Even when sieving has been accomplished carefully, some individuals will nevertheless fragment. Only head-end sections and whole worms should be enumerated. The initial sorting of specimens from sediment residue in the laboratory should be done at a 5-10 X magnification using a dissection microscope or

Table 2B. Pollution Tolerance of Selected Freshwater Annelids.

Taxa	Tolerance to Organic Wastes* T	F	I
ANNELIDA - OLIGOCHAETA			
TUBIFICIDAE			
Aulodrilus americanus		3	
A. limnobius		3	
A. pigueti		3	
A. pluriseta		3	
Bothrioneurum vejdovskyanum		2	
Branchiura sowerbyi	4		
Ilyodrilus templetoni		3	
Isochaetides curvisetosus		2	
Limnodrilus cervix	4		
L. claparedianus	4		
L. hoffmeisteri	5		
L. maumeensis	5		
L. udekemianus	5		
Potamothrix moldaviensis		3	
P. vejdovskyi		3	
Quistadrilus multisetosus	4		
Spirosperma carolinensis		3	
S. ferox		3	
S. nikolskyi		2	
Tubifex tubifex	5		

*Ranking from 0 to 5 with 0 being the least tolerant. T = tolerant; F = facultative; I = Intolerant.

lens. They can also be selectively hand-picked, fixed, and preserved in the field.

Leeches are also found attached to various substrates such as rocks, boards, logs, or almost any inanimate object littering both lentic and lotic environments or collected from prey organisms. Annelid specimens should be fixed in 5010% formalin, and transferred after 48 hours to 70% ethanol or 5-10% buffered formalin for storage. Undesirable shrinkage is kept to a minimum with this process. The use of alcohol as a fixative should be avoided

Table 2C. Pollution Tolerance of Selected Freshwater Annelids

Taxa	Tolerance to Organic Wastes* T	F	I
ANNELIDA - HIRUDINEA			
ERPOBDELLIDAE			
Erpobdella parva	4		
E. punctata	4		
Mooreobdella microstoma	4		
HAEMOPIDAE			
Haemopis grandis		3	
H. marmorata		3	
GLOSSIPHONIIDAE			
Alboglossiphonia heteroclita		3	
Gloiobdella elongata	4		
Helobdella stagnalis	4		
H. triserialis		3	
Glossiphonia complanata	4		
Placobdella multilineata		2	
P. ornata		3	
P. papillifera		3	
P. parasitica		3	
PISCICOLIDAE			
Myzobdella lugubris		3	
Piscicola punctata		3	

*Ranking from 0 to 5 with 0 being the least tolerant. T = tolerant; F = facultative.

because polychaetes, oligochaetes, and leeches initially preserved in alcohol without first being fixed in formalin tend to deteriorate and disintegrate. If the specimens of oligochaetes are to be cleared and they have been preserved in 70% alcohol, they should be placed in 30% alcohol and then in water for a short time to leach out the alcohol to enable placement into a tissue-clearing solution (e.g., Amman's lactophenol). Alcohol retards the clearing process of Amman's lactophenol.

To allow internal structures to be seen, oligochaete specimens should be cleared before specific examination. Temporary mounting media, Amman's lactophenol (l00g phenol, l00 mL lactic acid, 200 mL glycerine, and l00 mL water) or CMCP-9, or CMCP-10, can be used for rapid processing of specimens. Oligochaete specimens must be cleared and mounted on glass slides for examination under a compound light microscope capable of magnification up to 1000X (oil immersion). An 18 mm diameter, No. 0 or 1 round cover glass is appropriate because it will adequately accommodate nearly the size range of naidids and tubificids and the shape allows for maneuvering the specimens into the most desired position by gentle pressure and rotation of the coverglass. When preparing a temporary or permanent slide mount, an attempt should be made to place the specimen on its side, revealing both dorsal and ventral fascicles of chaetae. Permanent mounts of oligochaetes can be made following alcohol dehydration of specimens and clearing, using methyl salicylate or xylene, and mounting the specimens in a synthetic resin, such as Harleco's Coverbond or Canada balsam. Permanent mounts of oligochaetes are suitable for systematic study and may last over 20 years. Most leech specimens can be identified to species by examining the external features using a dissecting microscope (450X). Additional instructions for sorting, processing, and identifying polychaetes, naidid and tubificid oligochaetes, and leeches specimens can be found in a number of taxonomic guides (Brinkhurst 1986; Hiltunen and Klemm 1980; Stimpson, Klemm, and Hiltunen 1982; Klemm 1985a-c; Klemm at al. 1990).

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A Comparison of Macroinvertebrates Collected from Bottom Sediments in Three Lake Erie Estuaries

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Abstract

Macroinvertebrates were collected from bottom sediments from three Lake Erie tributaries as a part of EMSL's Biomarker Project. The objective of this paper is to compare the macroinvertebrate populations collected from the three water bodies and relate these populations to possible pollutional stresses and/or habitat characteristics. Three grab samples were collected with either a petite Ponar or a standard Ekman-on-a-stick at three different stations at each site. The sampling stations were chosen randomly from among the nine stations used for collecting fish at each site. In the Black River above a Coking Plant, 60-80% of the organisms were tolerant oligochaete worms but some pollution sensitive organisms were also present indicating organic enrichment but not toxic pollution. All of the individuals collected from below the plant were oligochaete worms (90%) and other organisms tolerant of both organic and toxic pollution. In Old Woman Creek, over 80% of the individuals collected were oligochaete worms and blood worms (midges) characteristic of organically enriched sediments associated with high oxygen demand. Toussaint Creek samples were characterized by a variety of midge larvae and many empty mollusk shells but few live mollusks. Less than 50% of the individuals were oligochaete worms. This may be a reflection of the sediment characteristics which consisted of gravel and clay with little of the muck substrate characteristic of the other two sites. The data indicate that all three sites are effected by organic enrichment and/or agricultural runoff, but the Black River macroinvertebrate community below the Coking Plant appears to be stressed by something in addition to organic enrichment.

Key Words: macroinvertebrates, bottom sediments, pollution, water quality, organic enrichment, biotic index.

Introduction

As part of EMSL-Cincinnati's Biomarkers Research Program, fish and macroinvertebrates were collected from the Black River, Old Woman Creek and Toussaint Creek during April 23-25, 1990. The project objective is to determine if biomarkers can be used to detect a wide range of pollutants. The Black River site was chosen as one study site because of polyaromatic hydrocarbons that have been detected in the sediment downstream from a coking plant and the resulting high incidence of liver cancer in brown bullhead that inhabit the site. Old Woman Creek and Toussaint Creek were sampled as possible control sites.

The purpose of this paper is to compare the macroinvertebrate populations collected from the three water bodies and attempt to relate these populations to pollutional stresses and/or habitat characteristics.

Methods

Nine fyke fish nets were set at each site along both east and west banks over a distance of approximately one mile. Each net was defined as a sampling station for fish. Three of these stations were randomly chosen as benthic macroinvertebrate collection stations on each water body. Three six inch Ekman or Ponar grab samples were collected at each station and preserved in 70% ethanol. The samples were returned to the laboratory, sorted and the organisms identified to the lowest taxonomic level possible. Pollution tolerance value for each taxa were taken from EMSL-Cincinnati's Biological Methods Manual (USEPA 1990) or Hilsenhoff (1987). Sediment and water samples were also collected at each station for chemical analysis.

Stations were analyzed using Hilsenhoff's (1977) modification (HBI) of Chutter's (1972) Imperial Biotic Index, Shannon-Weaver's mean diversity (d), and equitability (e). Although the HBI was designed to give a measure of the effects of organic pollution on the macroinvertebrate communities inhabiting stream riffles, it should be useful in analyzing grab samples collected from soft river substrates if care is used in interpreting the data. An HBI score of <1.75 would indicate excellent water quality, 1.76 - 2.50 good water quality, 2.51 - 3.75 fair water quality, 3.76 - 4.00 poor water quality, and >4.00 would indicate grossly polluted conditions. Mean diversity values of less than 1.0 are characteristic of gross pollution, values between 1.0 and 3.0 indicate fair to poor water quality, and values above 3.0 are common for clean water stations.

Equitability values above 0.5 are indicative of good water quality, 0.3 - 0.5 fair, and values below 0.3 indicate degradation of water quality. Stations within each site and the three water bodies (between sites) were compared using the Community Loss Index and Jaccard's Coefficient of Similarity (Plafkin et al. 1989). Trophic condition index (Howmiller and Scott 1977; Milbrink 1983) was also determined for each station but the results were not helpful in interpreting the data, possibly because most of the oligochaete worms could not be identified to species.

Station descriptions

Old Woman Creek - Station 1 was located 200 feet north of the RR bridge about 25 feet from the west shore. Substrate was muck and well rotted organic material; water depth was about 18 inches. Station 2 was located 100 feet west of the observation deck about 50 feet from shore. Substrate was muck with some clay; water depth was about 18 inches. Station 3 was located 500 feet south of the Highway 2 bridge and about 150 feet west of a high gravel bank near the east shore of the estuary. Substrate was muck and rotting leaves; water depth was about 16 inches.

Toussaint Creek - Station 1 was located 200 feet north of Highway 2 bridge about 50 feet from the east shore. Water depth was about six feet and the substrate was sandy and silty. Station 2 was located about one half mile south of Highway 2 bridge about 50 feet from the east shore just south of a small island. Water depth was about two feet and substrate was hard packed gravel with some clay and silt. Station 3 was located across the bay from Station 2 about 50 feet east of a very small island. Water depth was about three feet and substrate was muck, clay and hard packed gravel. A large ditch entered the bay about 50 feet north of the station.

Black River - Station 1 was located about four miles upstream from the mouth of the river about 30 feet from the west bank across from, and about 100 feet upstream from, the upper end of a large island. Water depth was about seven feet and the sediment was mostly fine silt. Station 2 was located about 100 feet downstream from the lower end of the large island about 20 feet from the bank. Water depth was about two feet and the sediment was muck, clay and leaves. Station 3 was about one half mile downstream from a major discharge from the coking plant at the bend of the river about 20 feet from the west bank at about river mile three. Water depth was 2 1/2 feet and the sediment was mud and silt.

Results and Discussion

Toussaint Creek. The Ponar samples collected at Station 1 (Table 1) yielded 13 taxa, two of which (see below) are not generally found in polluted waters and six of which are highly tolerant of organic pollution. Just over half (56%) of the individuals were oligochaete worms which is an indication of non-polluted conditions (Goodnight and Whitley 1960). The HBI of 3.76, the diversity index (2.3) and equitability (0.5) all indicate fair to marginal water quality. The large number of empty mollusk shells (123 individuals representing 8 species) indicate that conditions have been present, at least some time recently, for a diverse population of these organisms to develop. The midge larvae Ablabesmyia mallochi, which is very sensitive to metals contamination, and the gastropod Somatogyrus, which is not generally found in organically polluted waters indicate that this station is probably not, or only slightly, impaired by pollutants. Probably this is the least impacted station sampled during this study; therefore it was used as the reference station for this study.

The three Ekman samples collected at Station 2 (Table 1) contained only five living taxa, consisting of the midge Polypedilum scalaenum, which is generally restricted to unimpaired waters, two pollution tolerant oligochaete worms, and two facultative (wide range of tolerance) midge species. Less than half the individuals (36%) collected were oligochaete worms which indicates non impaired conditions (Goodnight and Whitley, 1960). The HBI of 3.33, the diversity index (1.8) and equitability (1.0) all indicate fair to good water quality conditions at this station. The presence of the midge Polypedilum scalaenum also indicates that toxic substances are probably not present in concentrations high enough to effect the biological integrity of the aquatic community. The lack of a diverse fauna is probably due to the difficulty of obtaining a sample because of the hard packed gravel and clay substrate. The Ekman sampler used was one with a handle so that it could be pushed with some force into the bottom or the samples could not have been taken here.

The three Ponar samples taken at Station 3 (Table 1) contained only seven living taxa but 14 taxa of empty mollusk shells. It seems odd that so many shells were collected without any live animals. Perhaps something had recently occurred that killed all the mollusks, but it is obvious that conditions in the recent past must have been conducive to the establishment of a diverse population. Only two taxa present (the midge Cryptochironomus sp. and the oligochaete worm Limnodrilus hoffmeisteri) are tolerant of pollution, however, these two taxa make up about 80% of the individuals collected. This station was very near a ditch that enters the bay here and it is possible that periodically toxic and/or organic substances may flow into the bay from this ditch causing stress on the benthic community. Our sampling may have occurred during the beginning of the recovery period. The samples were collected in the spring soon after planting and storm runoff from nearby agricultural lands may have entered the bay by way of this ditch. Krieger (1989) reported that agricultural herbicides used with corn and soybeans reach higher concentrations in rivers of northwest Ohio than in rivers anywhere else in North America. The Biotic Index of 3.73 indicates only a slight impact as do the diversity index (1.5) and equitability (0.5). The high percentage of oligochaete worms at this station as compared to the other two may be an indication that whatever has effected the benthic community was not limiting to the oligochaete worms and probably was not widespread throughout the bay.

Community Loss Index and Jaccard's Coefficient of Similarity indicate that the three stations are quite dissimilar with the greatest difference between stations 1 and 2. If station 1 is considered the control, station 2 shows a loss of 2.2 and station 3 shows a loss of 1.4. These differences would appear to be significant and one might expect that stations 2 and

Table 1. Macroinvertebrates Collected and Pollution Tolerance Values for Toussaint Creek. Intolerant taxa denoted by *.

Taxa	Number of Individuals Station 1	Station 2	Station 3	Pollution Tolerance Value
Chironomidae				
Coelotanypus concinnus	11			4
Cryptochironomus sp.			1	4
Cryptochironomus fulvus gr.	4		2	3
Procladius nr. bellus	30	5	2	3
Chironomus plumosus gr.	1			5
Ablabesmyia mallochi*	1			2
Cladotanytarsus sp.		15	2	3
Polypedilum scalaenum*		1	1	2
Tanytarsus guerlus gr.			1	3
Other Diptera				
Ceratopogonidae				
Nr. Probezzia sp.	2			3
Crustacea				
Isopoda				
Lirceus lineatus	2			3
Bryozoa				
Urnatella gracilis	P			3
Bivalvia				
Sphaeriidae	3			4
Gastropoda				
Somatogyrus sp.*	1			2
Oligochaeta				
Limnodrilus hoffmeisteri	7		1	5
L. maumeensis	1			5
Branchiura sowerbyi	1	2		4
Unidentified Oligochaeta	62	10	31	4
Total Individuals	126	33	41	
Total Taxa	13	5	7	
% Oligochaeta	56	36	78	
Biotic Index (HBI)	3.76	3.33	3.73	
Mean Diversity (d)	2.3	1.8	1.5	
Equitability (e)	0.5	1.0	0.5	

3 would be quite similar because the Community Loss Index between them is small, but Jaccard's Coefficient (<0.50) indicates otherwise. HBI scores for stations 1 and 2 and for 2 and 3 are statistically different from each other but HBI scores for stations 1 and 3 are similar. Probably most of the differences observed are ecological and not caused by pollution.

The data suggest that Toussaint Creek Bay is not affected to any great extent by pollution, however, the presence of many species of empty mollusk shells at stations 1 and 3 leads me to suspect that occasional instances may occur, either natural or man caused, that stress the aquatic community and temporarily affect the biological integrity of the bay in the vicinity of the canal that enters the bay on the west shore. Runoff from agricultural lands during storms may be a factor here as in most other northwest Ohio rivers (Krieger 1989). A total of 17 taxa consisting of 200 individuals (11.8/taxa) were collected in the nine grab samples taken from Toussaint Creek.

Old Woman Creek. The three Ekman samples collected at Station 1 (Table 2) yielded nine taxa, including the bryozoan Pectinatella magnifica which is known to be sensitive to toxic contaminants and five taxa which are highly tolerant to organic pollution. Of the 96 individuals collected 83 (86%) were oligochaete worms characteristic of organically enriched sediments. The HBI of 4.01 and the high percentage of oligochaete worms (Goodnight and Whitley 1960) indicate organic enrichment but the diversity index (2.3) and equitability (0.7) indicate fair to good water quality. The presence of the bryozoan Pectinatella magnifica at this station would indicate good water quality.

The three Ekman samples collected at Station 2 (Table 2) yielded ten taxa, none of which are known to be sensitive to pollution and seven which are highly tolerant of organic pollution. Of the 132 individuals collected, 107 (81%) were oligochaete worms characteristic of organically enriched sediment. The blood worm Chironomus plumosus, which is highly tolerant of sediments with high oxygen demand, was also common at this station. The HBI of 4.03 and the high percentage of oligochaete worms indicate organic enrichment but the diversity index (1.6) and equitability (0.4) would indicate fair water quality.

The three Ekman samples collected at Station 3 (Table 2) contained seven taxa, including the amphipod Gammarus pseudolimnaeus which is generally not found in waters containing toxic substances other than organic enrichment and four of which are highly tolerant to organic pollution. Of the 31 individuals collected, 23 (74%) were oligochaete worms characteristic of organically enriched sediments. The HBI of 3.84 and the presence of Gammarus pseudolimnaeus would indicate that toxic substances are probably not major limiting factors at this station. The percentage of oligochaete worms was less at this station than the other two and fell in the moderately polluted category of Goodnight and Whitley (1960). The diversity index (1.8) and equitability (0.6) indicate fair to good water quality.

Community Loss Index and Jaccard's Coefficient of Similarity show that stations 1 and 2 are quite similar and that station 3 is significantly different from the other two stations. The Community Loss Index values also indicate that station 3 has fewer taxa in common with either of the other two stations. HBI score for station 3 was also lower than for the other two stations, but the difference was not significant ($P=0.05$). However, HBI for Old Woman Creek stations 1 and 2 were significantly higher than for the reference station while station 3 was not significantly different ($P=0.05$).

The data suggest that Old Woman Creek Estuary may have been temporarily affected by storm runoff from agricultural lands (Klarer and Millie, 1989) and organic enrichment from decaying vegetation and that station 3 is slightly less effected than the other two. The limiting factor at this site would most likely be the highly enriched substrate consisting of muck and decaying vegetation probably accompanied by periods of low DO and high levels of nitrites resulting from storm runoff from nearby agricultural lands (Klarer and Millie, 1989). Using Goodnight and Whitley's (1960)

Table 2. Macroinvertebrates Collected and Pollution Tolerance Values for Old Woman Creek.

Taxa	Number of Individuals Station 1	Station 2	Station 3	Pollution Tolerance Value
Chironomidae				
Coelotanypus concinnus	4	6		4
Cryptochironomus fulvus gr	1	1		3
Procladius nr bellus	4	2	5	3
Chironomus plumosus gr	1	9		5
Dicrotendipes sp.	1			4
Other Diptera				
Ceratopogonidae				
Nr Probezzia sp.	2	7		3
Coleoptera				
Dubiraphia sp	1			3
Coleoptera				
Donacia sp.			1	3
Cruatacea				
Gammarus pseudolimnaeus*			1	2
Oligochaeta				
Limnodrilus maumeensis	2	2	1	5
L. hoffmeisteri	5	3	2	5
L. cervix		2	1	4
Branchiura sowerbyi		2		4
Ilyodrilus templetoni		1		4
Unidentified Oligochaeta	76	97	19	4
Bryozoa				
Pectinatella magnifica*	P	S	S	1
Total Individuals	96	132	31	
Total Taxa	9	10	7	
% Oligochaeta	86	81	74	
Biotic Index (HBI)	4.01	4.03	3.84	
Mean Diversity (d)	2.3	1.6	1.8	
Equitability (e)	0.7	0.4	0.6	

metric based on percent oligochaete worms present, all three stations would be considered polluted. A total of 15 taxa consisting of 259 individuals (17.3/taxa) were collected in the nine grabs taken from Old Woman Creek.

Black River. The three Ponar samples collected at Station 1 (Table 3) contained 18 taxa most of which are characteristic of slightly impaired or organically polluted waters. Two midge species, Dicrotendipes neomodestus and Harnischia curtilamellata, present at this station are usually not found under contaminated conditions. Of the 137 individuals collected 106 (77%) were oligochaete worms which would indicate moderately polluted or organically enriched sediments. The HBI of 3.92, the diversity index (2.4) and equitability (0.4) all

Table 3. Macroinvertebrates Collected and Pollution Tolerance Values for Black River.

Taxa	Station 1	Number of Individuals Station 2	Station 3	Pollution Tolerance Value
Chironomidae				
Harnischia curtilamellata*	1			2
Ablabesmyia mallochi*		3		2
Tanytarsus guerlus gr		2		3
Phaenopsectra prob dyari		2		3
Eukiefferiella claripennis		2		4
Hydrobaenus pilipes gr*		1		2
Cryptochironomus fulvus gr	7	1	11	3
Cryptochironomus sp	1	1	1	4
Diplocladius cultriger	1			4
Dicrotendipes neomodestus	1	2		2
Polypedilum ophioides	1			3
Glyptotendipes lobiferus	1	2	1	3
Orthocladius sp.			1	3
Cricotopus tremulus gr		2		3
Corichapelopia sp.			1	3
Procladius nr. bellus		1	9	3
Nanocladius distinctus		1		3
Other Diptera				
Hemerodromia sp	2	3		3
Chaoborus punctipennis	10	1	1	3
Nr. Probezzia sp.	3			3
Ephydridae	1			3
Unidentified Diptera		1		3
Coleoptera				
Elmidae				
Stenelmis sp.*		21		2
Dubiraphia sp.		4		3
Staphylinidae		1		2
Helodidae				
Scirtes sp		1		3
Noteridae				
Berosus sp		1		3
Ephemeroptera				
Caenis sp	2	2		3
Lepidoptera				
Bactra sp (?)		1		2
Hydracarina				
Trombidiformes		1		2
Odonata				
Argia apicalis		1		3

Table 3. Macroinvertebrates Collected and Pollution Tolerance Values for Black River. (Continued)

Taxa	Number of Individuals Station 1	Station 2	Station 3	Pollution Tolerance Value
Crustacea				
Isopoda				
Asellus communis		6		3
Bivalvia				
Pisidium casertanum		1	1	4
Gastropoda				
Physella vinosa			1	4
Bryozoa				
Lophopodella carteri*		P		1
Oligochaeta				
Limnodrilus hoffmeisteri	17	23	24	5
L. udekemianus		5	1	5
L. cervix	7	6	4	4
Potamothrix vejdovskyi*		2		3
Tubifex tubifex		2		5
Ilyodrilus templetoni		2		4
Quistadrilus multisetosus	1	3	1	4
Dero sp.	1			5
Nais elinguis	1			4
Nais communis		1		4
Enchytraeidae	1			4
Unidentified Oligochaeta	78	55	172	4
Total Individuals	137	164	229	
Total Taxa	18	35	13	
% Oligochaeta	77	60	88	
Biotic Index	3.92	3.62	4.00	
Mean Diversity (d)	2.4	3.7	1.5	
Equitability (e)	0.4	0.5	0.3	

* Intolerant taxa

indicate fair to poor water quality at this station which is located upstream from the major effluent from the coking plant. The mayfly Caenis sp., present at this station, is the most tolerant of the mayflies and is not a good water quality indicator.

The three Ponar samples collected at Station 2 (Table 3) yielded a surprising 35 taxa, many of which would not be expected in mud substrate where little current is present. Nine of the taxa are tolerant of pollution, one (Lophopodella carteri) is very sensitive to pollution (except in the statoblast stage) and seven others are not characteristically found in impaired waters. Almost exactly 60% of the individuals are oligochaete worms characteristic of organically polluted conditions which would indicate some organic enrichment, possibly due to decaying vegetation. It is not likely that toxic pollution is present in the sediment at this station because Potamothrix vejdovskyi, an intolerant oligochaete, was among the diverse worm fauna present. The diversity of midge and other insect groups would indicate that conditions are conducive to the development of a balanced benthic community. This may be due, however, to the possibility that these organisms are drifting into this station from some stream that may enter the river behind the island just upstream or from a spring entering the river from under the stream bank. The HBI of 3.62, diversity index (3.7) and equitability (0.5) all indicate fair to good water quality.

The three Ponar samples collected at Station 3 (Table 3) contained 13 taxa, eight of which are characteristic of polluted conditions. The other five species are all facultative and could be present in moderately polluted waters. Of the 229 individuals present, 202 (88%) were oligochaete worms indicating grossly polluted waters. It is interesting that the only deformed Procladius midge (Warwick, 1989) found during this study was collected from this station which is located one half mile downstream of the main effluent from the coking plant. The HBI of 4.00 indicates poor water quality as does the equitability (0.3) while the diversity index (1.5) is borderline between fair and poor conditions.

Community Loss Index and Jaccard's Coefficient of Similarity show that the three stations are considerably different from one another. Both stations 1 and 3 show significant community loss when compared with station 2. The HBI scores for the three stations are similar ($P = 0.05$), however the HBI scores for stations 1 and 3 are both significantly higher than for the control reference station.

The data suggest that the benthic community at Station 1, located upstream from most of the effects of the coking plant, does show some stress on the biota. Station 2 samples contain intolerant organisms (including riffle beetles) which are not characteristic of muddy substrates and some tolerant forms that are characteristic of organic pollution. Station 3 is noticeably degraded as compared to the other stations sampled during this study. A total of 47 taxa consisting of 530 individuals (7.2/taxa) were collected in the nine grab samples taken from the Black River.

Summary and Conclusions

The Community Loss Index and Jaccard's Coefficient of Similarity show that Old Woman Creek and Toussaint Creek macroinvertebrate communities are more similar to each other than either one is to the Black River but these similarities are not very great. There is no significant community loss between Old Woman Creek or Toussaint Creek and the Black River when composite data are compared. Based on the Community Loss Index, it would appear that Old Woman Creek and Toussaint Creek are both slightly less polluted than the Black River, but this is likely due to the diverse fauna collected from Black River station 2 as discussed above.

Toussaint Creek station 1 is considered the best control station because of its representative substrate and overall quality based on the combination of metrics used in this analysis. Using this as the reference station, Community Loss, Jaccard's Coefficient of Similarity and t-values based on a comparison of HBI scores for the other stations sampled are as follows:

Stations Compared	Community Loss Index	Jaccard's Coeff.	HBI t-values	
Toussaint 2	2.2	0.13	2.597	*
Toussaint 3	1.4	0.19	0.040	ns
Old Woman 1	0.3	0.44	4.590	*
Old Woman 2	0.6	0.47	4.531	*
Old Woman 3	1.6	0.13	0.241	ns
Black River 1	0.6	0.11	2.531	*
Black River 2	0.2	0.12	0.146	ns
Black River 3	0.7	0.18	3.983	*
Old Woman Creek Composite	0.4	0.37	3.073	*
Black River Composite	0.1	0.10	1.108	ns

*Significant at $p < 0.05$, df=4.

These metrics indicate that Toussaint Creek stations 2 and 3, Old Woman Creek Stations 1, 2, and 3 and Black River stations 1 and 3 are different from the reference station. The differences between the Toussaint Creek stations can be explained by substrate differences but the others could be related to pollution, including agricultural runoff (Krieger 1989). As might be expected Black River station 2 did not show a community loss (see discussion of the individual stations above). Based on Jaccard's Coefficient all the stations are significantly different from the reference station and all but Old Woman Creek stations 1 and 2 are vastly different. All of the HBI scores differ significantly (P=0.05) from the reference station except Toussaint Creek station 3, Old Woman Creek station 3, Black River station 2, and the Black River composite. The reason the Black River composite HBI scores did not differ from the reference station is probably because of the high variability due to station 2 Black River samples. As mentioned above, the dissimilarities between the reference station and stations 2 and 3 at Toussaint Creek and Black River station 2 may be attributable to environmental and/or physical factors. The other dissimilarities could be due to organic or toxic stresses. The Community Loss Index and Jaccard's Coefficient scores for the composite data indicate that Old Woman Creek is more like the reference station than is the Black River. The low Community Loss Index score and the low t-value for the composite Black River samples as compared to the reference station are mostly due to the diverse fauna collected at station 2 as discussed above.

Because the sediment samples that were collected for chemical characterization have not yet been analyzed, it is impossible to reach any real definitive conclusions based on the macroinvertebrate collections alone. However, the benthic macroinvertebrate grab collections seem to indicate that all three Old Woman Creek stations were organically enriched, with oligochaete worms making up over 60% of the individuals and the remaining taxa characteristic of waters with high oxygen demand. In Toussaint Creek only Station 3 located near a drainage ditch showed signs of stress. That may be due to periodic discharge of toxic and/or organic pollutants into the bay from this ditch, possibly during storms. Oligochaete worms made up 60% or more of the individuals collected from the Black River at all three Stations indicating organic enrichment or toxic substances in the sediment (Krieger 1990). However, a few sensitive taxa were found at Stations 1 and 2 indicating reasonably good water quality at these two stations. Station 3 samples contained about 90% worms and no sensitive taxa indicating a stressed community of benthic macroinvertebrates.

The complete absence of Hexagenia mayflies, the limited number of chironomids and gastropods, and the increase in oligochaetes in Lake Erie bays has been correlated with increased pollution in the lake (Edwards 1990, Reynoldson et al. 1989). Because no Hexagenia mayflies and only a few live gastropods were collected at any of the sites sampled during this study, it might be reasonable to assume that all of the sites were polluted.

Neither Old Woman Creek or Toussaint Creek appear to be good control sites. Grab samples collected from the Grand and Chagrin Rivers will be analyzed and the data compared with the Black and Cuyahoga Rivers to determine if either of them might be better control sites for the benthic phase of this study.

Acknowledgments

We would like to thank Larry Linns, Eastern District Office, U.S. EPA Region 5 for providing a boat and assisting with collection of the samples. This work was a part of Susan Cormier and Tim Neiheisel's Biomarker Research Project.

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A Comparison of the Results of a Volunteer Stream Quality Monitoring Program and the Ohio EPA's Biological Indices

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Abstract
Volunteer stream quality monitoring is increasing in popularity around the country, and organizations involved with the administration of volunteer stream quality monitoring programs are becoming interested in the effectiveness of their monitoring techniques. This research compares the results of the Ohio Department of Natural Resources (ODNR) volunteer-oriented Scenic Rivers Stream Quality Monitoring Program and the Ohio Environmental Protection Agency's (OEPA) biological assessments. The volunteer biological monitoring ("kick-seining") technique was performed on 12 Ohio rivers and tributaries, at 47 different sites, to coincide with the OEPA's monitoring agenda for the summer of 1989. Comparisons were made between the volunteer stream quality monitoring ratings and the OEPA's Index of Biotic Integrity (IBI) and Invertebrate Community Index (ICI). Sites which were rated "excellent" using the ODNR volunteer method tended to meet the OEPA's criteria for attainment of aquatic life uses for both the IBI and ICI. Sites which were determined to be "fair" or "poor" with the volunteer method corresponded to IBI and ICI scores falling in the non-attainment of aquatic life uses range. Although revisions in the sampling and rating system for the volunteer program could improve the predictive value of these results as compared to OEPA's indices, the volunteer technique assessments currently appear to have merit when interpreted in terms of aquatic life use attainment or non-attainment.

Key Words: volunteer monitoring, biological indices, stream quality, kick-seining, Ohio, Scenic Rivers.

Introduction
In 1983, the Ohio Department of Natural Resources (ODNR) developed the Ohio Scenic Rivers Stream Quality Monitoring Program with assistance from the Ohio Environmental Protection Agency (OEPA). This program uses volunteers to conduct simple stream quality assessments at designated monitoring stations on the state's ten Scenic Rivers. ODNR's stream quality monitoring technique involves assessments based on the presence or absence of 20 taxa of macroinvertebrates which are divided into three categories, according to each groups pollution tolerance level (Fig. 1).

Group One, the pollution intolerant organisms, includes mayfly and stonefly nymphs, dobsonfly, caddisfly and water penny beetle larvae, riffle beetles, and gill-breathing snails. Group Two macroinvertebrates, with intermediate pollution tolerances, include dragonfly and damselfly nymphs, beetle and cranefly larvae, scuds, crayfish, sowbugs, and clams. Group Three, the pollution tolerant organisms, consists of aquatic worms, pouch snails, black fly and midge larvae, and leeches. Many of the taxa used in the program encompass entire orders (i.e. mayflies - Order Ephemeroptera, caddisflies - Order Trichoptera) so identification is not refined.

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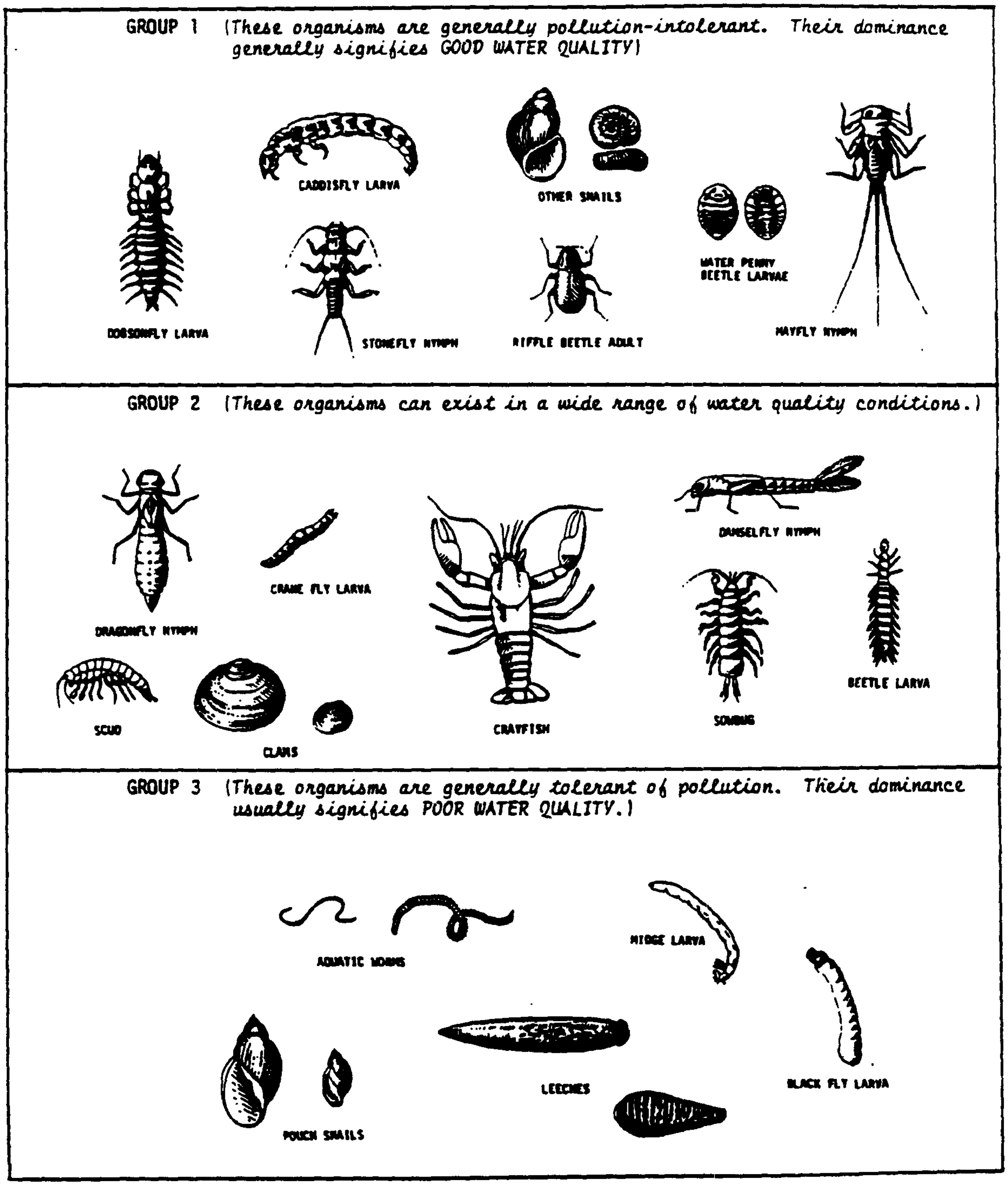

Figure 1. Ohio Department of Natural Resources Scenic Rivers Stream Quality Monitoring Program macroinvertebrate identification sheet with pollution-tolerance groupings.

In this Stream Quality Monitoring Program, volunteers collect macroinvertebrates from riffles using the "kick-seine" technique. Riffles, with little or no vegetation and stones up to 15 inches in diameter, are the type of habitat best suited to this method of sampling (Frost et al. 1971). The "kick-seine" technique involves disturbing the substrate upstream of the seine to dislodge the macroinvertebrates which cling to, and hide under the rocks and debris in the riffle. Once freed of the substrate, the macroinvertebrates are carried by the current into the seine. After a sample has been collected, the seine is taken to the stream bank where the organisms are hand-picked from the net and identified on site. Macroinvertebrates often exhibit patchy distributions in streams (Rabeni and Minshall 1977, Schwenneker and Hellenthal 1984). Therefore, volunteers are encouraged to take samples from a variety of habitats until they feel that no new taxa are represented in their samples. No set number of samples has ever been established for the program, however.

After all the samples have been collected, volunteers fill out an assessment form indicating the station sampled (according to OEPA river miles), water conditions such as clarity, algal bedgrowths, and odor, and the macroinvertebrate groups found (Fig. 2). For each macroinvertebrate taxon group located at the station, an estimated count letter code is entered on the assessment form. The letter codes A, B, and C represent 1 to 9, 10 to 99, and 100 or more individuals, respectively. Using estimated counts allows the ODNR staff to get an idea of population sizes while not placing the burden of counting the organisms on the program volunteers.

The final assessment score, referred to as the Cumulative Index Value, or CIV, is based only on the diversity of macroinvertebrates found and not the quantity. In the scoring system, each Group One taxon in the sample receives a point value of three, each Group Two taxon, a point value of two, and each Group Three taxon, a point value of one. The CIV is the sum of the points given to each category. The final step in the stream quality assessment is determining a qualitative rating for the station based on the CIV. Cumulative Index Values of over 22 are given an "excellent" rating. Scores between 17 and 22 are rated "good." "Fair" is 11-16, and a "poor" rating is given to scores of less than 11.

Once completed, assessments are sent to ODNR where they are entered into a computer database. The database allows for a quick review of the history of a given monitoring station to determine if the site has experienced any significant impacts over the years it has been monitored. It is believed that this may allow for early detection of degradation on the Scenic Rivers. The Ohio Department of Natural Resources stresses that the procedure "is not intended to pinpoint subtle changes in water quality, but rather the general condition of the river," and that "information which indicates potential decreases in water quality will be coordinated with the Ohio Environmental Protection Agency." (ODNR n.d.). The program is not intended to completely assess the source or degree of degradation, but rather provide an inexpensive and enjoyable way for the public to flag the attention of responsible enforcement agencies in the event that further study may be warranted.

The simplicity and accessibility of the program has made it popular among schools, conservation groups, scouts, and families. Since its beginning, the Stream Quality Monitoring Program has grown quite rapidly. During the 1990 monitoring season, approximately 3,000 volunteers monitored Ohio's Scenic Rivers. In addition to this considerable volunteer force, many Soil and Water Conservation Districts in Ohio have expressed interest in ODNR's method to develop volunteer stream quality monitoring programs for streams within their counties

STREAM QUALITY ASSESSMENT FORM

STATION ________ STREAM ____________________ SAMPLE # ________

LOCATION ____

COUNTY __________ TOWNSHIP/CITY __________ DATE ________ TIME ________

GROUP OR INDIVIDUALS ____________________ NO. OF PARTICIPANTS ________

DESCRIBE WATER CONDITIONS (COLOR, ODOR, BEDGROWTHS, SURFACE SCUM, ETC.)	HACH KIT RESULTS (if used) AND OTHER OBSERVATIONS

USE BACK OF FORM IF NECESSARY

WIDTH OF RIFFLE ____	BED COMPOSITION OF RIFFLE (%)
WATER DEPTH ____	SILT ☐ SAND ☐ GRAVEL (¼"- 2") ☐
WATER TEMP. (°F) ____	COBBLES (2"- 10") ☐ BOULDERS (> 10") ☐

MACROINVERTEBRATE TALLY	ESTIMATED COUNT LETTER CODE	A = 1 to 9 B = 10 to 99 C = 100 or more

GROUP 1 TAXA	LETTER CODE	GROUP 2 TAXA	LETTER CODE	GROUP 3 TAXA	LETTER CODE
WATER PENNY LARVAE		DAMSELFLY NYMPHS		BLACKFLY LARVAE	
MAYFLY NYMPHS		DRAGONFLY NYMPHS		AQUATIC WORMS	
STONEFLY NYMPHS		CRANE FLY LARVAE		MIDGE LARVAE	
DOBSONFLY LARVAE		BEETLE LARVAE		POUCH SNAILS	
CADDISFLY LARVAE		CRAYFISH		LEECHES	
RIFFLE BEETLE ADULT		SCUDS			
OTHER SNAILS		CLAMS			
		SOWBUGS			
NUMBER OF TAXA (*times*) INDEX VALUE 3		NUMBER OF TAXA (*times*) INDEX VALUE 2		NUMBER OF TAXA (*times*) INDEX VALUE 1	

CUMULATIVE INDEX VALUE ☐

STREAM QUALITY ASSESSMENT

EXCELLENT (> 22) ☐ GOOD (17-22) ☐

FAIR (11-16) ☐ POOR (< 11) ☐

PLEASE SEND THIS FORM TO:

Mr. John S. Kopec, Planning Supervisor
Division of Natural Areas and Preserves
Ohio Scenic Rivers Program
1889 Fountain Square Court
Columbus, Ohio 43224 Phone: (614) 265-6458

Figure 2. Ohio Department of Natural Resources Scenic Rivers Stream Quality Monitoring Program assessment form.

(Kopec 1989). Other states and private organizations are also patterning programs after ODNR's technique.

Generally, identification of invertebrates to only the order level of classification is considered to have limited ecological meaning. Species level identification is necessary for a more sensitive measure of water quality. (Resh and Unzicker 1975). This fact, and the increasing interest in ODNR's Stream Quality Monitoring Program, caused OEPA and ODNR staff to question quality assurance and quality control for the program.

To examine the accuracy of ODNR's stream quality monitoring technique, a source of reliable stream health information was needed for comparison. James Karr (1981) stated that it would be impossible, because of the complexity of stream ecosystems, to ever recognize all the potential factors that may impact biological communities. Although no techniques exist which can fully acknowledge all the processes at work in an aquatic ecosystem, biological monitoring integrates the effects of many processes that occur in streams. To assess stream health, the OEPA uses biological indices which have been closely studied and tested, making the OEPA's methods the best available source of stream health and biological integrity information in Ohio.

The OEPA monitors rivers and streams using three primary indices as criteria for assessment. The Index of Biotic Integrity, or IBI, originated by Karr (1981), is based on fish populations. Invertebrate samples are used to compile the Invertebrate Community Index, or ICI. The third index, the Index of Well-being, or Iwb, was not examined in this study. These indices are used to rate the relative quality of Ohio's rivers and are translated into ratings of "exceptional, good, fair, poor, and very poor." The reason for the use of more than one organism group (fish and invertebrates) is explained in the OEPA publication Biological Criteria for the Protection of Aquatic Life: Volume I, which states "The need to use both groups is apparent in the ecological differences between them, differences that tend to be complementary in an environmental evaluation" (Ohio EPA 1988).

In order to address the quality assurance/quality control issue for ODNR's Stream Quality Monitoring Program, this research examined the correlation between the OEPA's indices (IBI and ICI) and ODNR's CIV and also the agreement between ODNR staff- and volunteer-generated stream quality assessments. The general objective of this paper is to illustrate how accurately the results of ODNR's simple approach to biological monitoring can reflect stream health assessments based on more sophisticated approaches.

Methods and Materials

Over the summer of 1989 (late June to mid-September), the standard ODNR stream quality monitoring technique (as described above) was performed on 12 of Ohio's rivers and tributaries which were also being monitored by the OEPA (Fig. 3). The sites on these rivers represented a variety of habitat and impact types. With the help of the OEPA's staff, a sampling schedule was arranged which closely adhered to their agenda. This was done to help reduce the effects of seasonal or temporary variations in stream quality. All ODNR stream quality assessments were made within 0.8 river miles of the area sampled by the OEPA and all ODNR assessments were made within two weeks of OEPA's testing.

A 9 in. high, 18 in. wide rectangular frame 1/32 in. mesh dip net was substituted for the seine to allow for solo collections. This type of net and the standard 1/16 in. mesh seine are used interchangeably in ODNR's program to allow stream quality monitoring coordinators to make collections alone. At

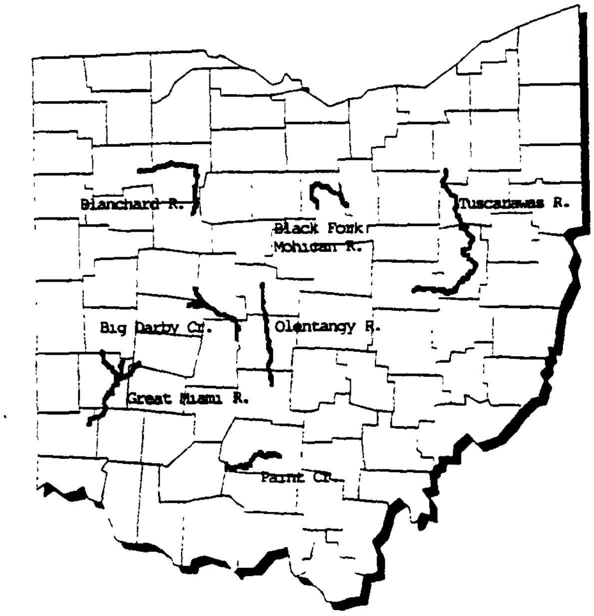

Figure 3. Ohio rivers monitored in study.

each site sampled, four regular samples were collected from areas approximately 9 ft. square and a search was conducted along the stream's edge for macroinvertebrates such as dragonfly naiads, which may prefer slower water velocity or vegetation. An index value was calculated for each sample and a CIV was calculated for the riffle. The CIV was then translated into a qualitative rating. Assessments were made on 37 different sites for comparison with the IBI, and many of those sites were monitored twice, resulting in 56 assessment records. For the comparison with the ICI, 30 assessment records from 30 sites were collected. The data were entered into a FoxBase Mac database.

In the spring of 1990, the OEPA finished processing all of its 1989 data, and their assessments were merged into a master database. The study sites were then examined for correlations between ODNR's stream quality monitoring results and the indices of OEPA, the IBI and ICI.

To examine volunteer accuracy, ODNR's volunteer monitoring database was searched for sites which were monitored both by staff members and volunteers within a three month period of time. Matched records in which one sample was taken in the early months of spring and the other in the summer were discarded, due to the notable changes in benthic community composition between these time periods. Spring CIVs are typically higher and are usually not comparable to summer CIVs. Over 200 usable matched records were located in the database.

Results and Discussion

Comparison of the IBI and ODNR's CIV

For sites rated "excellent" by ODNR's method, corresponding IBIs ranged from 30 to 57 (Fig. 4). This range includes IBIs which the OEPA would consider indicative of "fair" to "exceptional" conditions. The CIV ratings did not match exactly those of the OEPA. However, 86% of the corresponding IBIs did fall at a value of 40 or above, indicating attainment of aquatic life uses, as designated by the OEPA. For sites rated "good," the corresponding IBIs again showed a wide range, with approximately half the values indicating that sites did attain aquatic life uses, while the other half indicated that sites did not attain life uses. All "fair" and "poor" ratings were observed at sites where IBIs were less than 40, indicating non-attainment of life uses.

A primary reason for lack of complete agreement between the CIV and IBI qualitative ratings is the inherent differences between the indices. The IBI is an index based on fish collected from a 200 meter reach of stream and ODNR's CIV is based on macroinvertebrates sampled from a riffle only. However, another factor, drainage area, was found to affect the correlation. The OEPA designates sampling sites as headwater, wading, and boat sites, based on the drainage area. When the boat sites were eliminated

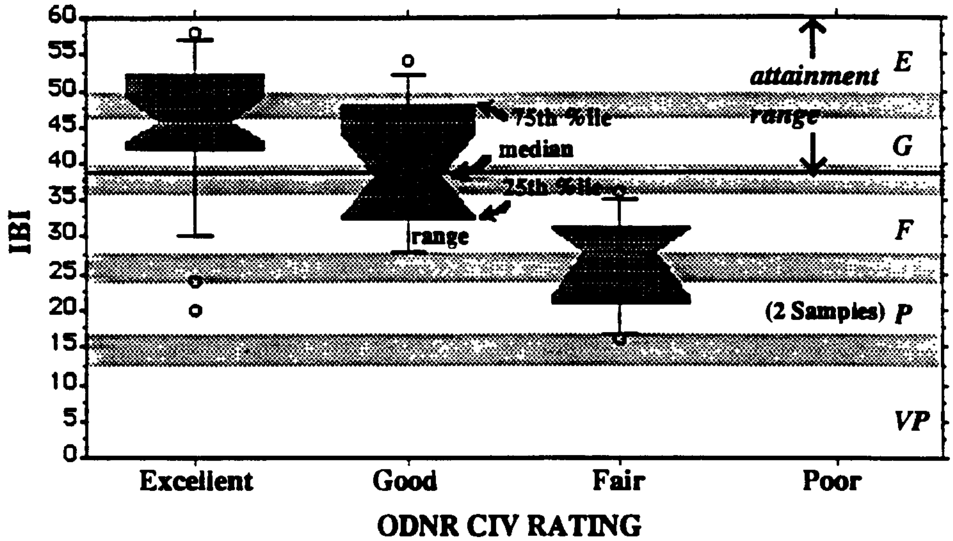

Figure 4. Notched box plots of Cumulative Index Value (CIV) qualitative ratings versus Index of Biotic Integrity (IBI) scores, 25th and 75th percentiles, IBI range, and IBI outliers (>2 interquartile ranges from median). IBI qualitative ratings (exceptional, good, fair, poor, and very poor) appear on the right vertical axis. Shading indicates approximate boundaries between ratings and the variability of the index.

from the comparison, the definition between ODNR's qualitative ratings and the corresponding IBIs increased (Fig. 5). The median IBI score for each corresponding ODNR rating fell in the correct qualitative range for the IBI, and the IBI ranges for the "excellent" and "good" ratings were shortened and more defined. The IBI range for sites rated "good" was still considerably large, but it was centered in the correct IBI qualitative range. For "fair" sites, all IBIs fell in the non-attainment range of less than 40. A box plot of those sites with drainage areas greater than 200 square miles further illustrates the impact of drainage area. (Fig. 6). Notice that, for sites of larger drainage, there is no detectable definition between sites rated "excellent" and "good" and the corresponding IBIs. The IBI ranges for the CIV ratings are notably similar.

Comparison of the ICI and ODNR's CIV

For sites rated "excellent" by ODNR's method, ICIs ranged from 41 to 57 (Fig. 7). This range includes ICIs which the OEPA would consider "good" to "exceptional." As in the IBI comparison, the CIV ratings did not exactly match the ICI ratings of the OEPA. However, all of the ICIs corresponding to the "excellent" rating did fall at a value of 35 or above, indicating attainment of aquatic life uses, as designated by the OEPA for the ICI. For sites rated "good," the corresponding ICIs showed a wide range, with approximately 62% of the values indicating attainment of aquatic life uses, while the other 38% of the values indicated non-attainment. ICI values were less than 35 at sites where "fair" ODNR results were observed, indicating non-attainment of life uses. No "poor" sites for

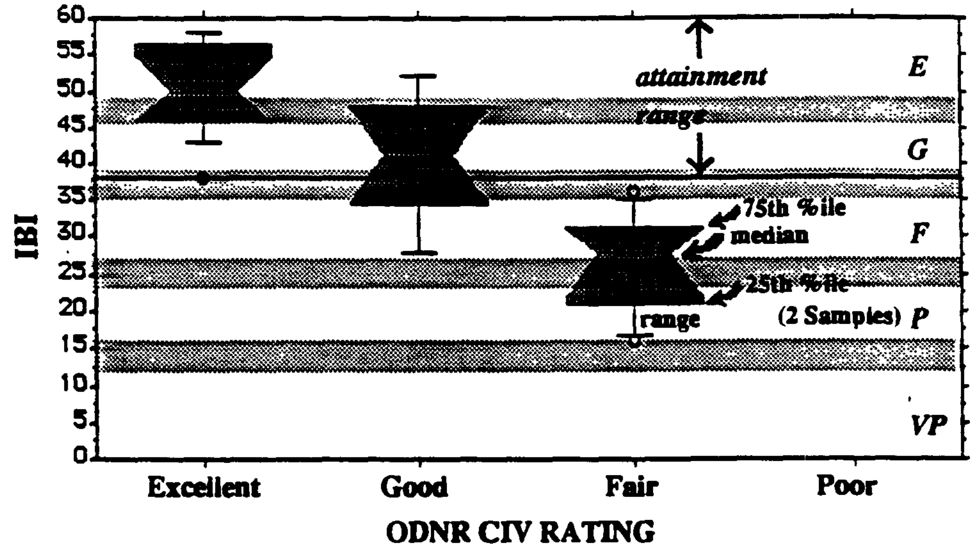

Figure 5. Notched box plots of Cumulative Index Value (CIV) qualitative ratings versus Index of Biotic Integrity (IBI) scores, 25th and 75th percentiles, IBI range, and IBI outliers (>2 interquartile ranges from median) for sites with drainage area ≤ 200 sq. mi. IBI qualitative ratings (exceptional, good, fair, poor, and very poor) appear on the right vertical axis. Shading indicates approximate boundaries between ratings and the variability of the index.

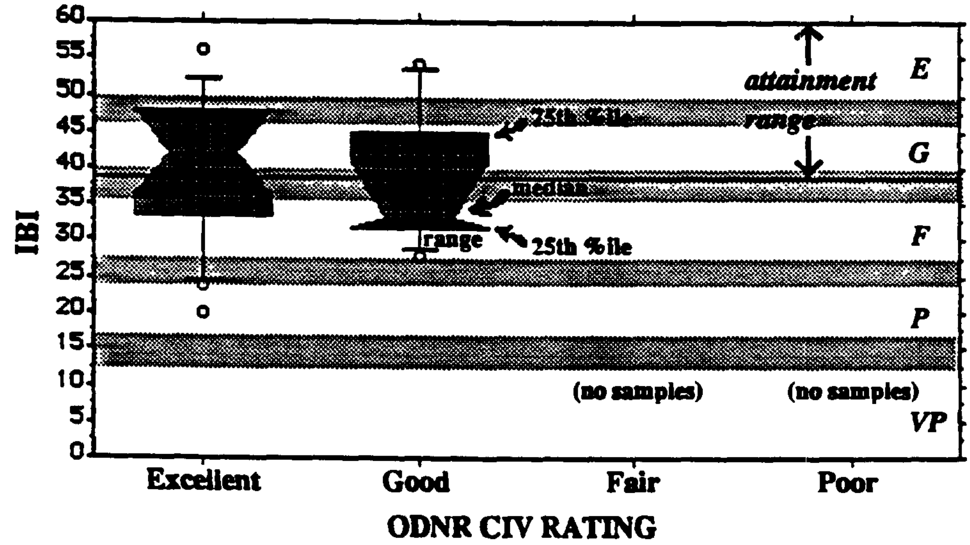

Figure 6. Notched box plots of Cumulative Index Value (CIV) qualitative ratings versus Index of Biotic Integrity (IBI) scores, 25th and 75th percentiles, IBI range, and IBI outliers (>2 interquartile ranges from median) for sites with drainage area > 200 sq. mi. IBI qualitative ratings (exceptional, good, fair, poor, and very poor) appear on the right vertical axis. Shading indicates approximate boundaries between ratings and the variability of the index.

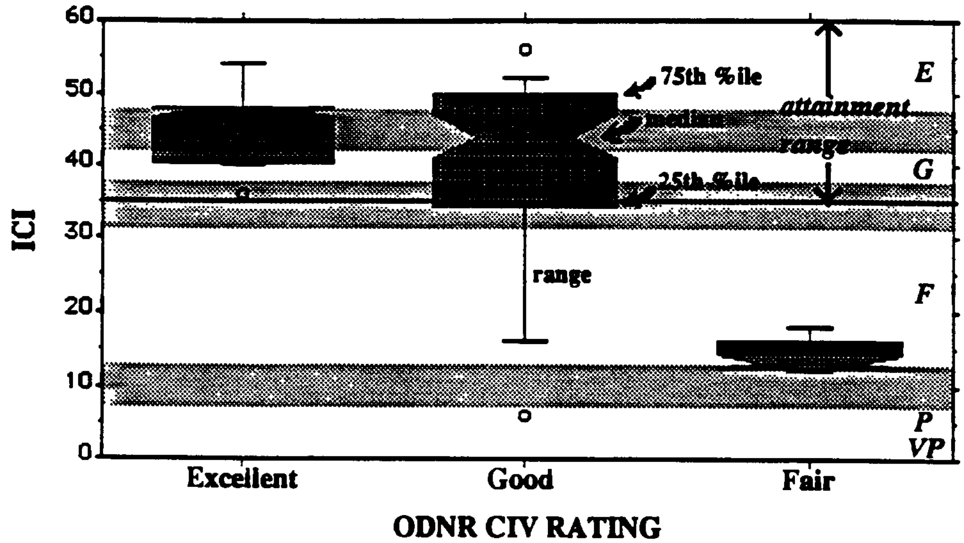

Figure 7. Notched box plots of Cumulative Index Value (CIV) qualitative ratings versus Invertebrate Community Index (ICI) scores, 25th and 75th percentiles, ICI range, and ICI outliers (>2 interquartile ranges from median). ICI qualitative ratings (exceptional, good, fair, poor, and very poor) appear on the right vertical axis. Shading indicates approximate boundaries between ratings and the variability of the index.

comparison with the ICI were present in the data set. Overall, there was a closer correlation (the ICI ranges for the CIV ratings were more defined) between ODNR's CIV ratings and the ICI than ODNR's ratings and the IBI. The "good" CIV rating still encompassed a large range of ICIs, however, and the actual CIV and ICI ratings did not always match.

The differences between ODNR and OEPA macroinvertebrate assessments may be due, in part, to the fact that the OEPA retains its collections for microscopic investigation and they are better able to locate and identify small early instar forms of these organisms. Another factor is that the OEPA researchers always make an attempt to sample a riffle, a run, and a pool area when performing their qualitative collection procedure. This could result in a higher diversity of organisms in their samples as compared to ODNR's samples, which are taken only from riffle areas. In addition, both the IBI and ICI incorporate a correction factor to adjust for drainage area impacts, while ODNR's technique does not. For the ICI/CIV comparison, drainage area impacts were not found to noticeably affect the correlation.

<u>Comparison of Volunteer and Staff Assessments</u>

Volunteer ratings tended to be higher than assessments made by ODNR staff members (Fig. 8). For sites rated "excellent" by staff, approximately 80% of volunteer CIVs fell in

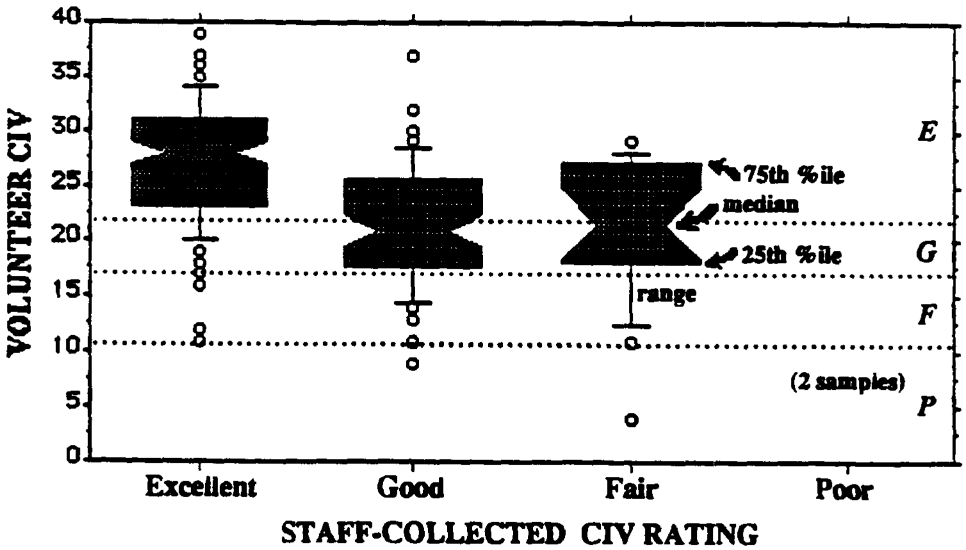

Figure 8. Notched box plots of ODNR staff ratings versus volunteer-generated Cumulative Index Values (CIVs), 25th and 75th percentiles, CIV range, and CIV outliers (>2 interquartile ranges from median). CIV qualitative ratings (excellent, good, fair, and poor) appear on the right vertical axis. Dashed lines indicate boundaries between ratings.

the excellent range, showing agreement. For sites rated "good" or "fair" by staff, the range of corresponding volunteer CIVs was wide, including CIVs which would be rated "fair" to "excellent." Differences between staff and volunteer ratings may be due to misidentification of organisms by volunteers, a misconception among program volunteers that water quality is always "excellent" in Ohio's Scenic Rivers (potential bias), or a greater sampling effort by volunteers as compared to staff members, who may rush through many reference sites in a day. In the Central Ohio area (ODNR's headquarters), stream quality monitoring coordinators have received better instruction on sampling strategy through frequent contact with program administrators and, as a result, the volunteer and staff assessments for this region showed closer agreement. This suggests that part of the reason for the general lack of agreement may be due to insufficient sampling by the ODNR stream quality monitoring coordinators, although all of the aforementioned factors probably contribute to the high variability of these results.

Summary

The qualitative ratings of ODNR's volunteer monitoring technique do not necessarily agree with the qualitative ratings of the OEPA. However, ODNR's CIV ratings do tend to reflect the attainment ("excellent" CIVs) or non-attainment ("fair" and "poor" CIVs) of aquatic life uses, as designated by the Ohio EPA, for both the IBI and the ICI. Hence, the assessments may be useful in screening sites at a basic level.

CIV ratings tend to reflect IBI ratings more accurately in streams and rivers with smaller drainage areas. Drainage area did not appear to have a marked effect on the correlation between the CIV and ICI, but further collection of data could amplify an otherwise undetectable effect. Adequacy of sampling with the use of ODNR's technique may also affect the correlation. The results of this research suggest that larger drainage areas may require a modified approach, although determining exactly what that approach should entail is beyond the scope of this project.

A review of ODNR's database revealed that program volunteers tend to overrate the health of Ohio's Scenic Rivers as compared to staff assessments. This is probably due to a lack of standardization in the number of samples collected and misidentification of the organisms. These problems could be solved through more thorough training and better communication between ODNR, the regional coordinators, and the volunteers. To improve on the program, a measure of sampling effort and better quantitative estimates could be incorporated.

It should be noted that the range of observed CIV ratings used for the comparisons in this paper is constricted. There were relatively few sites which were rated "fair" or "poor" using ODNR's "kick-seine" method. Further collection of data will be necessary before suggestions of revisions to the scoring criteria or rating system can be made.

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The Effects of Sediment Deposition on Insect Populations and Production in a Northern Indiana Stream

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Abstract
In 1986 the St. Joseph County, Indiana, Drainage Board began conducting routine maintenance operations in and along Juday Creek, a third-order tributary of the St. Joseph River. These activities, which include debris and snag removal from stream channels, have led to a large increase in sediment deposition into the lower reaches of the stream. Monthly benthic invertebrate samples were collected from June 1989 to June 1990 from a riffle area in Juday Creek and insect densities and secondary production rates during this time were compared to those from a previous study at the same site in 1981-82. Invertebrate density and production rate responses varied based on functional feeding group. Among filter-feeders, two species showed significantly lower mean annual densities in 1989-90 compared to 1981-82, two species showed significantly lower densities during several months in 1989-90 versus corresponding months in 1981-82, and only one species (Hydropsyche morosa) showed significantly greater density in 1981-82. Among collector-gatherers, mean annual densities were significantly higher for five of six species collected in 1989-90. Shredders showed mixed responses, with two species having significantly higher mean annual densities in 1981-82, and one species, Taeniopteryx nivalis, having higher densities in 1989-90. While production rates of three of the five species for which production rates were calculated increased in 1989-90, the net effect of the increased sediment deposition was a reduction in the combined production rates of the five species from 2765.1 mg/m^2/year in 1981-82 to 653.8 mg/m^2/year in 1989-90.

Key Words: sediment, water quality, secondary production, functional feeding group, filter-feeders, collector-gatherers, shredders, benthic macroinvertebrates

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Introduction

The effects of anthropogenic disturbances such as clear-cut logging, modification of riparian vegetation, and changes in land-use practices on streams and aquatic organisms are well documented (Dance and Hynes 1980, Swanson et al. 1982, Ward 1984). Physical and chemical attributes of streams affected by these activities include light penetration (McIntire and Colby 1978), water temperature (Hall and Lantz 1969, Holtby 1988), nutrient concentrations (Chauvet and Decamps 1989), and inputs of woody debris and sediments (Bryant 1983, Swanson et al. 1987). Such changes have been shown to affect algae (Minshall 1978), macrophytes (Hynes 1970), invertebrates (Newbold et al. 1980), fish (Thedinga et al. 1989, Ward and Stanford 1989) and other vertebrates (Hawkins et al. 1983, Spencer et al. 1991). However, impacts associated with more subtle or routine activities such as removal of snags and woody debris from streams are less understood. Because such operations likely represent common and widespread management practices in the midwestern United States, quantifying their impact on the biota is essential.

In 1986, the St. Joseph County (Indiana) Drainage Board began to perform maintenance operations in and along Juday Creek, a third-order tributary of the St. Joseph River. Included in these activities are removal of snags, debris, and trees that block water flow and that might result in flooding or pooling along the stream. Coincident with these practices in Juday Creek has been a large increase in sediment transport and subsequent deposition in downstream locations. Because data on insect production rates and populations were collected from this stream in 1981-82 (Schwenneker 1985) and 1985-86 (M.B. Berg pers. comm.), prior to drainage board activities, we had an opportunity to document changes in population densities and production rates of stream benthos that may have resulted from stream maintenance operations. Our objectives were to consider 1) the effects of increased sediment deposition on benthic macroinvertebrate populations; 2) whether changes in population densities were reflected by changes in invertebrate secondary production rates; and 3) the implications of these results for predicting invertebrate responses to sediment deposition.

Study Site

This study was conducted in a riffle area of the creek (41°43'N, 86°16'W, elevation = 206m) on land owned and maintained by the St. Joseph Co. Chapter of the Izaak Walton League of America. Juday Creek is important from a conservation perspective because it is one of only a few streams in Indiana known to support breeding trout populations. The upper segment flows through flat, agricultural land, and then makes its way through primarily residential areas. The lower segment, which includes the study location, flows through natural, deciduous woodlands and has a gradient of 1.3%. The site is heavily shaded from the late spring through the early fall, and the substrate is a mixture of sand, gravel, and cobble. Some of the physical and chemical data collected at this site in 1981-82 and 1989-90 are summarized in Table 1.

A silt trap is located approximately 100 m upstream from the site. It was built to protect the lower segment of Juday Creek from excessive sediment deposition. Prior to drainage board operations, approximately 18 yd^3 of sediment were removed from the silt trap every 1.5-2 years. Since 1988 the silt trap has been dredged once a year. In 1989, approximately 90 yd^3 of sediment were removed from the trap (J. Moore, pers. comm.). However, during the 1989-90 sampling period, the trap was completely filled after 4 months. Therefore, it provided little or no protection to the lower part of the stream for 8 months. During March and early April 1990, a large pulse of sediment entered the study area, resulting in extensive coverage of the gravel and cobble substrate with sand. This pulse was apparently a result of an unusually high number of stream maintenance activities during this period compared to previous years.

Materials and Methods

Benthic samples were collected monthly for a period of thirteen months during the course of two separate studies. The first was from September 1981 to September 1982 (Schwenneker 1985) and the second from June 1989 to June 1990. In both studies, ten random benthic bottom samples were collected each month from the riffle using a 0.09 m^2 Hess sampler with a mesh size of 333 μm. Because of extremely high water levels, samples could not be collected in January and March of 1982, and these two months were omitted from comparisons between years. Samples were preserved in 80% ethanol and transported back to the laboratory for processing and analysis.

In the laboratory, invertebrates were sorted from the substrate using sugar flotation (Anderson 1959) and then identified to species and instar or size class. Instar determinations were based on head capsule width, except for stoneflies and mayflies. These organisms were divided into size classes based on body length from the front of the head to the base of the cerci. Population densities were recorded for the most abundant species (excluding chironomids), and each was assigned to a functional feeding group (Merritt and Cummins 1984). Mean annual densities were compared between years using a repeated measures analysis of variance on log transformed data

Table 1. Comparison of physical and chemical characteristics of Juday Creek in 1981-82 and 1989-90.

	1981-82	1989-90
Temperature (°C)	2.5-17.0	1.5-20.5
Current Velocity (m/s)	0.3-0.5	0.4-0.65
Depth (cm)	20-30	20-40
Alkalinity (mg/l $CaCO_3$)	150	182
Nitrate (mg/l-N)	1.0	1.5-1.7
Orthophosphate (mg/l)	0.13	0.16
Conductivity (µmho/cm)	600	610-680

In addition, densities of each species were compared month by month between years (i.e., January 1982 versus January 1990) using a one-way ANOVA. Production rates were calculated for all species in the 1981-82 study, and for five species in the 1989-90 study, including Hydropsyche sparna Ross, Hydropsyche betteni Ross, Optioservus fastiditus (LeConte), Baetis vagans McDunnough, and Taeniopteryx nivalis (Fitch). Dry weights for each instar or size class of these organisms were obtained by drying at 70°C for 48 hours. Production rates were measured using either the instantaneous growth method for those species with distinguishable cohorts, or the size-frequency method for those with cohorts that could not be distinguished. Instantaneous growth calculations were made from computer programs developed by Schwenneker (1985) and Berg (1989). Size-frequency production calculations were made using the Aquatic Ecology-PC software package (Ekblad 1986). Because early instars often are inefficiently sampled, apparent increases in population densities are sometimes seen during cohort development. For production rate calculations, densities were back-calculated using the catch-curve method of Waters and Crawford (1973). The result of this correction was that if densities were lower at sampling time T-1 than at time T, densities at time T-1 were set equal to those at time T. Given the typical log-type decline exhibited by stream invertebrates, this correction represents a conservative estimate of densities (Schwenneker 1985).

Results

Population responses of individual species to sedimentation varied depending on functional feeding group. Among the six species of filter-feeders, four had lower mean annual densities in 1989-90, although only two of these differences were statistically significant (Table 2). Hydropsyche sparna and Chimarra obscura (Walker) showed the most dramatic density reductions in 1989-90 (over 80% and 95% respectively). Mean annual densities of Hydropsyche betteni and Cheumatopsyche petiti (Banks) were not significantly different between years, but each did show significant density differences in eight of the ten monthly comparisons (Fig. 1). During six of these months, H. betteni showed higher densities in 1981-82 compared to 1989-90, and C. petiti had significantly higher densities in 1981-82 during five months. Hydropsyche morosa (Ross) mean annual density was significantly higher in 1989-90 compared to 1981-82, and Simulium sp. had similar densities between years. All six filter-feeders had lower densities, four of which were significant ($p < .05$), in April and May of 1990 versus 1982.

The reduction in secondary production rates of filter-feeders in 1989-90 was much more pronounced than changes in population densities (Fig. 2). The production rate of H. sparna dropped more than 90% in 1989-90 from the 1981-82 rate. From 1981-82 to 1989-90, the production rate of H. betteni declined by more than 50%, while mean annual density was reduced about 30% in 1989-90.

Population densities of collector-gatherers showed different responses to the increased sediment deposition than the of filter-feeders. Five of the six species of collector-gatherers showed significantly greater mean annual densities in 1989-90 compared to 1981-82

Table 2. Mean annual densities (N/M^2) and p values of individual taxa in 1981-82 and 1989-90. * = $p < 0.05$; ** = $p < 0.01$.

	1981-82 Mean (1 SE)	1989-90 Mean (1 SE)	p
Filter-feeders			
Cheumatopsyche petiti	223.2 (18.2)	194.3 (30.6)	0.270
Chimarra obscura	44.2 (4.0)	2.2 (0.4)	**0.001
Hydropsyche betteni	258.8 (23.0)	187.9 (24.2)	0.097
Hydropsyche morosa	26.3 (2.4)	113.6 (18.9)	**0.001
Hydropsyche sparna	1648.7 (136.9)	293.7 (35.6)	**0.001
Simulium sp.	45.9 (5.4)	47.3 (13.4)	0.376
Collector-gatherers			
Antocha sp.	43.9 (3.3)	57.6 (5.2)	**0.001
Baetis vagans	17.3 (2.4)	32.7 (12.0)	**0.001
Macronychus glabratus	----------	34.0 (4.5)	-------
Optioservus fastiditus	22.1 (1.7)	144.0 (12.8)	**0.001
Stenelmis crenata	3.9 (0.7)	155.1 (13.0)	**0.001
Stenonema spp.	56.5 (5.1)	91.3 (10.7)	**0.001
Shredders			
Amphinemura delosa	66.6 (8.6)	3.4 (0.7)	**0.001
Taeniopteryx nivalis	6.5 (1.4)	38.8 (8.1)	**0.001
Tipula abdominalis	3.3 (0.5)	1.3 (0.3)	**0.001

(Table 2). The elmid beetle larvae Optioservus fastiditus and Stenelmis crenata (Say) had the largest increases in mean annual densities between years (650% and almost 4000%, respectively). Macronychus glabratus (Say) was collected so rarely in 1981-82 that its density was not reported by Schwenneker (1985). Therefore, we could not compare densities between years, although densities were clearly greater in 1989-90. Densities of some species of collector-gatherers dropped in April and May of 1990, although some declines could be explained partly by life history characteristics.

We compared secondary production rates between years for two species of collector-gatherers, Optioservus fastiditus and Baetis vagans. Production rates showed changes

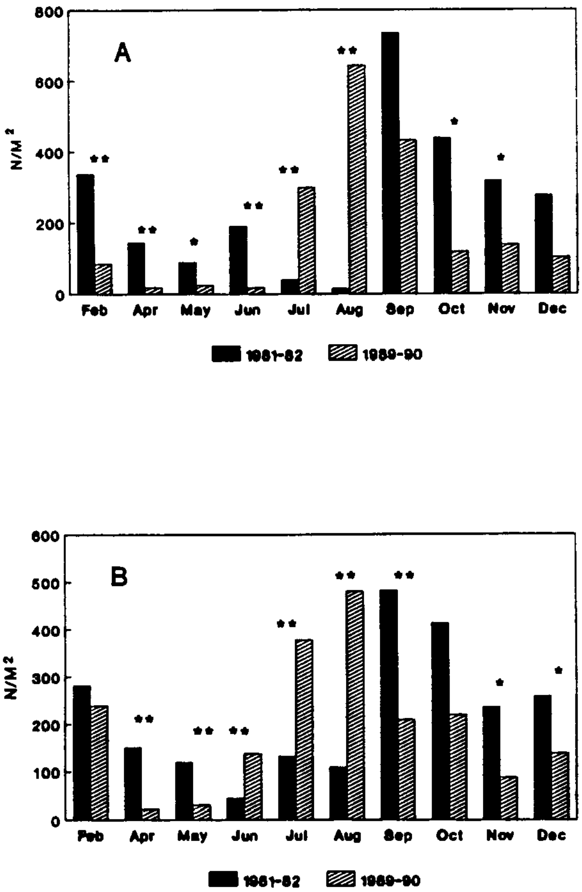

Figure 1. (A) Mean densities (N/M^2) by month for Hydropsyche betteni in 1981-82 and 1989-90. (B) Mean densities (N/M^2) by month of Cheumatopsyche petiti in 1981-82 and 1989-90. $^*=p<0.05$; $^{**}=p<0.01$).

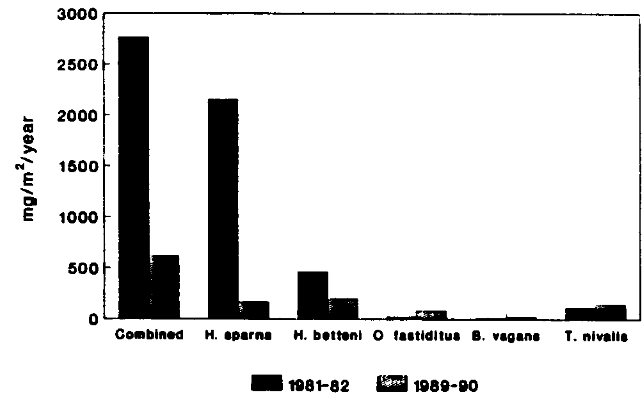

Figure 2. Combined (all 5 species) and individual species secondary production rates (mg/m^2/yr) in 1981-82 and 1989-90.

similar to those of the population densities for these species. Production rates of O. fastiditus and B. vagans were more than 300% and 200% greater, respectively, in 1989-90 than in 1981-82 (Fig. 2).

Population densities of shredders showed mixed responses between years (Table 2). Mean annual densities of Amphinemura delosa (Ricker) and Tipula abdominalis (Say) were significantly lower in 1989-90 than in 1981-82, Mean annual densities of Taeniopteryx nivalis, on the other hand, increased significantly in 1989-90. However, the secondary production rate of T. nivalis was only 20% higher in 1989-90 (Fig. 2), because of a greater mean individual biomass in 1981-82.

The predator functional feeding group is not represented because the only predaceous invertebrates at this location besides flatworms were the filter-feeding hydropsychid caddisflies. The only obligate scraper, Glossosoma intermedium (Klapalek), had greater densities in 1981-82 than in 1989-90 (8.91/m^2 vs. 6.6/m^2), but differences were not significant ($p=0.389$).

Three of the five species for which we

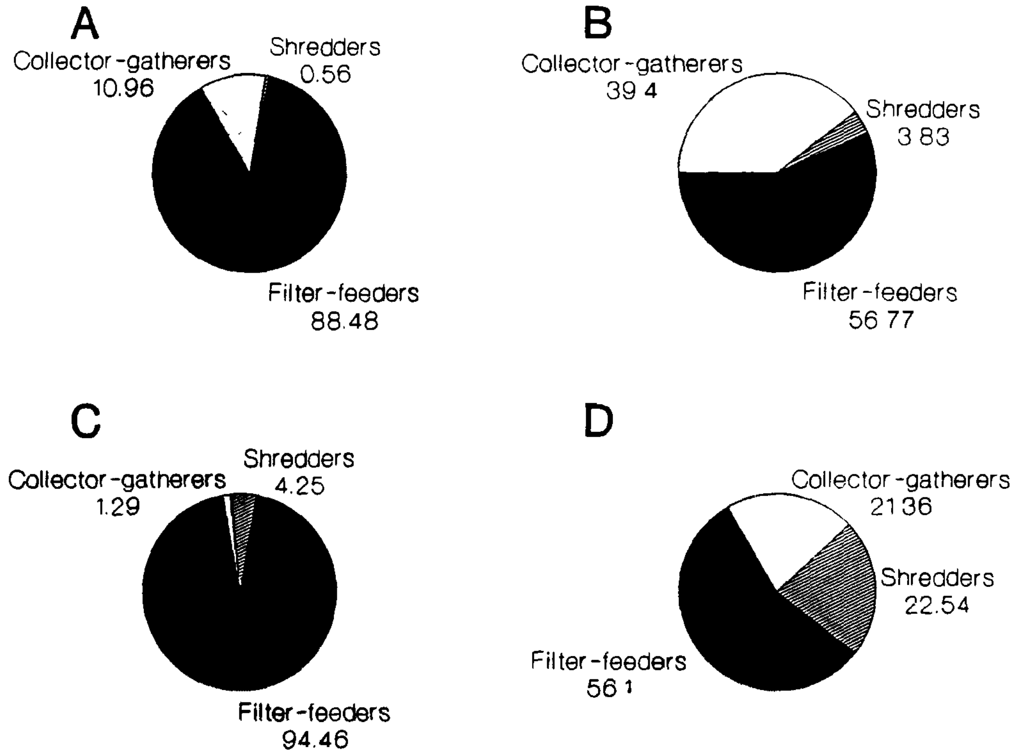

Figure 3. Percent contribution by functional feeding group to (A) 1981-1982 total densities, (B) 1989-90 total densities, (C) 1981-1982 secondary production rates, (D) 1989-90 secondary production rates. Relative values are calculated using only five species in Figure 2.

calculated secondary production rates had higher rates in 1989-90 compared to 1981-82. However, summing the production rates for all five species resulted in a substantial decline in production rates in 1989-90 compared to 1981-82 (Fig. 2). Of these five species, the two filter-feeders in 1981-82 contributed 88.48% of the numbers and 94.46% of the combined secondary production rate (Fig. 3). In 1989-90, these filter-feeders declined in both densities and production rates, resulting in a large decline in total secondary production rates. The three species that increased in production rate in 1989-90 versus 1981-82 were those that contributed little to total production rate. The result is a shift from a community dominated by filter-feeders in both numbers and production rate in 1981-82 to a community in 1989-90 in which collector-gatherers and shredders increased in importance in terms of relative contribution to both numbers and production (Fig. 3).

Discussion

It seems likely that the shift in invertebrate community structure and decreased secondary production rates observed in 1989-90 was the result of increased sediment deposition into the study area. Differences cannot be explained by changes in the physicochemical characteristics of Juday Creek between years (Table 1). Other authors have noted that reduction or elimination of the overhead canopy can result in abrupt changes in abundances of stream invertebrates (Behmer and Hawkins 1986, Newbold et al. 1980). However, the riparian vegetation in this part of the stream has remained undisturbed. Attributing differences between years to increased fish predation also is unsupported. Furthermore, data collected in 1985-86 showed that insect species abundances were similar to those from 1981-82 in this section of the stream (M.B. Berg, pers. comm.). The county drainage board began maintenance operations along Juday Creek soon after the 1985-86 study. Almost immediately, sediment deposition into the lower portion of Juday Creek increased substantially. The result has been severely reduced population densities for some species and higher densities for others.

Functional feeding group classification proved to be a good predictor of a species' population response to the sediment deposition. Filter-feeder population densities were the most severely reduced (Table 2, Figure 1). Reduction of filter-feeder densities due to greater sediment deposition is consistent with their biology and habitat requirements. These organisms require a solid and somewhat stable substrate on which to spin their nets (Hynes 1970, Minshall 1984), and are considered intolerant of heavily silted and sandy areas where they lose their attachment to the substrate (Marsh and Waters 1980, Tebo 1955). In addition, large amounts of suspended inorganic sediments can clog nets and interfere with the feeding mechanisms of the net-spinners (Nuttall and Bielby 1973). These results generally are consistent with those found in other studies. The net-spinning caddisfly Arctopsyche grandis (Banks) is highly sensitive to sedimentation due to the filling of interstitial spaces of rocks (Cline et al. 1982, McClelland and Brusven 1980). Barton (1977) found reduced densities of Hydropsyche slossonae Banks (48%) and Cheumatopsyche sp. (66%) in an Ontario stream immediately downstream from a highway construction site compared to upstream locations. He suggested that damage to benthos only occurs when stones are buried by sediment, which was the case in our study. Gurtz and Wallace (1986) and Benke et al. (1984) found lower production rates of net-spinning caddisflies on sand when compared with more stable substrates. Other studies also have found that filter-feeders are intolerant of sediment additions (Cherry et al. 1979, Nuttall and Bielby 1973, White and Gammon 1976).

In contrast to the filter-feeders, mean annual densities of all collector-gatherers increased significantly in 1989-90 from the 1981-82 levels (Table 2). Although most of the deposited sediment was inorganic sand during the 1989-90 study, the amount of organic material deposited in the riffle area probably increased as well. This would result in a greater food supply for the collector-gatherers, which forage along the substrate for food particles (Berkman et al. 1986, Merritt and Cummins 1984). Elmid beetles showed the greatest rise in numbers (Table 2). Although studies have shown that Optioservus and Stenelmis prefer larger, solid substrates (Cummins and Lauf 1969, Marsh and Waters 1980, Rabeni and Minshall 1977), these organisms are known to tolerate fine sediments to some extent (Brown 1987, White and Gammon 1976). The reason for the increased density of Macronychus glabratus in 1989-90 is unclear, since this species is almost always associated with wood substrate (Brown 1987, Hynes 1970). The amount of woody debris in the stream was not quantified in 1981-82 or in 1989-90. Stenonema also is somewhat tolerant of silt (Dance and Hynes 1980, Jones and Clark 1987), and could benefit from an increase in deposition of organic matter. Investigators have found that Baetis nymphs generally prefer larger substrates and drift in the presence of large quantities of sediment (Culp et al. 1986, Wagner 1989, White and Gammon 1976). However, numbers of the European species Baetis rhodani (Pictet) increase as sediment deposition increases (Nuttall and Bielby 1973, Scullion and Edwards 1980). Wallace and Gurtz (1986) found that although production rate of Baetis sp. was greatest on cobble and gravel, individuals of this genus were found in moderate numbers in sandy areas. Culp et al. (1986) showed that while sediment transport reduced B. tricaudatus Dodds densities by 67%, sediment deposition actually decreased drift rates of this species. As with the other collector-gatherers in this study, the potential increase in food material transported into the study area may have offset any negative effects on B. vagans associated with the loss of stable substrate. However, densities of O. fastiditus, Antocha sp., and Stenonema spp. all decreased substantially in April and May of 1990, coinciding with the sediment pulse that covered the study site. It is possible that continued heavy sediment deposition could eventually cause population declines in many of these collector-gatherers.

The population responses of the shredder functional feeding group varied depending on the species (Table 2). Taeniopteryx nivalis appeared to benefit from the increased sediment deposition. T. nivalis nymphs are generally found in leaf packs or other debris along the stream margins (Sephton and Hynes 1984, Stewart and Stark 1988). This species may not be affected by sediment that covers the cobble and gravel substrate as long as there are sufficient leaf packs in the stream. During this study, there were many leaf pack accumulations both in the main channel and along the margins and backwater areas. Like the collector-gatherers, T. nivalis may have benefitted from a potential increase in available detritus in the study area. Because adults emerge in February and the nymphs diapause deep in the substrate until September (Stewart and Stark 1988), this species would not have been affected by the sediment pulse that occurred in the spring. In contrast, Tipula abdominalis had significantly lower densities in 1989-90 compared to 1981-82 (Table 2). Cummins and Lauf (1969) found that Tipula caloptera Loew preferred coarse substrates, but showed a wide tolerance for finer sediments, including silt. They suggest that these larvae are probably found in microhabitats of finer sediments and organic material between and behind coarse sediments. If T. abdominalis has similar tolerances, then this species should be moderately affected by the increased deposition. Amphinemura delosa densities also were significantly lower in 1989-90 (Table 2). Another congeneric species, A. sulcicollis (Stephens), is known to move from leaves to stone as it develops (Hynes 1976). If A. delosa exhibits a similar habitat shift, then a reduction in numbers would be expected with increasing sediment deposition. However, Scullion and Edwards (1980) found that A. sulcicollis was tolerant to mine discharge siltation. The lack of tolerance of A. delosa in our study may represent species-specific differences in tolerance or may be related to the amount of material deposited.

Most studies that examine the effects of sediment deposition on benthic invertebrates look only at changes in population densities or relative changes in species composition (Barton 1977, Nuttall and Bielby 1973, Scullion and Edwards 1980). The few studies that have looked at changes in biomass found it to be a better measure of benthos response to sedimentation than density and diversity (Letterman and Mitsch 1978, Marsh and Waters 1980). We have found no studies that examine the effects of sediment deposition on the secondary production rates of stream invertebrates. Studies have compared invertebrate secondary production rates between logged and unlogged areas (eg. Wallace and Gurtz 1986), but have difficulty separating the relative effects of sediments, increased algal growth, and water temperatures.

There are many advantages to calculating secondary production rates rather than looking only at changes in abundances. Secondary production incorporates a measure of individual growth as well as population density, and provides a measure of the functional importance of a species to stream energy flow and nutrient processing (Short et al. 1987). From a management perspective, invertebrates are an important component of fish diets and may limit fish production (Benke 1984, Tebo 1955). Our results suggest that changes in secondary production rates between years give a clearer picture of what is happening to the benthos in Juday Creek than changes in abundance. Because eight species show greater densities in 1989-90 compared to six species with greater densities in 1981-82, examination of numbers alone suggests that the only impact of the sediment on the invertebrate community was a change in relative abundance of species. However, the species that had reduced densities in 1989-90 are those that contributed the most to invertebrate secondary production rates in 1981-82. Those that had increased densities in 1989-90 contributed little to secondary production in 1981-82 (Figure 2). The result was that the combined production rates for five species decreased from 2765.1 mg/m^2/year in 1981-82 to 653.8 mg/m^2/year in 1989-90. This 78% reduction in secondary production rate represents a substantial decline in the amount of food available to support higher trophic level organisms such as fish. The response of Taeniopteryx nivalis populations also represents a good example of the value of calculating production rates. While densities of this species increased approximately 6 times in 1989-90 from 1981-82, production rates increased only 20% (Fig. 2). The large increase in mean densities of this species was mostly offset by lower individual growth rates.

Our results suggest that a functional feeding group classification of organisms offers a method for predicting the downstream impacts of stream maintenance activities. If a community is dominated by filter-feeders, then substantial impacts associated with an increase in sediment deposition may be expected. If collector-gatherers contribute the majority of invertebrate production, then moderate increases in sediment transport and deposition may actually enhance population densities and production rates through increased import of organic material. However, heavy, sustained sediment deposition will probably have a negative impact on many of these species as well. In addition, secondary production rates are a better measure of invertebrate community response than diversity and population density. Finally, many studies have documented that the benthos recovers rapidly when the source of sediment is eliminated and high flows are allowed to wash the sediment downstream (Barton 1977, Cherry et al. 1979, Tebo 1955). If silt traps are properly maintained and cleaned out before filling with sediment, then it is possible that negative impacts associated with instream maintenance operations can be reduced.

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Agricultural Impacts on the Fishes of the Eel River, Indiana

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Abstract
The Eel River of northern Indiana is a major tributary of the Wabash River. It is approximately 177 km (110 mi) in length with an average rate of descent of 0.457 m/km (2.41 ft/mi). Approximately 79% of its 210,800 ha (814 m^2) drainage basin is devoted to row-crop agriculture. The fish communities and habitat were studied during the summer of 1990. Fish were collected from 25 sites located throughout the Eel River and some of its tributaries. A 3/16 inch mesh, 30 ft by 4 ft seine was effective in collecting small fish including darters. Backpack electrofishing was also used at most stations on two separate dates. Historic records of the fish communities were examined and, when possible, converted into Indexes of Biotic Integrity values so that changes over time could be estimated. Habitat evaluation included a mainstem reconnaisance, a habitat survey (HEP) conducted at all collecting stations, and a synoptic turbidity survey on July 16 and 17, 1990. Estimates of the amount of woodland were made from conventional analysis of enlarged infrared photographs. Analyses of existing suspended sediment data were used to evaluate possible impacts of nonpoint-source influence from agricultural fields as well as historic records of fish kills and chemical spills within the Eel River watershed. The 1990 fish community was generally better than the community found in 1982. However, this improvement is probably temporary and the result of a series of recent years when both river discharge and suspended sediment concentrations were lower than normal. From a longer time perspective the fish community is degraded, with many species which were common 50 years ago now either absent or very severely reduced. Rainbow darter, orangethroat darter, bluebreast darter, and stonecat were not collected at all. Sculpin, greenside darter, blackside darter, silver shiner, rosyface shiner, longear sunfish, and smallmouth bass were very restricted in distribution.

Key Words: non-point source, habitat, suspended sediment, fish community, Indiana

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Introduction

In 1982 populations of smallmouth bass (Micropterus dolomieui) were found to be virtually lacking by Braun and Robertson (1982) who collected from the same sites as Taylor (1972). Exerting roughly equivalent effort and similar methods, Taylor (1972) collected 98 smallmouth bass while Braun and Robertson (1982) found only 3. During the 1980's smallmouth bass populations in the lower part of the Eel River were augmented by stocking fin-clipped fingerlings (5130 fish on 10-28-83; 5000 on 9-17-85; and 6960 on 4-17-86). A limited number of stations were more intensively sampled and additional tributaries were also investigated.

The current study was planned to provide information about the fish communities at all of Taylor's sites and a few additional sites. It included an evaluation of instream and near-stream habitat from the standpoint of agricultural nonpoint sources of pollution and their possible influence on those fish communities. This report is a condensation and extension of a larger report to the Indiana Department of Environmental Management (Gammon and Gammon 1990).

The Study Area

The Eel River is a major tributary of the Wabash River in northern Indiana. Originating in northwest Allen county near Ft. Wayne, it flows southwest for approximately 177 km (110 miles) through Kosciusko, Whitley, Wabash and Miami counties into the Wabash

River at Logansport in Cass county. Its rate of descent is approximately 0.457 m/km (2.41 ft/mile) with a lower rate in the upper third and a slightly higher rate in the lower 20 km.

This area originally contained glacial lakes and swampy wetlands, but it was extensively ditched and drained prior to 1900 for agricultural use. Approximately 79% of its 2,148 km2 (814 m2) drainage basin area (Hoggatt 1975) is devoted to rowcrop agriculture, primarily corn and soybeans. Most of the smaller tributaries and the upper river have been channelized to facilitate drainage. Low mill dams have been constructed at various locations, many of which are currently in a state of disrepair except in Logansport. That dam severely restricted the movement of Wabash River fishes into the Eel River and facilitated evaluations of impacts.

Materials and Methods

The study included a) a reconnaisance float trip of the entire river, b) sampling each station twice by electrofishing, c) sampling most of these same stations once by seining, and d) a habitat survey (HEP) at each station. Secchi transparency and temperature were routinely measured on each occasion. In addition, synoptic short-term profiles of turbidity, temperature, and dissolved oxygen concentration were determined on three separate dates.

Single stations were located on lower Twelve Mile, Paw Paw, Squirrel, Beargrass, and Sugar Creeks, and also upstream and downstream of Columbia City on Blue River. The remaining 16 stations were located on the mainstem of the Eel River. A few mainstem stations (Taylor's 2B, 2, and 3) and Squirrel Creek were not seined because of inappropriate seining habitat.

Seining was conducted with a 30-foot by 4-foot seine having 3/16 inch mesh weighed down by a heavy steel chain tied to the bottom. This method was very effective at capturing darters and minnows. Three seining passes along 20 meters of shoreline constituted each seine sample.

Electrofishing utilized a Safari Bushman 300 backpack shocker carried in a canoe or while wading, depending on place and depth. Each electrofishing sample was about 20 minutes in duration along approximately 400 meters of shoreline. This method was effective in capturing larger fish such as redhorse and suckers and species which prefer nearshore cover such as sunfish and bass.

All captured fish were identified to species, weighed and measured, then released back into the river. Those fish not easily identified in the field were preserved in formalin and returned to the laboratory for identification (Trautman 1981).

Fish data were analyzed using the Iwb and the IBI. The 1990 Iwb values were based upon the average of two electrofishing catches at each station. The rationale of this community parameter is presented by Gammon (1980), who recommended multiple collections at each site.

The Iwb was calculated as:

$$Iwb = 0.5 \ln N + 0.5 \ln W + Div.no. + Div.wt.$$

Where, N = number of fish captured per km; W = weight in kg of fish captured per km; Div.no. = Shannon diversity based on numbers; Div.wt. = Shannon diversity based on weight.

The IBI methodology has been thoroughly discussed by Karr (1981 and 1987), Karr et al. (1986 and 1987), and Angermeier and Karr (1986). Regional applications are summarized by Miller et al. (1988).

Table 1. Scoring criteria used to determine IBI for Eel River fish collections.

Metric	Score 1(worst)	3	5(best)
Fish species (total)	0-9	10-19	≥20
Darter species	0-1	2-3	≥4
Sunfish Species	0-1	2-3	≥4
Sucker Species	0-1	2-3	≥4
Intolerant Species	0-1	2-3	≥4
No. Individuals	0-100	101-200	≥201
Percent individuals as:			
Green sunfish	11-100	6-10	0-5
Omnivores	45-100	21-44	0-20
Insect. cyprinids	0-20	21-44	45-100
Top carnivores	0-2	3-10	≥11
Hybrids	4-10	2-3	0-1
Diseased	6-10	2-5	0-1

Table 2. Habitat assessment scoring criteria (HEP).

Parameter	Condition Excellent	Good	Fair	Poor
PRIMARY INFLUENCE				
Substrate and Instream Cover				
1. Substrate/cover	16-20	11-15	6-10	0-5
2. Embeddedness	16-20	11-15	6-10	0-5
3. Water velocity	16-20	11-15	6-10	0-5
SECONDARY INFLUENCE				
Channel Morphology				
4. Channel Alter	12-15	8-11	4-7	0-3
5. Scour/Deposition	12-15	8-11	4-7	0-3
6. Pool/Riffle Ratio	12-15	8-11	4-7	0-3
TERTIARY INFLUENCE				
Riparian and Bank Structure				
7. Bank stability	9-10	6-8	3-5	0-2
8. Bank vegetation	9-10	6-8	3-5	0-2
9. Bank cover	9-10	6-8	3-5	0-2

The original criteria for determining IBI (Karr, et. al., 1987) were modified slightly for the Eel River (Table 1). The scaled metrics are those used in studies of the Sugar Creek system (Gammon et al. 1990a) and an agricultural analysis of several streams in west-central Indiana (Gammon et al. 1990b). They differ in some details from the criteria used in other studies. The 1990 IBI values were based upon the combined catches from electrofishing and seining. The IBIs calculated on data from earlier Eel River studies may be influenced to an unknown degree by the somewhat different methodologies used to collect fish. Taylor (1972) used a combination of electrofishing and rotenone, while Braun and Robertson (1982) used more intensive electrofishing. We have elected to use the same criteria regardless of stream order.

Habitat was quantitatively evaluated at each mainstem collecting site, except for the most downstream site near the Logansport dam and Taylor's site 1, using a habitat evaluation procedure (HEP; Plafkin et al. 1989) adapted from Platts et al. (1987). HEP quantifies 9 habitat characteristics summarized in Table 2. The total score for each site was based upon data from 10 transects at each site spaced 25, 50, or 100 feet apart.

In addition, several other physical measurements were taken whenever fish collections were made during special longitudinal surveys. Stream turbidity was measured with a secchi disc or a Minispec20 nephelometer. Water temperature and dissolved oxygen were measured using a YSI meter. Water velocity was measured with a Gurley pygmy meter. ALI distances were measured optically using a Leitz rangefinder.

Estimates of the amount of woodland were based on conventional analyses of enlarged LandSat infrared photographs taken on May 2, 1981. These were obtained from the U.S.

Geological Survey (ESIC), EROS Data Center, Sioux Falls, SD.

The drainage area perimeter was determined using topographic maps of tributaries. This scaled map was superimposed over the infrared photographs on a light table. Plots of land with permanent tree cover were outlined on the topographic map.

Using a light table, the marked topographic map was traced onto a fine grid. Individual grids with more than 50% woodland was calculated. Land use in a few tributaries was not determined because of insufficient coverage of LandSat infrared photographs.

Results

A total of 6,635 fish comprising 46 species were captured by electrofishing and seining. Forty species and 4154 individuals (63%) were taken by seining alone. Electrofishing catches also yielded 40 species, but only 2481 individuals or 37% of the total.

Bluntnose minnow (Pimephales notatus) was very common comprising 40.9% of the total number seined, while sand shiner (Notropis stramineus), spotfin shiner (N. spilopterus), striped shiner (N. chrysocephalus), silverjaw minnow (Ericymba buccata), and creek chub (Semotilus atromaculatus) together contributed another 37%.

The electrofishing catch was more evenly distributed with common shiner (N. cornutus) and white sucker (Catostomus commersoni) each contributing about 15% to the catch. Substantial numbers of the following were also collected: creek chub (9.3%), bluntnose minnow (9%), rock bass (Ambloplites rupestris; 7.4%), and northern hog sucker (Hypentilium nigricans; 7.1%).

Smallmouth bass (Micropterus dolomeiui) adults and subadults were mostly found in the lower 50 miles of the Eel River and only in Paw Paw and Twelve Mile Creeks. Catch rates were higher in the lower 30 miles of river and attenuated from RM 30 to RM 51.7. Three of 12 smallmouth bass 250 mm and longer were fin clipped, indicating that they were stocked fish. Two of these were collected by electrofishing at RM 37.8(1) near Roann and the other at RM 27.3(6B) near Chili. Young-of-the-year smallmouth bass were taken only in the extreme lower part of the Eel River and in Paw Paw and Twelve Mile Creeks.

Largemouth bass (M. salmoides) formed a minor component of the catch. Fair numbers of small spotted bass (M. punctulatus) were scattered throughout the mainstem Eel and in Paw Paw and Twelve Mile Creeks. This species had not been present since they could easily have been misidentified as small largemouth bass. Spotted bass young-of-the-year were found even in the otherwise poorer habitat of the upper 30 miles above South Whitley. This species has been shown to be tolerant of high turbidity and sedimentation (Gammon 1970).

Rock bass was taken at all stations except Squirrel Creek. Longear sunfish (Lepomis megalotis) were most common at the upper mainstem stations and in the Blue River and were sporadic in the lower river. Green sunfish (L. cyanellus) also occurred at most sites, but was more abundant in the upper mainstem and in the Blue River. Substantial numbers of bluegill (L. macrochirus) were also taken more regularly in the upper mainstem Eel from RM 63.5 to RM 80.

The most abundant catostomid was white sucker with greatest numbers in the upper mainstem from RM 63.5 to RM 80 and in the Blue River, Sugar Creek, and Beargrass Creek. They were uncommon in the lower 60 miles of the mainstem. Northern hogsucker was widely distributed throughout the mainstem and most tributaries. Spotted sucker (Minytrema melanops) was found in good numbers only in the pool above the Logansport dam.

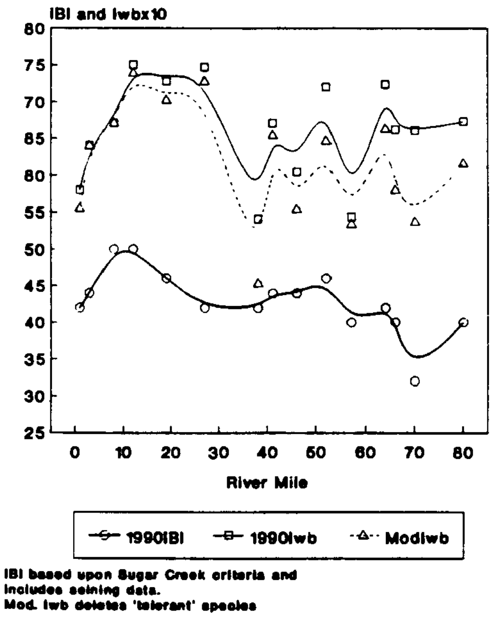

Fig. 1. IBI, Iwb, and modified Iwb values for 1990 Eel River fish communities.

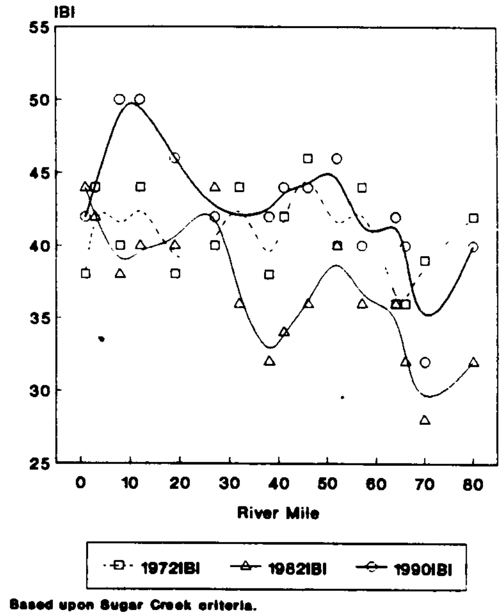

Fig. 2. IBI profiles of mainstem Eel River fish communities for 1972, 1982, and 1990.

Golden redhorse (Moxostoma erythrurum) was the most common of the three redhorse species, but it was not all that abundant. It was absent between RM 56.5-80, as well as, from all tributaries including Blue River. Black redhorse (Moxostoma duquesnei) was almost as common as golden redhorse, but was mostly restricted to the lower 30 miles of the mainstem. Greater redhorse (Moxostoma valenciennesi) is a rare species throughout Indiana and most of its range, but a healthy population thrives in the Eel River system. It was particularly abundant in the lower 20 miles of river, but was also found in Paw Paw and Squirrel Creeks.

The distribution of smaller species of minnows and darters is best illustrated by the seining catches. Bluntnose minnow (Pimephales notatus) was the dominant species, occurring throughout the mainstem and tributaries. Common shiner was even more frequently encountered by electrofishing and was also widely distributed throughout the Eel River system.

Spotfin shiner and sand shiner mostly occurred in the lower 50 miles of the mainstem. Creek chub was common only in the tributaries. Redfin shiner (Notropis umbratilus) and rosyface shiner (Notropis rubellus) were most common in the lower river, but also occurred in Sugar and Twelve Mile Creeks. River chub (Nocomis micropogon) was regularly taken by seine and electrofishing mostly downriver from RM 65. A few bigeye chub (Hybopsis amblops) were also present in the lower river.

Among the darters, only johnny darter (Etheostoma nigrum) was common and widespread. Blackside darter (Percina maculata), greenside darter (E. blennioides) and eastern sand darter (Ammocrypta pellucida) were found only in the lower river. Dusky darter (P. sciera) was taken only from upper Eel River (RM 88.0) and Beargrass Creek.

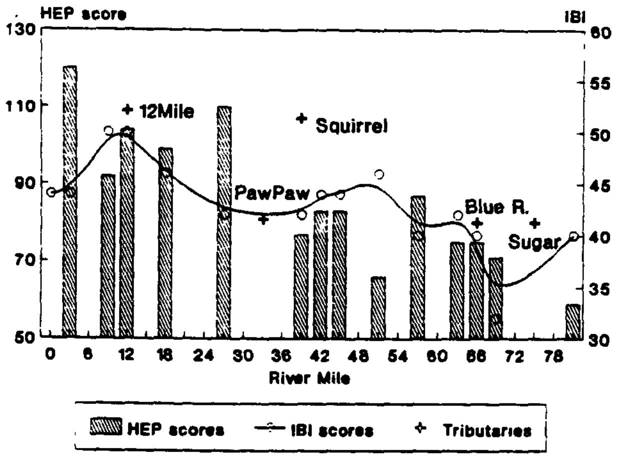

Fig. 3. IBI and HEP values for the mainstem Eel River and tributaries.

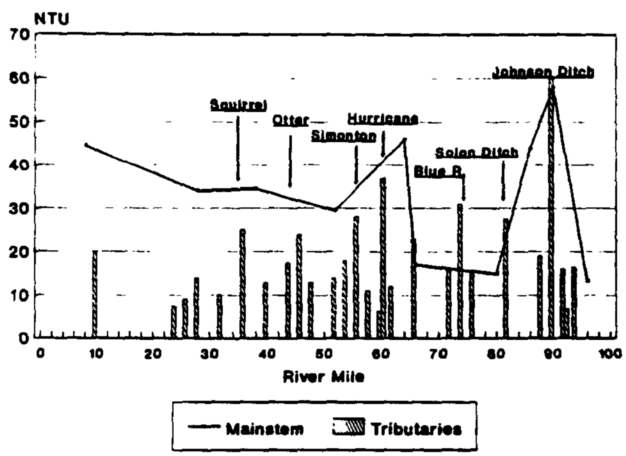

Fig. 4. Turbidity (NTU) for the mainstem Eel River and its tributaries on July 16 and 17, 1990.

Fantail darter (E. flabellare) was collected only at RM 63.5). Mottled sculpin (Cottus bairdi) was taken only at RM 56.5 and RM 63.5).

Important community index values are summarized in Table 3. IBI values were also calculated on less extensive data sets provided by Braun et al. 1984, 1986) on five collections of fish from each of three stations; 2B (RM 3.3), 3B (RM 8.3) and 3 (RM 46.4) during the years 1984 and 1985. The mean IBI values at stations 2B, 3B, and 3 were 39.6, 42.0, and 43.6 in 1984 and 43.2, 41.2, and 42.9 in 1985, respectively.

The IBI and Iwb profiles for the Eel River mainstem are shown in Figure 1. An additional modified Iwb is also shown, wherein four tolerant species were deleted prior to calculation, carp, bluntnose minnow, creek chub, and green sunfish.

All three profiles indicate somewhat depressed fish communities in the lower river, probably because of the ponding effect of the dam, followed by relatively good communities from RM 8 to RM 25. From RM 30 to RM 80 there is considerable variation from place to place, but the communities are generally depressed, especially at RM 70.

In Figure 2, the 1990 IBI profile is repeated and compared to IBI profiles based on Taylor's (1972) and Braun et al.'s (1982) series of collections. The 1990 fish communites are clearly much better than they were in 1982. However, both profiles indicate better communities in the lower river than in the upper river. In 1972 there was less difference in the lower mainstem but equal variation between stations.

Habitat Evaluation

Habitat scores were generally lower in the upper part of the watershed and higher

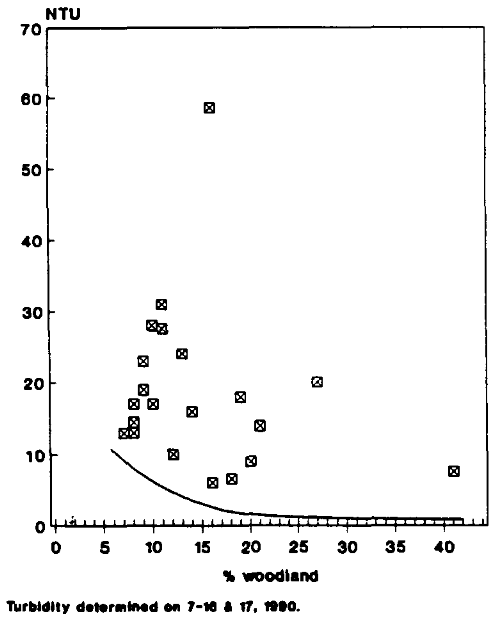

Figure 5. Turbidity (NTU) of Eel River tributaries in relation to percent woodland in their drainage basins.

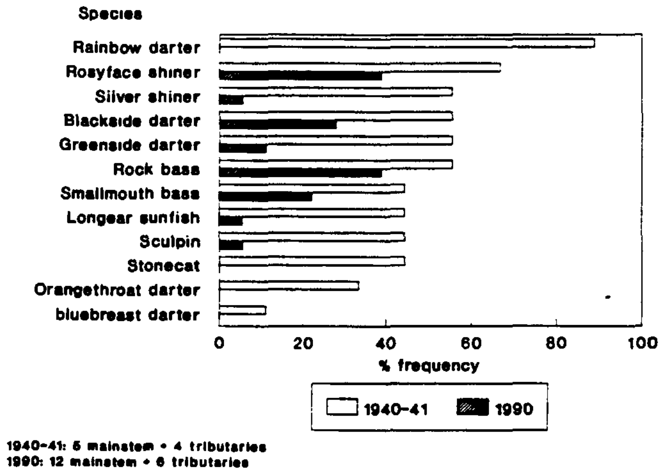

Figure 6. Frequency of occurrence of some species collected by seining in 1990 compared to 1940-41.

downstream. Upstream from South Whitley, habitat features were uniformly low in quality and homogeneous because of past channelization and recent deforestation of both banks. Habitat scores of tributaries were generally higher than the mainstem reaches into which they flowed (Figure 3). An excpetion was Paw Paw Creek which was somewhat lower.

<u>Turbidity and Landuses</u>

Turbidity determination during the synoptic surveys on July 16-17, 1990 are portrayed graphically in Figure 4. Scattered showers fell throughout northern Indiana during the week previous to the turbidity determinations. It is not known to what extent these results may be affected by differential rainfall. Tributaries which were disttinctly more turbid than others included Squirrel Creek, Otter Creek, Simonton Creek, Hurricane Creek, Blue River, Solon Ditch, and Johnson Ditch. A bridge was under construction in the Simonton watershed, but

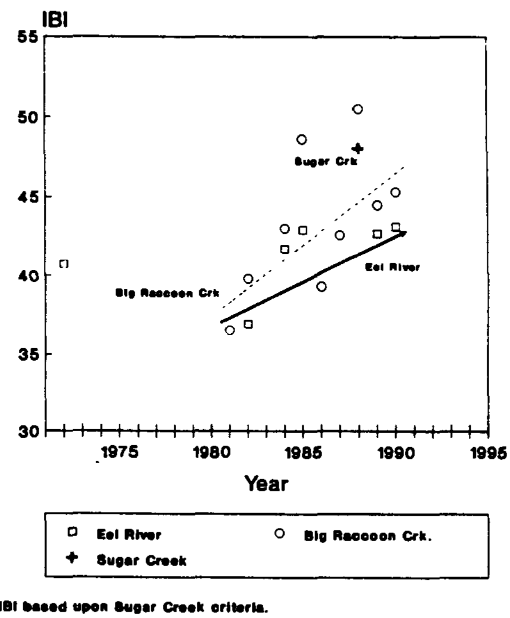

Figure 7. Changes in the IBI values for the Eel River compared to Big Raccoon Creek and Sugar Creek.

Table 3. Fish community indicies for Eel River stations.

Station Taylor	RM	1990 Iwb	No. Spec. elec. 1972	1982	1990	IBI 1972	1982	1990
1B	1.0	5.8	12	13'	15	38	44	44
2B	3.3	6.4	14	14	14	44	42	44
3B	8.3	6.7	10	11	12	40	38	50
4B	12.0	7.5	17	14	17	44	40	50
5B	19.0	5.3	14	17	18	38	40	46
6B	27.3	7.5	11	12	16	40	44	42
7B	32.0	5.4	17	10	--	44	36	--
1	37.8	6.7	14	8	10	38	32	42
2	41.4	6.1	18	9	17	42	34	44
3	46.4	7.2	16	11	13	46	36	44
4	51.7	5.4	10	12	16	40	40	46
5	56.5	7.2	18	13	10	44	36	40
6	63.5	6.6	13	8	13	36	36	42
7	66.0	6.6	15	9	13	36	32	40
8	70.3	6.6	12	6	16	39	28	32
11	79.8	6.7	19	9	17	42	32	40
Tributary Stations								
Twelvemile Creek								44
Paw Paw Creek								40
Squirrel Creek								40
Beargrass Creek								40
Sugar Creek								40
Blue River - upstream from Columbia City								40
Blue River - downstream from Columbia City								44

animals were also pastured in the stream and some corn fields extended to stream banks.

The turbidity of mainstem water was high in the upper river mainly because of highly turbid Johnson ditch. The water cleared considerably after passing through two mainstem gravel pits at RM 84 then again became progressively more turbid as it flowed downstream.

During this same period the turbidity gradually increased in the mainstem from the upper river to the lower river, although there were localized sharp increases in turbidity downstream from both Johnson ditch and South Whitley. Earlier in the summer (June 12, 1990) when water levels were higher the turbidity (NTU) was 45 in the lower 60 km (40 mi) of river and between 46-48 in the upper river. In some streams lateral erosion can be a major source of sediment and turbidity, but scoured banks were a very limited component of the lower portions of the Eel River mainstem. However, they were highly evident in the channelized upper portions. The entire upper 50 km (33 mi) of the Eel River has been stripped of its trees and bushes along both banks. During this study the trimmings had been removed from the river and were piled along the shore for burning.

Woodlands were readily determined from the infrared photographs, but other kinds of permanent vegetation such as brushlands, pastures, and winter wheat were indistinguishable from one another. estimates of woodland ranged from only 7.0% in the Beargrass Creek watershed to 40.9% in the Weesaw Creek watershed. There was a greater percentage of landuse in agriculture south of the mainstem and in the upper two-thirds of the Eel River watershed than north of the mainstem and in the lower third. There was an inverse relationship between the percentage of tributary watersheds in woodland and the measured turbidity (Figure 5).

Discussion

The Eel River in 1990 was found to support fairly diverse fish communities throughout most of the watershed, although the upper reaches had depressed populations and reduced numbers of species. Many species of juvenile fish were caught, with larger numbers at stations 1B and Twelve Mile Creek. This is an indication that reproduction for many species was successful during the past couple of years.

Several usually common species which Braun and Robertson (1982) did not collect were found in good numbers in 1990: river chub, bigeye chub, several species of shiners including silver shiner, spotfin shiner, rosyface shiner, redfin shiner, and blackside and johnny darters.

Some species present in 1972 were found only rarely or not at all in 1990. These species included mottled sculpin, blacknose dace, unidentified madtom species, suckermouth minnow, largemouth bass, and carp.

It is difficult to evaluate long-term changes in abundance of any single species of fish because of the different collecting methodologies. The comprehensive study of Gerking (1945) used the seine as the primary collecting gear and our effort in 1990 was comparable. Gerking collected from five mainstem sites and four tributaries. We collected from 12 mainstem sites and six tributaries.

A comparison of percent frequency of occurrence from these studies indicates rather drastic reductions for many species populations of sediment sensitive fish (Figure 6). Rock bass, johnny darter, and eastern sand darter appear to be distributed much as they were 50 years ago. However, many species have suffered drastic declines including rainbow darter (E. caeruleum), orangethroat darter (E. spectabile), and bluebreast darter (E. camurum) which may have been totally eliminated from the river.

Changes over time in populations of clams and mussels parallel those of fish. Henschen (1988) concluded that while the Eel River once supported a diversity of mussel species throughout its length, its currently reduced population is mostly confined to the lower river in Cass and Miami Counties.

Changes in the Fish Community over Time

The IBI offers one way of addressing questions about how the overall fish community has changed over time and how it compares to fish communities in other streams. The mean IBI values for the Eel River mainstem stations declined from 40.7 in 1972 to 36.9 in 1982. This increased substantially to 43.1 in 1990. The IBI values estimated from data of the studies of Braun et al (1984, 1986) and Braun (1990) generally corresponded to improving trend noted in the 1980's.

The overall Eel River fish community appears to have improved rapidly from the degraded community found in 1982.

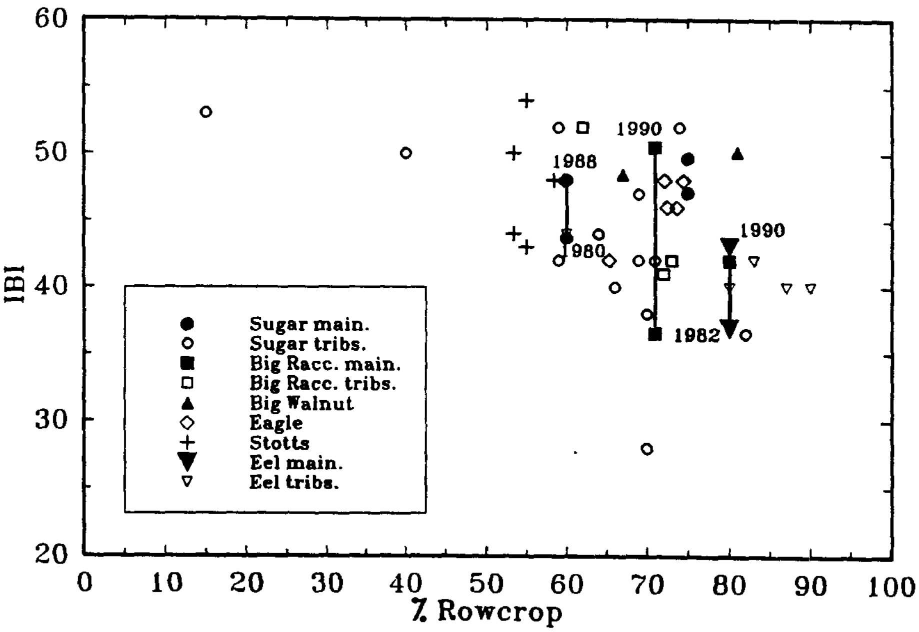

Fig. 8. IBI values of fish communities in Indiana streams primarily influenced by nonpoint source pollution in relation to percent of the watersheds in rowcrop.

IBI values from two other streams are also shown for comparison in Fig. 7 for Sugar Creek (Parke and Montgomery Counties) and Big Raccoon Creek (Putnam County).

We noted the same kind of improvement during the 1980's in Big Raccoon Creek, another stream system influenced primarily by agriculture (Gammon et al. 1990; Gammon 1990). In Big Raccoon Creek the mean IBI was lowest in 1981 (IBI=36.5) and highest in 1988 (IBI=50.5). The low IBI values from 1981 through 1984 probably resulted from poor reproduction and survival during unusually high water in the summers of 1979, 1981, and 1982. Darters, sunfish, and bass were virtually absent during those years, but increased significantly by the end of the decade.

The unusually high IBI value found in 1988 was associated with extremely low flows and a prolonged drought. Fish were undoubtedly concentrated and, therefore, much more vulnerable to capture.

The Sugar Creek system has also been examined during the past decade (Gammon and Riggs 1983; Gammon et al. 1990). Estimates from 1979 and 1980 data also indicate lower IBI values at that time than in 1988. This stream is less influenced by agriculture.

All of the data presented by Gammon et al (1990) together with the more recent assessments are summarized in Fig. 8. All three of the stream systems examined over the past decade or so have shown improved fish populations, the extent of the change, indicated by the vertical lines. Big Raccoon Creek changed the most and Sugar Creek the least. The pattern of changes suggests that in watersheds with intensive agriculture, e.g. Eel River, the scope for improvement may be more limited than in watersheds with somewhat less intensive agriculture, e.g. Big Raccoon Creek.

Nevertheless, a series of years with high runoff and increased non-point source pollution can depress fish communities to equally low levels.

Other factors which may modify the recovery of fish populations in the Eel River system include the absence of high quality tributaries to serve as refugia for sensitive species during unfavorable years and the dam blockage at Logansport which reduces recolonization by species from the Wabash River.

The Potential Influence of Habitat and Turbidity

Much of the upper Eel River is characterized by low HEP values, e.g. channelized stream beds, poor riffle/pool development, and a lack of instream structure. In addition, riparian trees have been removed recently from many older previously channelized sections of the river.

The bottom substrate usually included much fine sediment, as indicated by the low embeddedness scores for almost all of the mainstem stations and most tributaries. Turbidity was high for virtually the entire summer. At Roann we saw a layer of mud two centimeters deep on top of a flat boulder after higher water had subsided in a pool.

The lower 48 km (30 mi) of Eel River contained much better habitat than the upstream reaches. Beds of water willow (Dianthera) were mostly limited to the lower 64 km (40 mi) of the mainstem Eel. This section also had fairly good riparian protection and good instream habitat.

Habitat in the tributaries generally scored higher than the mainstem. Twelve Mile Creek, with 26.5% of its watershed in forest, contained the best habitat, followed by Squirrel Creek. The Blue River is approximately the same size as the Eel River where the two streams converge. With only 11% permanent vegetation cover, its turbidity readings were among the highest recorded. Fish from this stream, and Paw Paw Creek, were commonly infected with blackspot disease (Simon 1989).

Potential Negative Effects from Point-Source Pollution

Agricultural point-source pollution in Indiana often occurs because of accidents or careless handling of animal wastes and farm chemicals. Spilled materials, animal wastes applied to fields, and the contents of waste holding lagoons may be flushed into ditches and streams following rain storms. Fish kills reported to the Indiana Department of Environmental Management (IDEM) since 1969 include five incidents on Paw Paw Creek and single kills on Twelve Mile, Pony, Beargrass, and Clear Creeks.

There were 39 additional reports of spilled materials which are not known to have resulted in fish kills, but which may have exerted sublethal damage. Most of the materials were fertilizer and animal wastes, which include wastes generated by chickem, turkey, veal, and swine rearing operations.

All of the known causes of ifsh kills and most of the spills reported within the Eel River watershed are agriculturally based. The actual number of fish kills and spills is unknown, but would certainly far exceed the number of reported cases.

In the decade following passage of the Clean Water Act of 1972, it was estimated that municipal BOD loads decreased by 46% and industrial BOD loads decreased at least 71% (U.S. Environmental Protection Agency, 1982). Most of the communities in the area have improved waste treatment and reduced BOD concentrations by at least 50%. Some previously unsewered communities now have a central treatment system. It is likely that any negative influences from these point sources are masked by the magnitude of non-point source impacts.

Weather and Nonpoint Source Pollution

Unlike point source pollution, nonpoint sources of influence such as occurs from plowed fields are most severe during storm events. The discharge of rivers is roughly proportional to the amount of rainfall, hence, non-point sources are most severe when rainfall is great and river discharge is high. Conversely, non-point sources are reduced during periods of dry weather. Fish populations are negatively affected by non-point sources during the reproductive period and in the months immediately after hatching, spring and summer.

From October 1974 through September 1980 the U.S. Geological Survey Water Resources Division determined daily sediment loads for the Eel River near Logansport (Anonymous 1974 through 1980). Data from the Eel River and rivers throughout Indiana is analyzed and discussed by Crawford and Mansue (1988). They estimated that for the Eel River the mean annual suspended sediment yield was 178 tons/square mile/year and the flow-weighted mean annual suspended sediment concentration was 89 mg/l (median = 53 mg/l). These values are high for the northern moraine/lake portion of Indiana which Crawford and Mansue found to have the lowest sediment yield. Only that part of the Eel River watershed north of the mainstem resides within the moraine area. The portion of the watershed situated south of the mainstem is located in the Tipton Till Plain where both parameters were generally much larger.

Monthly data from May through August for the years 1974 through 1980 was used for regression analysis of suspended solids concentration on discharge. The regression equation obtained was then used to estimate the suspended solids concentration for the months May through August for the years following 1980 (Figure 9). Suspended solids concentrations were highest during May and June when relatively high discharges occurred during half of the years since 1974. "Wet" summers of relatively high suspended solids concentration include the years 1974, 1975, 1980, 1981, 1982, and 1986. "Dry" summers when Eel River water was relatively clear include the period from 1976 through 1979, 1983 through 1985, and 1987 through 1988.

During "dry" summers the effects of point sources of pollution such as from population centers would theoretically increase, but nonpoint source pollution should be less than normal. For streams influenced mostly by NPS the fish communities following a sequence of "dry" summers should improve. The Eel River fish communities did improve somewhat, but less than might have been expected compared to fish communities in Big Raccoon Creek.

The 1990 fish communities may be as good as the Eel River is able to support considering present land use. The summers of 1989 and 1990 were relatively "wet". Therefore, reproductive success and survivorship through the first year of life would be expected to be lower than normal. It is likely that the 1991 fish communities will be poorer than they were in 1990 and the prognosis for improvement in the future is bleak unless changes in land-use are implemented.

Summary

The Eel River is essentially a linear stream. Its drainage basin is long and narrow and its tributaries are generally small first and second order streams. Improving landuse in these tributaries will be necessary in order to improve the mainstem of the Eel River. Thorough surveys of all tributary watersheds should be conducted using both Geographic Information System (GIS) technology and ground study.

Twelve Mile Creek, Paw Paw Creek, and, possibly, Squirrel Creek appear to be less influenced by agriculture than other tributaries. These tributaries may act as refugia for sensitive species during periods of stress and serve as species reservoirs to replenish the mainstem during more benevolent times. They should receive special attention to ensure that: a) the streamside riparian buffer zone is maintained, b) tilled fields do not impinge on the stream itself, c) hogs and cattle are not pastured directly in the streams, d) appropriate forms of conservation tillage are encouraged, e) animal wastes are properly disposed.

Several other tributaries appear to be more environmentally degraded than others. Otter Creek, Simonton Creek, Hurricane Creek, Blue River, Solon Ditch, and Johnson Ditch delivered higher than average sediment loads to the Eel River during the survey of July 16 and 17, 1990. While this survey is only a brief "snapshot" in time, it nevertheless suggests that these streams may have greater than average negative impacts on the Eel River system. They should also receive the same items of attention listed above.

Streams in the upper watershed are referred to and used as drainage ditches. Nevertheless, these streams are permanent "creeks" and should support normal aquatic life. Their rehabilitation could contribute positively toward the improvement of the lower mainstem. The creation of a "green belt" riparian corridor would also contribute toward a greater ecological diversification.

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Selenastrum Algal Growth Test: Culturing and Test Protocol at the Illinois EPA

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Abstract
The availability of quality test organisms is of fundamental concern in conducting any regularly scheduled biomonitoring activities. The Selenastrum algal assay requires a continuous supply of pure log-phase algae. The most convenient means to meet this demand is through the establishment of in-house cultures. Upon receipt of a pure Selenastrum "starter" culture from an outside source, laboratory stock cultures are initiated. The algae is aseptically transferred to a series of culture flasks containing synthetically prepared algal medium. Once pure algal cultures have been established, a regime of routine cell transfers will provide the laboratory with a steady supply of log-phase algal cells suitable for testing purposes. Back-up reserve cultures are stored on agar slants and plates. Testing of municipal and industrial effluents at the Illinois EPA using Selenastrum algae follows USEPA test protocol. Through experience running the test and repeated attempts to get confident results, the testing has been refined and the integrity of the analysis is ensured. Various techniques are employed in the testing that serve to tighten the USEPA protocol and may be of interest to other regulatory bioassay personnel.

Keywords: Selenastrum algae, log-phase, aseptically, agar slant, agar plate.

Introduction
At the present time, the Illinois EPA (IEPA) is the only state run bioassay laboratory in USEPA Region V conducting the Selenastrum capricornutum algal growth test. The algal growth test is conducted in the Toxicity Testing Unit (TTU) which is one of two units in the Office of Ecotoxicology (OE). OE serves as a support laboratory for the various control divisions within the IEPA (air, land, water, and public water supplies). IEPA uses USEPA protocol, Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (USEPA 1989) as a guideline for culturing and conducting tests. TTU maintains a continuous supply of in-house stock cultures for use in bioassays. Various techniques are employed in culturing and testing that serve to tighten USEPA protocol.

Establishing and Maintaining Selenastrum Stock Cultures
The Selenastrum algal assay calls for healthy log-phase-growth cells which are harvested from resident in-house stock cultures. The TTU laboratory maintains a continuous supply of these log-phase Selenastrum cultures in quantities sufficient to meet all testing demands. The culturing process is relatively straight-forward and consists of six basic components: 1) general culture setup/ conditions; 2) algal nutrient culture medium; 3) aseptic technique; 4) routine cell transfers; 5) back-up/reserve cultures; and, 6) quality assurance/quality control considerations.

1. General Culture Setup/Conditions.
The Selenastrum cultures are maintained in an environmental chamber at 25 ± 1°C under a continuous "cool-white" fluorescent illumination of 400 ± 40 ft-c (4306 ± 431 lux). The algal cells are kept in a constant state of suspension through the use of a mechanical shaker at approximately 100 cpm (cycles per minute). The culture flasks are arranged on the shaker table and allowed to incubate anywhere from four to seven days, depending upon current testing schedules. This incubation interval

Table 1. Nutrient Stock Solutions For Maintaining Algal Stock Cultures (adapted from USEPA 1989).

Nutrient Stock Solution	Compound	Amount dissolved in 500 mL Distilled H_2O
1	$MgCl_2 \cdot 6H_2O$	6.08 g
	$CaCl_2 \cdot 2H_2O$	2.20 g
	H_3BO_3	92.8 mg
	$MnCl_2 \cdot 4H_2O$	208.0 mg
	$ZnCl_2$	1.64 mg[a]
	$FeCl_3 \cdot 6H_2O$	79.9 mg
	$CoCl_2 \cdot 6H_2O$	0.714 mg[b]
	$Na_2MoO_4 \cdot 2H_2O$	3.63 mg[c]
	$CuCl_2 \cdot 2H_2O$	0.006 mg[d]
	$Na_2EDTA \cdot 2H_2O$	150.0 mg
	$NaNO_3$	12.75 g
2	$MgSO_4 \cdot 7H_2O$	7.35 g
3	K_2HPO_4	0.522 g
4	$NaHCO_3$	7.50 g

[a]$ZnCl_2$ - Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to Stock #1.
[b]$CoCl_2 \cdot 6H_2O$ - Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to Stock #1.
[c]$Na_2MoO_4 \cdot 2H_2O$ - Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to Stock #1.
[d]$CuCl_2 \cdot 2H_2O$ - Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to Stock #1.

provides plenty of viable, log-phase cells suitable for bioassay purposes.

2. Algal Nutrient Culture Medium.

Upon receipt of a Selenastrum "starter" culture from an established outside source, in-house stock cultures are initiated by aseptically transferring a portion of the cells to freshly prepared algal nutrient medium. The culture medium consists of a mixture of various macro- and micronutrients prepared in four separate stock nutrient solutions using the reagent grade chemicals listed in Table 1.

The nutrient medium is prepared by adding 1 mL of each of the four stock solutions, in order as listed in Table 1, per liter of distilled water. The solution is mixed well and then pH-adjusted to 7.5 ± 0.1 by dropwise addition of 0.1 N NaOH or HCL, as appropriate. The medium is then immediately filtered through a pre-washed 0.2 μm pore diameter membrane at a vacuum pressure of approximately 8 psi. The algal nutrient medium is then ready to be dispensed into the various culture flasks and inoculated as needed. Any leftover portions of the sterile medium may be stored in a refrigerator at 4°C until needed. Care should be taken, however, to seal off the storage vessel well so as to prevent loss of water by evaporation. Evaporation losses will alter the concentration of macro-micronutrients in the final medium, thus compromising its quality for use in culturing purposes.

3. Aseptic Technique.

Extreme care is exercised to prevent contamination of the cultures by other microorganisms. All glassware products used in the culturing process are thoroughly cleaned, sealed with aluminum foil, and sterilized at 121°C in an autoclave. All pipet tips used in handling the algal cells during routine cell transfer procedures are of the disposable type, and they too are autoclaved at 121°C. The algal nutrient culture medium is cold-sterilized before use by passing it through a 0.2 μm pore diameter membrane filter, as described above. Despite these efforts, contamination problems do occur from time to time. Contaminated cultures are either discarded or used as food for Ceriodaphnia cultures.

4. Routine Cell Transfers.

To meet scheduled testing demands, the TTU laboratory maintains a continuous source of log-phase Selenastrum cells. This is achieved through a series of routine cell transfers from existing stock cultures to various aliquots of fresh algal nutrient medium. An inoculum is prepared from a four - to seven - day stock culture by concentrating the cells of the culture through a centrifugation process. The algal cell concentrate is then diluted with distilled water to provide an initial density of approximately 10,000 cells/mL in the culture flasks. A Coulter Counter[R] (model ZM) is utilized in cell density determinations for both the stock cultures and the final inoculum.

Once prepared, 1 mL of inoculum is aseptically transferred to each of three 500 mL Erlenmeyer culture flasks containing 250 mLs of fresh algal medium each. After inoculation, the flasks are situated in the environmental chamber on a mechanical shaker for incubation purposes. An incubation period of four to seven days renders plenty of healthy, log-phase Selenastrum cells ready for harvest and use in testing and/or other purposes. Routine cell transfers are carried out twice per week, with each transfer staggered 3-4 days apart. This arrangement will provide a continuous supply of log-phase cells suitable for biomonitoring purposes.

The volume of stock cultures required depends on the test loads involved and any other targeted uses for the algae (ie., food source for Ceriodaphnia cultures, etc.). The TTU laboratory meets all of its current algae demands by inoculating 1.5 - 2 L of fresh culture medium weekly.

5. Back-Up/Reserve Cultures.

As mentioned above, contamination of the stock cultures seems to be inevitable from time to time. It is therefore essential to have in place some type of a back-up/reserve system for storing clean, pure Selenastrum stocks that may be called upon to rejuvenate "dirty" or "fouled" cultures. The TTU laboratory meets this objective through the use of a system of agar slants and plates. The agar medium is prepared with the same stock nutrients, in the same amounts, as the standard liquid algal medium. The only difference is that the stock nutrients are dissolved in a 1-2% Bacto[R]Agar solution. The agar nutrient medium is mixed up in an AgarMatic[R]bench top agar sterilizer, which in turn is linked up to a PourMatic™automatic plate dispensal system. Thus, the agar medium is mixed, sterilized, and poured into plate form all in one process. Any excess medium is then hand-poured into test tubes for use as slants. A large batch of plates and slants are poured all at once, the bulk of which is then stored in a refrigerator at 4°C until needed.

At scheduled intervals of approximately once a month, several fresh agar plates are "streaked" with Selenastrum cells from existing stock cultures. A 10 μl inoculating loop is used to transfer the cells from the liquid stock cultures to the agar, where they are streaked out into quadrants on the plated medium. The plates are then arranged on a rack situated in a partially enclosed glass box shelter in the environmental chamber for incubation purposes. The glass box, along with rubber bands used to seal the lids on the petri dishes, serves to break up the airflow patterns of the chamber around the immediate vicinity of the plates, thereby minimizing dessication problems of the media. The plated cultures need air exchange for proper growth, but too much airflow will only serve to dry out the plates completely, rendering them useless for storage purposes. An incubation period of 1-2 weeks yields several distinct Selenastrum colonies that may then be targeted for transfer to fresh liquid nutrient medium, thereby rejuvenating active stock cultures.

Agar slants are also streaked up from time to time as needed, but serve primarily in a secondary backup role. After incubation, the slants displaying healthy Selenastrum colonies are pulled from the environmental chamber and stored in a refrigerator at 4°C for up to several months. In this way, they may serve as a "backup" to the backup cultures.

6. Quality Assurance/Quality Control Considerations.
At each cell transfer, the stock cultures are examined microscopically for species verification purposes and to look for any signs of microbial contamination. This information, along with general observations on the overall condition of the cells themselves, is then recorded in an algal culture logbook. This enables the TTU laboratory to keep a running history of culture activities and any special problems/ solutions encountered. Stock cultures are also subjected to monthly NaCl reference toxicant tests. EC50 point estimates are calculated for each reference test, and these values are then plotted on a standard reference toxicant control chart for quality control purposes (Figure 1). These steps are taken to ensure the quality and suitability of the Selenastrum stock cultures for use in biomonitoring activities.

Selenastrum Algal Growth Test Protocol
Samples received by TTU for algal bioassays consist of municipal and industrial effluents and their ambient receiving waters (upstream of the effluent outfall). For the purposes of the IEPA, Illinois is divided into seven regions. All regions, except Region 4 (Field Operations Services in Champaign, IL) and Region 5 (Field Operations Services in Springfield, IL), ship samples to OE via bonded courier (e.g., Emery Worldwide). Regions 4 and 5 hand deliver samples to OE. All samples are received in the laboratory and testing started within 28 hours of sampling. A chain of custody is maintained by field and laboratory personnel to ensure the samples are not tampered with.

When samples arrive in TTU they are logged in, warmed to the proper temperature, aerated, and initial water chemistries are performed. Initial water chemistries consist of alkalinity, hardness, chlorine, and ammonia determinations. Measurement of these parameters helps resolve the cause of toxicity. When the samples have been warmed and aerated, dilutions are poured. A 0.5 dilution series is used. Temperature, pH, conductivity, and dissolved oxygen are measured on each dilution to determine if these parameters are within the range for normal growth of Selenastrum. A 250 mL portion of each dilution is then poured off for the algal bioassay.

Each 250 mL dilution is enriched with 250 μL of each of the four nutrient stock solutions (with EDTA). To reduce the possibility of contamination in the algal bioassay, aseptic techniques are employed throughout the test. All glassware is washed with non-phosphate detergent and rinsed with tap water, acetone, hydrochloric acid, tap water, and distilled water. Glassware and pipet tips are autoclaved at 121°C.

Each dilution (with nutrients) is filtered through a 0.2 μm membrane filter. This filtration removes any indigenous algae from the dilutions. Following filtration, 150 mL of each dilution is measured in each of three 125 mL Erlenmeyer test flasks (three flasks per dilution with 50 mL of diluent per flask). The entire test consists of three test flasks in each of the following concentrations; control, 0%, 6.25%, 12.5%, 25%, 50%, 100%.

The test flasks are inoculated with 1 mL of log-phase-growth Selenastrum (4 to 7 days old) to provide an initial cell density of 10,000 cells/mL ($\pm$10%). At IEPA the algal cells are not "washed" prior to inoculation (there is no need to remove EDTA from the test cells since the test nutrients contain EDTA). The required volume of stock culture needed to inoculate the test flasks is calculated as follows:

$$\frac{\text{number of test flasks} \times \text{volume of test solution/flask} \times 10{,}000 \text{ cells/mL}}{\text{cell density (cells/mL) in the stock culture}}$$

= volume (mL) of stock culture required

Test flasks are covered with aluminum foil for autoclaving. After the flasks are inoculated, the

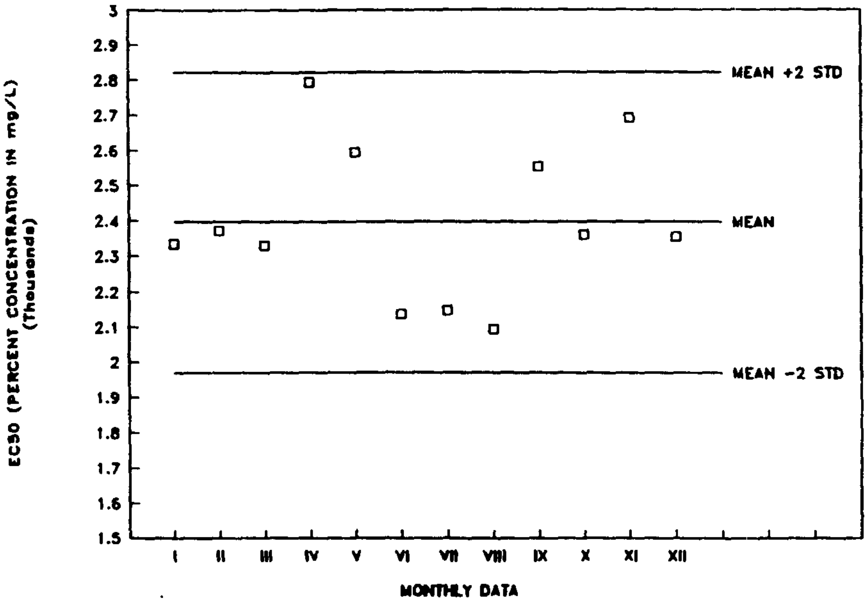

Figure 1. Illinois EPA Reference Toxicity Test (NaCl).

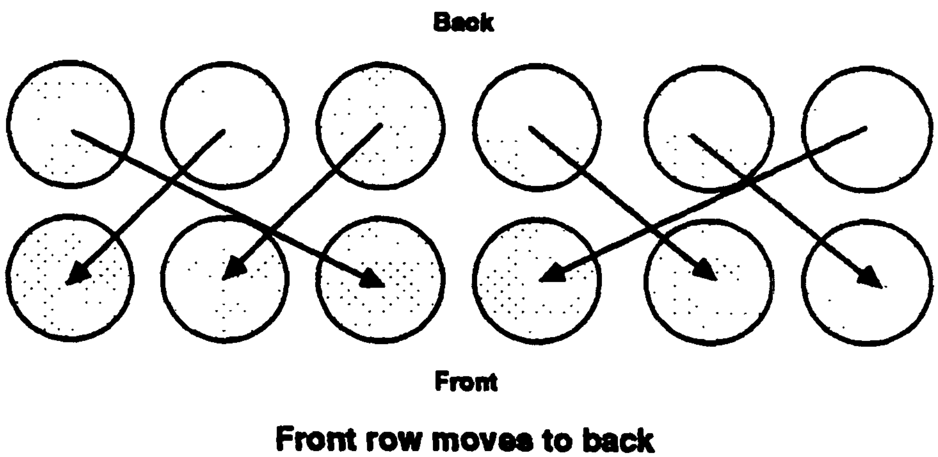

Figure 2. Rotational pattern.

aluminum foil is removed and replaced with 100 mL plastic beakers for incubation. Cell density is checked in at least three flasks within two hours of inoculation. The test flasks are randomized on the holding tray prior to incubation. The flasks are rotated at 24, 48, and 72 hours using a standard rotation pattern (Figure 2) to help ensure that all test containers receive equal amounts and intensity of light and even temperature throughout the 96 hour incubation.

The algal incubator is kept at a constant temperature of 25 ± 1°C. Lighting is turned on approximately four hours before the start of the test to allow the lights to reach equilibrium. During the test the flasks are rotated mechanically at 100 cycles per minute continuous rotation. Light intensity during the test is 400 ± 40 ft-c (4306 ± 431 lux). Light lux is measured at the beginning of the test and at the end of the test. The pH of the 0% and the 100% is also measured at the beginning and at the end of the test.

Test termination is at 96 ± 2 hours. Algal cell density in each flask is measured using a Coulter Counter^R Model ZM. Test cultures are diluted with Isoton^R (a sodium chloride electrolyte solution) and counted directly on the Coulter Counter^R. Three cell counts are taken for each aliquot and the mean cell volume is averaged for the three counts. Each test flask is mixed thoroughly following USEPA procedure. For IEPA purposes, the counts from each test culture must have less than 10% variability.

Test results are considered acceptable if the average cell counts in the control flasks are greater than 2 x 10^5 cells/mL and control variability does not exceed 20%. When using stock nutrient solutions without EDTA, obtaining average cell counts greater than 200,000 cells/mL was not a problem, however keeping control variability below 20% was difficult. Without EDTA, cell counts in the controls ranged from 400,000 to 800,000 cells/mL. Variability in the three control flasks was as high as 79%. variability in control flasks inoculated with stock nutrient solutions containing EDTA consistently remained below 20%.

EDTA can lower toxicity of a sample by complexing heavy metals. EDTA facilitates algal growth by increasing the availability of micronutrients. Based on the control flask variability (when EDTA is not used) the decision was made at IEPA to conduct the Selenastrum algal bioassay with EDTA in the stock nutrient solutions. Adverse effects on Selenastrum cell growth, expressed in LOEC and NOEC values, are obtained using Dunnett's Procedure. Statistics are analyzed using an in-house written computer program.

Conclusion

The Selenastrum algal bioassay is a useful aquatic toxicity test, and is an important component of IEPA's testing program. In addition to detecting phytotoxic contaminants, the bioassay could identify wastewaters which are nutrient rich and biostimulatory. By incorporating a freshwater primary producer (Selenastrum) into the bioassay regime, toxicity could be detected which is not detected by tests using primary consumers (Ceriodaphnia dubia, at IEPA) or secondary consumers (Pimephales promelas, at IEPA).

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Effects of Acute Sublethal Levels of pH on the Feeding Behavior of Juvenile Fathead Minnows

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Abstract
This study was conducted to determine the impact of acute sublethal pH levels on the feeding behavior of juvenile fathead minnows. Eighteen to 24 day-old juveniles were fed live or dead brine shrimp under light or dark conditions in order to identify the role of the senses of vision, chemoreception, and mechanoreception in feeding at different pH's. Feeding trials were conducted at various pH combinations; 5.0, 7.0, 10.0; 4.5, 7.0, 11.0; and 3.5, 7.0, 11.5. Total mortality was observed at pH 3.5 and 11.5. Appetitive behavior was present at all pH levels as evidenced by frequencies of occurrence of feeding ranging from 93-100%. No relationship was observed between pH and the number of fish feeding. The fathead minnow is chiefly a visual feeder and vision was not affected at any pH level as 99.9% of all brine shrimp, live and dead, were consumed in the light. Significantly fewer brine shrimp were consumed in the dark than in the light and significantly fewer brine shrimp were consumed in the dark at the lower pH's than in the dark at pH's 7.0+. No measurable impact on feeding behavior was observed at pH 7.0 and 10.0+. Chemoreception was stressed at low pH levels. The ability of chemoreception and mechanoreception to successfully function in consort in capturing living prey in the dark at low pH levels was noticeably impacted. The effect of low pH on mechanoreception was not determined.

Keywords: pH, fathead minnow, feeding behavior

Introduction

The science of behavioral toxicology is a recently developed diagnostic approach to measuring and recording observations of behavior that reflect biochemical and ecological responses of organisms to environmental contamination (Little 1990).

Introduction

The science of behavioral toxicology is a recently developed diagnostic approach to measuring and recording observations of behavior that reflect biochemical and ecological responses of organisms to environmental contamination (Little 1990). Behavioral activities are rapidly becoming recognized as highly sensitive indicators of sublethal toxicity (Diamond et al. 1990, Little and Finger 1990). A variety of behaviors has been used to study sublethal toxicities including ventilation and cough frequencies, feeding activities, temperature preference, predator avoidance, swimming performance, schooling behavior, and pH detection and avoidance (Hill 1989). However, while it is readily acknowledged by investigators that differing behavior activities involve a diversity of sensory-motor pathways and physiological processes (Sandheinrich and Atchison 1990), little attention has been given to the impacts that toxicants selectively impart to specific senses or sensory pathways. Although the

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embryo-larval-juvenile life cycle stages are accepted as being the most sensitive for toxicity tests (McKim 1977), little consideration has been given to the impacts of sublethal toxicants upon the sensory systems of these life cycle stages, many of which exhibit a gradient of sense organ development from the time of hatching until the completion of successful behavior formation (Noakes and Godin 1988).

Although acid stress and depressed pH conditions have been studied at length from many different perspectives (Zischke et al. 1983, Leino et al. 1987, Mills et al. 1987, Jansen and Gee 1988, among others), and are receiving much local press in relation to acid precipitation, little attention has been given to the effects of acid stress on fish behavior (Jones et al. 1985). Lemly and Smith (1985) summarized the literature supporting fathead minnows as being among the most acid sensitive fishes. Jones et al. (1985), in pursuing the effects of sublethal pH levels on the behavior of arctic char, reported acid stress to suppress chemoreception. Lemly and Smith (1985, 1987) found acidification to significantly affect the ability of fathead minnows to detect or respond to chemical stimuli. Jones et al. (1985) described this chemo-suppression to likely result from the reduced stimulatory nature of amino acids at reduced pH's and the damage of epithelial tissues (olfactory epithelium) by acidic conditions. Lemly and Smith (1987) suggested that increased olfactory mucous thickness in response to lowered pH prevented normal stimulus-receptor interaction and/or that chemical interaction at the subcellular level was impaired because of stearic/ charge changes at the receptor cells. Whatever the explanation, these observations are of potentially profound importance to environmental biologists in that they represent avenues for unrecognized massive larval mortalities among those fish species that are dependent upon chemoreceptors of chemoreceptors/mechanoreceptors in the formation of exogenous feeding behavior.

The purpose of this study was to determine the effects of different acute pH levels on the senses of vision, mechanoreception and chemoreception in the feeding behavior of juvenile fathead minnows, Pimephales promelas.

Methods and Materials

Test Fish

Fathead minnows used in the project were obtained within 12 hours of hatching from the U.S. EPA Newtown Fish facility, Newtown, Ohio, on 3 July, 1990. Fish were maintained in 2 1 finger bowl in ASTM water at 24 +/- 1C and were fed freshly hatched brine shrimp twice daily, 0800 and 1700 h. At the onset of the pH trials, the minnows were 18 days old and averaged 11.58 mm in total length (range 10.1 mm - 13.0 mm). All experimentation was conducted at the Graduate Center for Toxicology at the University of Kentucky, Lexington, KY.

pH Test Solutions

pH solutions in 3.0 1 aliquots were prepared using ASTM water with Nitric acid to produce low pH levels and Sodium Hydroxide to produce high pH levels. A pH of 7.0 was achieved by adding either Nitric acid or Sodium Hydroxide as required. An Orion pH meter was used to determine pH levels in producing the desired pH concentrations. Acid pH's tested were 5.0, 4.5, 4.0, and 3.5. Basic pH's were 10, 10.5, 11.0, and 11.5. An acidic, a basic, and a pH 7.0 concentration, was used daily for four consecutive days in the following sequence: Day 1 - pH's 5.0, 7.0, 10.0; Day 2 - pH's 4.5, 7.0, 10.5; Day 3 - pH's 4.0, 7.0, 11.0; Day 4 - pH's 3.5, 7.0, 11.5.

Sense Organ Isolation

Each daily combination of pH test solutions was applied to four different feeding regimes, living brine shrimp fed in the light, living brine shrimp fed in the dark, dead brine shrimp fed in the light, and dead brine shrimp fed in the dark (see Table 1 for design).

Table 1. Frequency of occurrence of juvenile fathead minnows eating at least one brine shrimp during live-dead, light-dark, feeding trials at varying pH levels.

pH Level		5.0			7.0			10.0	
Light-Live	5/5	5/5	4/5	5/5	5/5	4/5	5/5	4/5	4/5
Light-Dead	3/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Dark-Live	4/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Dark-Dead	5/5	5/5	5/5	5/5	5/5	3/3	5/5	5/5	5/5
		(93%)			(98%)			(95%)	
pH Level		4.5			7.0			10.5	
Light-Live	5/5	5/5	5/6	5/5	4/5	5/5	5/5	5/5	5/5
Light-Dead	5/5	4/5	4/5	5/5	5/5	5/5	5/5	5/5	5/5
Dark-Live	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Dark-Dead	5/5	5/5	5/5	5/5	5/5	4/5	5/5	5/5	5/5
		(95%)			(95%)			(100%)	
pH Level		4.0			7.0			11.0	
Light-Live	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Light-Dead	5/5	5/5	5/5	4/5	5/5	5/5	5/5	5/5	5/5
Dark-Live	5/5	5/5	5/5	5/5	5/5	5/5	4/5	4/5	5/5
Dark-Dead	5/5	5/5	4/4	6/6	5/5	5/5	4/4	4/4	4/4
		(100%)			(98%)			(96%)	
pH Level		3.5			7.0			11.5	
Light-Live	Total			No			Total		
Light-Dead									
Dark-Live	Mortality			Test			Mortality		
Dark-Dead									

Test Procedure

A total of 180 minnows were selected at approximately 1600 h the day before a trial. The fish were separated into 12 groups of 15, each group of which was placed in a 500 ml beaker containing water of a specific pH concentration (daily test combinations described above). The fish were then placed in an environmental chamber at 25.0 C with an 8 hour dark period (2200 to 0600 h) for acclimation until approximately 1300 h the following day. Five fish from each pH concentration were then placed in each of three 150 ml finger bowls containing fresh mixtures of the test pH's for replicate trials. The fish were allowed to acclimate in the finger bowls for ten minutes in either light or dark before food was added. Light feeding trials with live and dead brine shrimp were conducted prior to similar dark feeding trials.

Feeding

Brine shrimp (Salt Lake City variety) were raised in the laboratory and fed immediately following 24 hours incubation. The brine shrimp used in the feeding trials were the same variety and size used to raise and maintain the minnows. Average brine shrimp length was 0.7 mm. Fifty live or dead brine shrimp for each fish subsample were selected

Table 2. Number and Percent of brine shrimp remaining following juvenile fathead minnow live-dead, light-dark feeding trials at varying pH levels.

pH Level		5.0			7.0			10.0	
Light-Live	0	0	0	0	0	0	0	0	0
Light-Dead	0	0	0	0	0	0	0	0	0
Dark-Live	4	0	0 (2.7%)	0	0	0	5	5	3 (8.7%)
Dark-Dead	9	14	8 (20.7%)	8	3	0 (7.3%)	2	0	0 (1.3%)
		(5.8%)			(1.8%)			(2.5%)	
pH Level		4.5			7.0			10.5	
Light-Live	0	1	2	0	0	0	0	0	0
Light-Dead	0	0	0	0	0	0	0	0	0
Dark-Live	17	22	23 (41.3%)	1	1	3 (3.3%)	0	0	0
Dark-Dead	15	5	21 (27.3%)	9	0	0 (6.0%)	2	0	0 (1.3%)
		(17.2%)			(2.3%)			(0.3%)	
pH Level		4.0			7.0			11.0	
Light-Live	0	0	0	0	0	0	0	0	0
Light-Dead	0	0	0	0	0	0	0	0	0
Dark-Live	30	8	36 (49.3%)	0	0	0	2	0	0 (1.3%)
Dark-Dead	1	3	7 (7.3%)	1	0	1 (1.3%)	3	2	2 (4.7%)
		(14.2%)			(0.3%)			(1.5%)	
pH Level		3.5			7.5			11.5	
Light-Live		Total			No			Total	
Light-Dead									
Dark-Live		Mortality			Test			Mortality	
Dark-Dead									

with a 10 cc syringe and counted using a dissecting microscope. Each of nine syringes was loaded immediately prior to the feeding exercise and the fifty brine shrimp added to each group of five fish following the ten minutes acclimation to the test pH's. Feeding time for all tests was ten minutes. Brine shrimp for the dead feeding trials were killed by treatment in an ultrasonic bath for two to four minutes. Fish were aspirated from the test dishes immediately following the feeding trial and isolated in holding dishes. While the number of brine shrimp ingested by each individual fish could not be determined, the number of fish having consumed at least one brine shrimp was recorded using a dissecting microscope. Each feeding test dish was examined with the aid of a dissecting microscope and the number of brine shrimp remaining following the feeding trial was counted. All fish used in a feeding exercise were excluded from further feeding.

Results

Survival

All fish survived every trial except those at pH 3.5 and 11.5 in which 100 percent mortality was observed.

Frequency of Occurrence of Feeding

The number of fish ingesting at least one brine shrimp during the feeding trials ranged from 93 to 100 percent for all pH levels (Table 1). No relationship was observed between number of fish feeding and light or dark or live or dead food conditions. No relationship between frequency of occurrence of fish feeding and decreasing or increasing pH levels was observed. The average frequency of occurrence of feeding by fishes in pH 7.0 water, 97.7%, was the same as that (97.7%) for fish in the increasing pH level trials and only slightly greater than that (96.1%) for fish in the decreasing pH level trials (Table 1).

Light-Dark Feeding versus pH Level

With the exception of three brine shrimp at pH 4.5, all food organisms (99.9%), live or dead, were consumed during light feeding trials, at all pH levels (Table 2). In the dark, however, live or dead brine shrimp remained after feeding in 15 of 18 trials. The number of brine shrimp remaining in each dark trial ranged from 1.3% to 49.3% (Table 2). At pH 7.0, slightly more dead brine shrimp remained in the dark than live brine shrimp, 5,0% and 1.1%, respectively. At the lower pH's 5.0, 4.5, and 4.0 combined, more live brine shrimp (31.1%) remained in the dark than dead shrimp (18.4%). The number of live and dead brine shrimp remaining at the higher pH's was generally similar to that of pH 7.0 (Table 2). Significantly fewer live brine shrimp were consumed at pH 4.5 and 4.0 than at the higher levels while significantly fewer dead brine shrimp were consumed at pH 5.0 and 4.5 (Table 2).

Discussion

Based upon the high frequency of occurrence of feeding (93+%) by juvenile fathead minnows at all pH levels during all test regimens, appetitive behavior was concluded to be present to the lethal pH levels of 3.5 and 11.5. Hill (1989), in a chronic study of low pH effects on feeding behavior of smallmouth bass, also observed no loss of appetitive behavior at pH 4.2. Mortality at pH 3.5 in this study was consistent with the report by Mount (1973) that most lethal pH values recorded from laboratory data occur below 4.0. No similar data were found regarding high pH mortality, although Carlender (1969) reported the fathead minnow to have a broad tolerance for pH. The persistence of appetitive behavior through all pH levels was further supported by the minnows eating all except three (99.9%) live and dead brine shrimp fed during the light feeding trials. Consequently, since appetitive behavior persisted throughout the study, any observed reductions in feeding activity were considered to be the result of the selective impairment of sense organs by the different pH levels, or the behavioral inactivation of the feeding response during certain environmental conditions of the test (i.e.,dark), or possibly a combination of both these features.

According to the feeding patterns observed in this study, the fathead minnow is predominantly a visual, daylight feeder. The removal of all except three brine shrimp, live and dead, in the light at all pH levels identified the eyes, or the eyes in conjunction with the senses of chemoreception and mechano-reception, as the major sense organs involved in early life stage feeding. Klemm (1985) reported the fathead minnow to be primarily omnivorous and to possess large black eyes,presumably functional if heavily pigmented, at the time of hatching. The eye must play a strategically greater role in early life stage feeding as indicated by the recommendations by Birch et al. (1975) and Klemm (1985) that fathead minnow fry at least 6 days to 28 days old be fed live, freshly hatched (small) brine shrimp during the day, while older individuals may be fed frozen brine shrimp or varying forms of dry chow. This recommendation suggested a greater visual feeding success on living prey by larval-juvenile individuals and more successful visual-chemoreceptive feeding behavior in older individuals.

The eyes did not appear to be functionally impacted by different pH levels in this study. All brine shrimp, living and dead except for three individuals, were consumed in the light at all pH levels. However, the small test chambers (150 ml) with concomitant short reaction distance and number of brine shrimp per trial (50) might have alleviated any eye stress that was present or developing. Hill (1989) reported low pH levels to impair visual acuity, coordination, and agility, subsequently resulting in lower growth in chronic trials with smallmouth bass. The question of acute versus chronic study interpretations is brought into focus at this point. Hill's (1989) suggestion that acute bioassays may be too short to detect certain biological parameters, such as growth and survival, is not contested. However, in meeting the objectives of studies such as this, acute sublethal tests, especially feeding, may be more sensitive than chronic growth studies (Sandheinrich and Atchison 1990). That the fathead minnow is not an adept nocturnal feeder was supported by the number of uneaten live and dead brine shrimp in dark feeding trials at all pH's. The presence of more dead (5.0%) than live (1.1%) brine shrimp following dark feeding trials at pH 7.0 indicated a slightly greater ability or behavioral preference by the fathead minnow to select living food over dead food in the dark. However, at the lower pH's during the dark, feeding was greatly reduced and more dead brine shrimp were consumed than live shrimp.

Chemoreception was considered to be impaired at pH 5.0 when 20.7% of the dead brine shrimp remained, worsened at pH 4.5 when 27.3% remained, and then inexplicably improved at pH 4.0 when 7.3% of the shrimp remained. The marked improvement in dark-dead feeding at the lowest pH could not be explained. Feeding on dead food in the dark was considered to be entirely a function of chemoreception since stimuli for visual and mechanoreceptive senses were not present. Consequently, since appetitive behavior was known to exist, this decreased feeding on dead food in the dark strongly suggested chemoreceptive inhibition. Yoshii and Kurihara (1983) reported that bluegill without functional lateral lines did not produce successful feeding strikes in the dark based on chemoreception alone. The omnivorous feeding capability of certain species such as bluegill sunfish and fathead minnow might employ the sense of chemoreception at levels not yet described and different than other species. However, the findings by Jones et al. (1985) that pH 5.0 suppressed chemo-orientation in the Arctic char, and Lemly and Smith (1985, 1987) that pH 6.0 eliminated fathead minnow responses to chemical stimuli supported the initial conclusions drawn in this study that chemoreception was impaired by the lower pH levels.

Living prey in the dark seemed to represent the maximum sensory challenge in feeding, especially for the visual feeding fathead minnow. Consequently, low pH exhibited its greatest impact on feeding on live brine shrimp in the dark. Although live brine shrimp were successfully preyed upon in the dark at pH 7.0 and higher, only slightly more than 50% were captured at the lowest pH's. Live food under dark conditions would suggest the involvement of the combined senses of chemoreception and mechanoreception in successful feeding. Enger et al. (1989) and Montgomery (1989) presented evidence substantiating the role of mechanoreceptors in detecting moving prey. Montgomery (1989) further described the role of mechanoreceptors as operating synergistically with vision in daylight planktivory and singly in total darkness. No mention was made by Montgomery of any receptor involvement with mechanoreceptors in dark feeding. Hara

(1986) summarized the extensive literature reviews on the role of chemoreception in feeding behavior and identified the first step in the feeding sequence as arousal to the presence of food which is primarily mediated by olfaction. Atema (1980) excepted the most visual fish species, i.e., anosmic sticklebacks, from the above behavior and suggested that accompanying senses such as mechanoreception and vision may also be involved in initial prey recognition. Should these sensory assumptions be correct and chemoreception was impaired to a reduced level of effectiveness in establishing the presence of living food, then several explanations regarding the role of mechanoreception in dark feeding become likely. First, mechanoreceptors act synergistically with chemoreception in dark-live feeding and the impairment of one sense automatically reduced the success of the other; secondly, mechanoreceptors also were impaired by the lowered pH rendering them incapable of successfully detecting and/or locating the moving prey; or thirdly, mechanoreceptors alone are incapable of successfully detecting and locating living prey in complete darkness in strongly visually directed species.

Summary
Fathead minnow feeding behavior was not observed to be affected by any non-lethal pH in the light. The interaction of vision with chemoreception, or mechanoreception, or both, produced successful feeding on live brine shrimp at all pH levels. Likewise, in the dark at pH's of 7.0 and higher, the senses of chemoreception and mechanoreception operated successfully in feeding on live food (98.9%) in the dark. However, at low pH's in the dark, chemoreception and mechanoreception were impaired in detecting and locating living prey. While chemoreception was observed to be stressed at low pH's, no evidence of such an effect on mechanoreception was detected. Future studies using streptomycin sulfate (Montgomery 1989) or cobalt (Karlsen and Sand 1987) to ablate mechanoreceptors might provide valuable insights into the role of mechanoreceptors in initiating as well as concluding the feeding response.

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