LCMR PROGRESS REPORT JULY 1, 1993

I. Aquatic Invertebrate Assessment Archive: Wildlife #60

Program Manager: Judy Helgen Telephone # 296-7240
Water Quality Division
Minnesota Pollution Control Agency
520 Lafayette Road North
St. Paul, Minnesota 55155-4194

A. M.L. 91, Ch. 254, Art. 1, Sec. 14, Subd. 9(f) Appropriation: $130,000
Balance: $ 0

Aquatic Invertebrate Assessment Archive: This appropriation is from the Minnesota Environment and Natural Resources Trust Fund to the Commissioner of the Pollution Control Agency, in cooperation with the Science Museum of Minnesota, to continue work on a record system for aquatic invertebrates, and to select unimpaired aquatic reference sites for monitoring of invertebrates for analysis and development of biological criteria of pollution.

B. Compatible data: During the biennium ending June 30, 1993, the data collected by projects funded under this section that have common value for the natural resource planning and management must conform to information architecture as defined in guidelines and standards adopted by the Information Policy Office. In addition, the data must be provided to and integrated with the Minnesota Land Management Information Center's geographic data bases with the integration costs borne by the activity receiving funding under this section.

C. Match Requirement: None

II. Narrative:

The requirement to protect the biological integrity of the nation's waters is driving the mandate for development of biological criteria for pollution assessment, criteria that will include invertebrates. In this project, work on representative unpolluted aquatic reference sites will help set the stage for future development of Minnesota-specific biological criteria. The pilot invertebrate database, funded by LCMR during FY 1989-1990, will be further developed to centralize and analyze historical work on invertebrates, as well as the new work on reference sites to be selected. This long-term database will be made accessible, both as a resource and as a repository, to agencies, researchers and educators involved in biological assessment.

III. Objectives:

A. Development of the database as the central repository for records on aquatic invertebrates.

A1. Narrative:

The pilot database will be expanded to include more invertebrate groups, ~~records from historical collections,~~ records from new reference site work, and information on tolerance values.

A2. Procedures:

The data base is being designed to communicate with national and local data storage systems, while allowing the local users to add their own additional coding for field sites, collections or species. BIOS STORET taxonomic codes for invertebrate groups not currently on the database will be accessed and added to the database. If NOAA/NODC changes the code system, we will build a conversion file into the database. Records from MPCA's biomonitoring invertebrate data from 1977-1979 will be added and analyzed. Records from new reference site work will be added. We will collect information on existing, quantitative invertebrate assessments already done in Minnesota.

The database is being developed with major input from people in the data management group in MPCA Water Quality Division, and input from staff in the DNR Heritage and Nongame program, U.S. Fish and Wildlife Service, LMIC, and people working on museum collection inventory databases. Habitats will be coded with U.S. Fish and Wildlife National Wetlands Inventory habitat codes, DNR DOW lake codes and river codes as used by MPCA. Center of area points will be obtained using ARCINFO programs from LMIC and MPCA.

A3. Budget:

Amount Budgeted:	$50,000
Spent by 6/30/92:	$25,000
Spent by 1/1/93:	$35,000
Spent by 6/30/93	$50,000

A4. Timeline for tasks:

	Jul 91	Jan 92	Jul 92	Jan 93	Jun 93
Expand data base for other taxa	**********				
~~Access MPCA records~~		**********			
Central info on quantitative ~~historical~~ collections			**********		
Analyze ~~historical~~ reference site data mathematically				**********	
Add records from reference site work				**********	
Access tolerance values	**********				

A5. Status: The database developed for aquatic invertebrates will be used in proposed future work for biological criteria development in cooperation with U.S. EPA. Records of the invertebrate groups for the 35 reference wetlands sites will be fully entered in the database by early fall 1993, after final data has been delivered to MPCA. We will be using a modified BIOS coding system for species until the new federal coding system is available. Over 11,000 records from Dr. Gunderson's collection of aquatic invertebrates, primarily from wetlands, have been entered. We will be interacting with Dr. Gunderson after the end of this project to continue developing report forms and new uses of the database. At a recent conference on biological monitoring, a clear need was expressed for centralized regional species information, at the least lists of species expected for habitats in the region. In the future, it is my hope that the current work will contribute to an upper midwest regional database for aquatic invertebrates.

A6. Benefits:
Production of an ongoing centralized repository for information on Minnesota's aquatic invertebrates will be useful to agencies, researchers, and educators involved in biological assessment. At present, records from wetlands research, for example, are widely scattered and sometimes inaccessible. There are already some extensive historical collections with data that can be analyzed with the new tools for biological assessment, and provide us information on recent historical conditions. The database will become the repository for new records, as from reference site work. It will assist coordination of efforts in environmental assessment.

B. Initiate selection of aquatic reference sites for baseline invertebrate data on unstressed wetlands ~~and streams~~.

B1. Narrative:
We need baseline information on species composition in representative unpolluted habitats so we can develop biological criteria in the future.

B2. Procedures:
Reference sites will be selected from typical areas within the region for which biocriteria will be developed. Such sites should represent the "least impaired" condition, with minimal human disturbance. Reference sites will be representative, of appropriate habitat type and size, and preferably located in a refuge of some kind. Particularly valuable candidates for reference sites are ones with historical records of the biota. This is why we need information on invertebrate collections made in the state especially any that were quantitative and gave precise location information. The selection procedure will begin with selection of ecoregion or watersheds within ecoregions, then selection of candidate sites from aerial and ground surveys and inclusion as candidates sites with preexisting invertebrate data. Final selection will be based on habitat evaluation from site visits. Protocols for stream habitat evaluation have been developed by MPCA based on MN DNR and Ohio EPA's methods. Any existing wetlands evaluation protocols will be modified for our purposes. Standardized sampling methods will be used or developed. For streams, EPA has guidelines on biological assessment from rapid qualitative to quantitative assessment. Wetlands methods are under development, and we can participate in the development of good sampling methods. A variety of methods are needed to assess various wetland invertebrates, so methods selected will depend on which invertebrate groups will be needed for calculating a biotic index or other measures.

B3. Budget:

Amount budgeted:	$68,000
Spent by 6/30/92:	$43,000
Spent by 1/1/93:	$63,000
Spent by 6/30/93:	$68,000

B4. Timeline for Products/Tasks:

	Jul 91	Jan 92	Jul 92	Jan 93	Jun 93
Plan pilot monitoring	**********				
Evaluate methods	**********				
Prepare/acquire gear	**********				
Test sampling methods	*********	********			
Select candidate sites	**********	**********			
Select pilot sites to sample		*********			
Begin site monitoring		********			
Analyze samples*			**********	**********	
Analyze pilot site data				********	

* The amount of sample analysis depends on finding additional funds for a student assistant.

B5. Status: A total of 35 wetlands were selected for this project, 32 as reference sites and 3 with history of some impact. The sites are isolated wetlands, mostly in publicly-owned lands in 17 counties in the Central Hardwood Forest Ecoregion in Minnesota. Some sites are grassland/prairie and many are wooded or partly wooded. As reference sites, they are in the least impaired condition, with no polluting runoff or physical disturbance.

The wetlands sites were sampled in 1992 with a variety of methods producing over 800 biological samples primarily of invertebrates but also including amphibians and vegetation. Eleven water chemistry parameters (385 analyses) and six sediment chemistry parameters (210 analyses) were analyzed. The biological collections are being verified by experts in the particular taxa. This large data set will be the basis for additional work on the analysis of reference wetlands.

An overview of the findings will be provided to the LCMR in the early fall of 1993. Proposed funding by a Cooperative Agreement between EPA and MPCA will fund more analysis of biological samples and a thorough statistical analysis of the biological data in relation to the chemical and physical data. This will provide baseline information on the reference condition and the basis for evaluating the methods used. This is important because EPA is interested in development of protocols for assessing wetlands condition based on biological assessment. Please see the long form B.5. status report to LCMR July 1, 1993 for more detail.

B6. Benefits:
This project will help initiate longterm recording of key species or communities in best-condition representative aquatic habitats. The information will be used as a basis for the development of biological criteria for detecting pollution based on Minnesota's aquatic invertebrates. It will provide a framework for understanding longterm biodiversity changes, during rehabilitation and recovery, or during worsening pollution or environmental stress.

C. Develop the database as a usable resource.

C1. Narrative:
We need to program into the database the mathematical tools for analyzing invertebrate data to provide a basis for judging the biological integrity of the habitat. A version of the database that can be made available to other users will be developed, and complete documentation will be written.

C2. Procedure:
The database will be programmed with tools for the calculation of various indices for analyzing biomonitoring collections, such as ratios of sensitive taxa to total, species richness, community similarity, diversity and biotic indices that will permit us to analyze existing or new quantitative invertebrate collection data. We may develop Minnesota-specific or ecoregion-specific ratios and indices. Development of the database will continue with the method used now: a close interaction of users and the database programmer.

C3. Budget:

Amount budgeted:	$12,000
Spent by 1/1/93:	$ 2,000
Spent by 6/30/93:	$12,000

C4. Timeline for Products/Tasks:

	Jul 91	Jan 92	Jul 92	Jan 93	Jun 93
Program database for math analyses	**********	**********			
Interact with potential users	**********	**********			
Develop applications version			**********	**********	
Write documentation	**********	**********	**********	**********	**********

C5. Status:

The aquatic invertebrate database has been revised, making it easier to work with. Report functions have been developed, but more are needed. MPCA has recently purchased FOXBASE for use in the data management group in Assessment and Planning, and we expect to make more use of this program both on the Mac and IBM PC computers. Other states are using PC's for their biological assessment data rather than the mainframe computer, so a precedent has been set. The applications version of the database is the version in use by Dr. Gunderson. Because of the difficulties encountered with the federal BIOS coding system changes in the original program are being made. Mathematical indices will be used in the EXCEL spreadsheet with data exported from the database to EXCEL.

C6. Benefits:

Developing the capability to perform mathematical analyses on biomonitoring collection data is essential for interpretation of the data, and for future development of biological criteria based on reference site work. Development of an application version of the database will make it available to others doing biological assessment.

IV. Evaluation: A technical report on the data from this project will be delivered to LCMR in the fall of 1993. The project has stirred the interest of U.S. EPA and continuation of the work is possible under proposed EPA funding.

Future work to derive from this project:

1. work on landscape and descriptive data analysis (EPA July-Sept. 1993);
2. a Cooperative Agreement proposed to EPA for 1993-1994 (6 months' work) to cover additional analysis and multivariate techniques to relate the biological data to the chemical and physical data; and
3. proposed work with EPA (3 months in 1993-4) on development of metrics appropriate for wetlands assessment and protocols to use for different levels of information needed on wetland condition.

Reports to derive from the Aquatic Invertebrate Assessment Archive:

1. A short technical report to LCMR on the data analyzed under LCMR funding to include: the biological data, the water and sediment chemistry data and the reference site descriptions.
2. There will be a short report to EPA on the landscape analysis aspect and overview of analysis done in July-Sept. 1993.
3. A major report to EPA for the proposed Cooperative Agreement work in which analyses are completed, and multivariate techniques are applied to the biological, chemical and physical (landscape) data.
4. A paper will be submitted for publication in a scientific journal sometime in 1994, assuming the Cooperative Agreement is funded.

V. Context:

A. Records of Minnesota's aquatic invertebrates are widely scattered. The database under development is the only one for this state. It may be unique to the nation.

B. The database will be built upon excellent work already done by biologists and others. The reference site work will be developed in cooperation with other projects, such as the MN County Biological Survey, where appropriate. New work on assessment sampling will be done, as the project moves in the direction of biological criteria development.

C. There is no other source of funding to continue this project. The pilot aquatic invertebrate database under development now is funded by LCMR to Dr. Helgen through the Science Museum of Minnesota. To fulfill the mission of stewardship to clean water as evidenced by the invertebrates, this project will require future funding from LCMR, so we can record trends in species changes, and develop and test Minnesota-specific analytical tools for assessing changes.

D. Program title for FY 1989-1990: Aquatic Invertebrate Education and Database. Budget for current biennium: $60,000.

E. Biennial Budget System Program Title and Budget: title not available, budget shown here is tentative.

	FY 1992	FY 1993
Salary/fringes 1 FTE	40,000	40,000
Temporary assistants	3,000	3,000
Consultant, contract	3,500	3,500
Travel (instate)	500	500
Travel (outstate)	1,000	1,000
Supplies	2,000	2,000
Indirect costs	15,000	15,000
Total	65,000	65,000

VI. Qualifications:

Program Manager: Dr. Judy Helgen, Research Scientist II
Water Quality Division, Minnesota Pollution Control Agency

B.A. Zoology, Mount Holyoke College; M.A. Zoology, Columbia University;
Ph.D. Zoology, University of Minnesota
Publications on invertebrates:

1. Fall 1990. J.C. Helgen. The trouble with mussels. Imprint, Bell Museum of Natural History. Vol. VII, 4-5.
2. 1989. Helgen, J.C. Larval mosquitoes as vulnerable prey: Chaoborus predation Canadian Journal of Fisheries and Aquatic Sciences. 46: 1642-1650.
3. 1988. Helgen, J.C., N.J. Larson, and R.L. Anderson. Responses of zooplankton and Chaoborus to temephos in a natural pond and in the laboratory. Arch. Environ. Contam. Toxicol. 17:458-471.
4. 1987. Helgen, J.C. The Distribution of the Crayfishes of Minnesota. Report submitted to SMM and DNR December 1987, to be published as a DNR Technical Report.
5. 1985, 1987 one of several authors on two papers, one on toxic impact of ammonia, the other of pentachlorophenol on stream biota. Papers in Environmental Pollution and Aquatic Toxicology.
6. Helgen, J.C. 1987. Feeding rate inhibition in crowded Daphnia pulex. Hydrobiologia 154: 113-119.
7. Helgen, J.C. 1977. Rotifers in Lake Itasca. Minnesota Academy of Sciences Journal. Vol. 43.

Grants: 1985-1987 $5800 joint grant from Science Museum and DNR Nongame Program to survey crayfish in Minnesota.
1987 $2000 Faculty Summer Research Grant at St. Olaf College, for research on Chaoborus predation on mosquitoes.
1989-1991 $60,000 grant from LCMR for Aquatic Invertebrate database.

Advisory Committees: Scientific Peer Review Panel for research conducted on Metropolitan Mosquito Control pesticides; and Scientific Advisory Committee for Science Museum's new St. Croix Field Station on the St. Croix River, Exotic Species Task Force.

VII. Reporting Requirements:

Semiannual status reports will be submitted not later than January 1, 1992, July 1, 1992, January 1, 1993 and a final status report by June 30, 1993.

LCMR PROGRESS REPORT JULY 1, 1993 - Detailed

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C. Match Requirement: None

II. Narrative:

The requirement to protect the biological integrity of the nation's waters is driving the mandate for development of biological criteria for pollution assessment, criteria that will include invertebrates. In this project, work on representative unpolluted aquatic reference sites will help set the stage for future development of Minnesota-specific biological criteria. The pilot invertebrate database, funded by LCMR during FY 1989-1990, will be further developed to centralize and analyze historical work on invertebrates, as well as the new work on reference sites to be selected. This long-term database will be made accessible, both as a resource and as a repository, to agencies, researchers and educators involved in biological assessment.

One objective of the Federal Water Pollution Control or Clean Water Act of 1977 and 1987 is to "restore and maintain the chemical, physical and biological integrity of the nation's waters" (Sect. 101). Biological integrity can be defined as "the ability to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitat of the region" (Karr and Dudley, 1981). There is a growing concern over the losses of species worldwide, and public tolerance of pollution often resonates more strongly to biological losses or changes than to chemical information. Analyzing the aquatic biota, invertebrates as well as fish, can provide a better way of diagnosing the health of an aquatic habitat than single chemical measurements.

In the recent U.S. EPA document on biological criteria development, EPA offers guidance for acceptable approaches, but "States are to establish assessment procedures, conduct field evaluations, and determine criteria values to implement biological criteria in State standards and apply them in regulatory programs" (U.S. EPA 1990a, p.6). In its new document on water quality standards for wetlands, EPA includes numeric biological criteria development as a future direction (U.S. EPA 1990b).

This version of the proposal workplan describes in more detail the Aquatic Invertebrate Assessment Archive, Wildlife #60, to be funded in fiscal years 1991-2 by the Minnesota Environment and Natural Resources Trust Fund. The original proposal has been revised in response to the suggestions of the peer review panel. The proposal no focuses on two main objectives, one to continue development of the pilot aquatic invertebrate database, funded by LCMR in FY 1989-90, the other to initiate work on representative, unpolluted aquatic reference site invertebrates, for the purposes of developing biological criteria for pollution detection.

Invertebrates will be especially useful for establishing biological reference conditions because they are closely tied to their aquatic habitats, and occur abundantly in fishless habitats, where the fish biotic integrity index (Karr, 1981) can't be used. Many of the insects which hatch and leave the site spend most of their life cycle in juvenile stages under water, where they depend on other invertebrates or vegetational material to grow. Aquatic crustaceans, annelids and mollusks, the "non-insect invertebrates," have their entire life cycle in the water. These latter groups will therefore also be important in an index of wetland biotic integrity. Expertise for the identifications will be provided by Dr. Helgen and by outside experts when needed, and as the budget allows. At MPCA there are a few biologists with past experience with invertebrate identifications.

The assignment of pollution tolerance values is difficult because of gaps in our knowledge: we need more information on the response of the native species to different types of pollution, not just to reduced oxygen levels as in the Hilsenhoff index for stream invertebrates (Hilsenhoff, 1987). Metrics should include species which are sensitive to environmental stressors. There is a growing information base on acute and chronic responses to various invertebrates to pollutants, accessible via the EPA AQUIRE database, and in the EPA toxicity criteria documents summarizing toxicological data on various taxa from the scientific literature. However, the number of species used in laboratory toxicity tests is extremely low compared with the 11,000 species of known freshwater invertebrates in the U.S.

This project requires a long development time. Assessment of reference sites and analysis of the collections will needed beyond the next biennium. Considering that the state has over 91,000 river miles, 12,000 lakes and 5 million acres of wetlands, careful choices will have to be made on reference site selections within ecoregions. At present, biological monitoring of fish and invertebrates is not part of the present or near future routine water quality monitoring program at MPCA, although biological monitoring of the fish in the Minnesota River basin will continue in cooperation with the DNR. In strategic planning, biological criteria development has not yet been highlighted as a goal. Those of us who believe strongly in the need to assess human impacts on aquatic biota as part of our compliance to the Clean Water Act need to help provide direction for future planning. We need to push for eventual use of biological standards in monitoring programs and in regulation. Broad discussions are just beginning about planning for future monitoring programs that may include biological monitoring.

Biological assessment in the future can be used for 1) preassessment analysis, before some new treatment or alteration is allowed in the habitat, 2) recovery assessment, or tracking of improvements after pollution abatement, 3) problem area identification, detecting pollution problems that might not be detected by routine chemical monitoring, and 4) to assist, by routine or regular monitoring of stations, in describing the status of the water quality of the state. Other states have detected many impaired sites by biological criteria alone (see Ohio EPA, 1988). Biological assessment can also be valuable for certification actions (U.S. EPA 1990b).

III. Objectives:

A. Development of the database as the central repository for records on aquatic invertebrates.

A1. Narrative:

The pilot database will be expanded to include more invertebrate groups, ~~records from historical collections,~~ records from new reference site work, and information on tolerance values.

Expansion of the database now under development will allow us to input records from a broader range of taxonomic groups, and have it ready for entering reference site records. The analysis of MPCA's and other historical invertebrate collections, primarily from running water, will enable us to connect records to existing water quality information, and to test some of the analytical methods for biological assessment. Whether these stations could be selected as reference sites is unknown at present. The user version of the database will be adapted for other scientists, as well as citizens' monitoring or volunteer or schools programs, as needed. The reference site work would result in establishing baseline metrics for selected wetlands sites, and provide information for developing Minnesota-based biological standards using wetlands invertebrates. Very little has been done nationally on procedures for developing such biocriteria, so we will be part of that development.

A2. Procedures:

The data base is being designed to communicate with national and local data storage systems, while allowing the local users to add their own additional coding for field sites, collections or species. BIOS STORET taxonomic codes for invertebrate groups not currently on the database will be accessed and added to the database. If NOAA/NODC changes the code system, we will build a conversion file into the database. Records from MPCA's biomonitoring invertebrate data from 1977-1979 will be added and analyzed. Records from new reference site work will be added. We will collect information on existing, quantitative invertebrate assessments already done in Minnesota.

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A3. Budget: Amount Budgeted: $50,000
Spent by 6/30/92: $25,000
Spent by 1/1/93: $35,000
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A4. Timeline for tasks:

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Expand data base for other taxa	**********				
~~Access MPCA records~~		**********			
Central info on quantitative ~~historical~~ collections		**********			
Analyze ~~historical~~ reference site data mathematically				**********	
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A5. Status: The database developed for aquatic invertebrates will be used in proposed future work for biological criteria development in cooperation with U.S. EPA. Records of the invertebrate groups for the 35 reference wetlands sites will be fully entered in the database by early fall 1993, after final data has been delivered to MPCA. We will be using a modified BIOS coding system for species until the new federal coding system is available. Over 11,000 records from Dr. Gunderson's collection of aquatic invertebrates, primarily from wetlands, have been entered. We will be interacting with Dr. Gunderson after the end of this project to continue developing report forms and new uses of the database. At a recent conference on biological monitoring, a clear need was expressed for centralized regional species information, at the least lists of species expected for habitats in the region. In the future, it is my hope that the current work will contribute to an upper midwest regional database for aquatic invertebrates.

A6. Benefits:
Production of an ongoing centralized repository for information on Minnesota's aquatic invertebrates will be useful to agencies, researchers, and educators involved ih biological assessment. At present, records from wetlands research, for example, are widely scattered and sometimes inaccessible. There are already some extensive historical collections with data that can be analyzed with the new tools for biological assessment, and provide us information on recent historical conditions. The database will become the repository for new records, as from reference site work. It will assist coordination of efforts in environmental assessment.

B. Initiate selection of aquatic reference sites for baseline invertebrate data on unstressed wetlands ~~and streams~~.

B1. Narrative:
We need baseline information on species composition in representative unpolluted habitats so we can develop biological criteria in the future.

Reference aquatic sites will be selected from representative areas within the ecoregion for which biological criteria will be developed. Such sites should represent the least impaired condition, with minimal human disturbance, and a good distribution of the native invertebrates. The first reference sites that will be selected for monitoring will be wetlands rather than streams for the following reasons: 1) many wetlands are fishless, so the fish biotic index won't apply, 2) there is a need for development of biological criteria assessing wetlands, especially for nonpoint source pollution detection and wetlands protection programs, and 3) Dr. Helgen has some field research experience on wetlands invertebrates (Helgen, 1989; Helgen, Larson and Anderson, 1988; Helgen, 1987). In addition, this project will be analyzing MPCA's existing historical invertebrate data from rivers and streams, and work on the stream fish biotic index for the Minnesota River basin continues. The invertebrate work done there through EPA should be available to MPCA when completed.

B2. Procedures:
The procedure for selection of reference sites will begin with National Wetlands Inventory maps overlaid with boundaries for the ecoregion of concern, aerial photos, and USGS topographic maps. Candidate sites with the least amount of human disturbance, especially ones in a refuge, will be selected. We will decide on how many classifications or types to have as reference sites. Sites will be selected preferably in the mid-range of known environmental or landscape gradients. Criteria for selection of reference sites will be developed as part of this project, building upon criteria already in use for streams (U.S. EPA, 1990a; Mass. DEQE, 1989; Ohio EPA, 1988; Fiske and Langdon, 1986; Hughes et al, 1986). U.S. EPA's suggested criteria for streams can be modified for wetlands: 1) no human disturbance, 2) stream size, 3) type of stream channel, 4) location in refuges, 5) existence of historical biological data. Historical

invertebrate records can add to our knowledge of the unimpaired condition, as historic fish records have.

For wetlands reference sites, modifications of criteria used for stream reference sites will be developed, based on criteria proposed by U.S. EPA, and upon approaches to wetlands assessment by others (Audobon Soc., Mass. 1989; Audobon Soc., National, 1990; Brooks and Hughes, 1988). The steps outlined by Hughes et al (1986) will be used, with some modifications: first determine human disturbances, quantify habitat size features (watershed, discharges), characterize water source (stream channel, gradients, wetland hydrology), and locate refuges for reference sites. Then determine how typical the sites are, determine zoogeographic factors which might affect species richness at the sites, and finally rank sites by level of disturbance, so degraded or atypical sites can be eliminated as reference candidates for dominant wetland types. In the future, biological criteria for special habitats like bogs and vernal pools could be valuable (see Audobon Soc., Mass. 1989).

Site visits to candidate sites will be necessary to determine the level of human impact, whether there is undisturbed, well-developed wetland vegetation, some idea of the site's history, and qualitative evidence of good vertebrate and invertebrate communities. The final selection of pilot reference sites would be based on a habitat evaluation. Protocols for stream habitat evaluation have been developed at MPCA (Pat Bailey, Water Quality Division), based on the Minnesota DNR and Ohio EPA's evaluation methods. Existing wetlands habitat evaluation procedures will be modified for the purpose of choosing sites suitable as references for aquatic invertebrates and other aquatic species.

During the process of reference site selection, we will need to examine the aquatic use classifications used by MPCA (Minn. Rules No. 1990 ch. 7050.0200), how these relate to reference sites and whether changes or refinements in use classifications should be proposed as we move towards biological criteria development.

Sampling Methods and Analytical Tools

The issues of spatial and temporal variability of biological data will have to be addressed before the sampling phase begins. Because of the important seasonal changes in densities and species of invertebrates, sampling of reference sites will take place in three seasons to select the best season for effective monitoring of impacted waters. Obviously, a biological standard developed in the spring cannot be applied to a site monitored in the fall. The variability of quantitative sampling by date is usually high for invertebrate samples. White many of the ratios and indices in use do not require population density information, quantitative sampling has not been ruled out for this project. The variance in the data can be reduced by aggregating taxonomic groups from species up to family, but then the sensitivity of the response to a stressor or pollutant would be reduced, if only some members of the aggregated group are sensitive. In certain invertebrate groups, e.g. chironomids, there are wide differences in environmental sensitivities, and an impact on some species could be masked by aggregation of the data.

Spatial variability can be great, especially for biological data. In a study of many lakes, the biological parameters were least in concert within the region, compared with the physical or chemical parameters (Tim Kratz, pers. comm.). This is a very real problem, and where possible, reference sites in the mid-range of known environmental gradients should be selected, and several sites within a region should be sampled. There is also spatial variability within the habitat, which means it will be necessary to sample the habitat strata in a repeatable way.

Most agree it is extremely important to have a variety of measures of the biota, and not rely on just one or two indices. This is especially true in the exploratory stages of biological standards development. Standard wetland sampling techniques for invertebrates are activity traps and core samples (Brooks and Hughes, 1988), but these techniques sample only limited classes of invertebrates. Depending on which species we select for wetland indices of biological integrity, and which analyses we use or develop, a variety of sampling techniques will be needed. Sweep/dip-netting would establish presence and relative abundances of species such as mollusks, insects and crustaceans. Funnel trap samplers sample vertically migrating zooplankton, floating funnel cones capture surface breathing insects (Kyle Thompson pers. comm.), minnow traps capture crayfish and adult aquatic beetles. Sampling methodologies will have to be standardized eventually, as criteria are developed and tested.

Sampling techniques used for streams for calculating the Hilsenhoff Biotic Index (HBI) of organic pollution are well developed (Hilsenhoff, 1987). The stream riffle is sampled with the kick net technique, until at least 100 organisms < 3 mm length are collected by sorting on a randomly numbered grid. Specimens are identified to genus or species and the numbers of each are multiplied by the assigned tolerance values (0 = intolerant, 5 = most

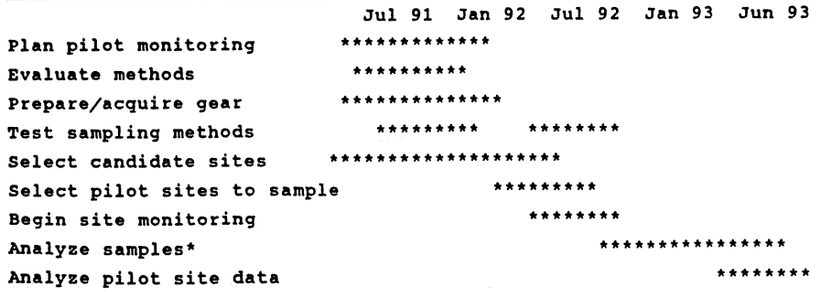
tolerant). The calculated Biotic Index will range from 0 (excellent water quality) to 10 (very poor water quality). This index is based on arthropods, mostly insect larvae, and the tolerance values reflect responses to changes in oxygen in the water. Hilsenhoff (1988) has also developed a rapid assessment family level biotic index. This approach, with different sampling techniques and taxonomic groups, is one that will be applied to wetlands.

U.S. EPA has developed three levels of bioassessment protocols (Plafkin et al, 1989). The level II and level III protocols have a variety of metrics to use as standards for monitoring: taxa richness, family biotic index, EPT to chironomids ratio, scraper/filterer ratio, ratio of shredders/total, percent that is dominant family, EPT index and community similarity index (three similarity indices are recommended). Monitored sites are scored on the basis of percent comparability to the reference site condition, except for community similarity indices which already include a comparison with the reference condition. These protocols are derived for stream riffle areas only at present. We will adopt an approach similar to that in the EPA protocols, one that derives a variety of metrics for comparison to reference sites and will work for the development of new biotic indices for wetland invertebrates.

B3. Budget:

Amount budgeted:	$68,000
Spent by 6/30/92:	$43,000
Spent by 1/1/93:	$63,000
Spent by 6/30/93:	$68,000

B4. Timeline for Products/Tasks:



* The amount of sample analysis depends on finding additional funds for a student assistant.

B5. Status: A total of 35 wetlands were selected for this project, 32 as reference sites and 3 with history of some impact. The sites are isolated wetlands, mostly in publicly-owned lands in 17 counties in the Central Hardwood Forest Ecoregion in Minnesota. Some sites are grassland/prairie and many are wooded or partly wooded. As reference sites, they are in the least impaired condition, with no polluting runoff or physical disturbance.

All the sites were analyzed in stages in 1992 by several sampling methods: coring sediments for chironomids (midges, 5 cores/site, 175 cores), artificial substrates put out three weeks for chironomids (5/site, 175 substrates), activity traps for mobile invertebrates and tadpoles (10/site, 2 pooled to = a sample, 175 samples), standardized dipnet samples (2/site, 70 samples of the pan portion, 70 of the remainder vegetation), algae and zooplankton samples (2/site, 70 each type, not analyzed under this project funding), and vegetation collected in linear transects every 10 meters (average 5 bags of vegetation/site around 175 samples). There are over 800 biological samples being analyzed.

Water chemistry samples were taken during spring and summer site visits. The 11 parameters are listed in the table below, for a total of at least 385 chemical analyses. Dissolved oxygen (DO) was measured at all sites in the summer before 8:00 a.m., to obtain the minimum DO reading after overnight respiration occurred. In the future, for the purpose of determining water quality standards, information is needed on the "natural" oxygen fluctuations in wetlands. Sediment cores were taken (3/site, 105 cores) and pooled and frozen for analysis as previously described (January 1993 Progress Report) and the parameters analyzed are given in the table below, for a total of 210 sediment analyses not counting moisture. The oxalate-extractable iron and aluminum content will be useful as an indicator of the phosphorus-retention capacity of the wetland sediments. This information is important because wetlands may not be able to act as "sinks" for nutrient pollution from the land.

Table 1. Biological and physical parameters for reference wetlands analysis. Data analysis carried out with LCMR funding is not starred. Data analysis to be carried out or completed after June 30, 1993, under proposed funding by U.S. EPA is indicated by *. Most of the chironomid and vegetation analysis has been done with LCMR funding. All of the landscape analysis will be done under EPA funding.

Biological parameters	Water chemistry
Caddisflies	Calcium
Chironomids (midges) from 4 methods*	Chloride
Coleoptera (beetles) and Hemiptera (bugs)	Conductivity (field)
Dragonflies and damselflies	Nitrite/nitrate
Fingernail clams	Nitrogen total
Leeches	Orthophosphate
Mayflies	Oxygen minimum (field)
Other diptera, other groups	pH (field)
Snails	Phosphorus total
Amphibian densities	Total suspended solids
Dominant vegetation*	Turbidity
Taxa richness (genus level)*	
Invertebrate community structure*	
Metrics and indices*	

Landscape analysis*	Sediment chemistry
Elevation	Aluminum
Percent grassland	Chloride
Percent tilled cropland	Iron
Percent wooded/shrub	Moisture content
Site area	Nitrogen total
Watershed area	Organic carbon
	Phosphorus total

This project hired a student worker for about a year to assist with the field work and lab analysis of invertebrates, and a temporary worker was hired for a few weeks to pick the chironomids. Two students from Gustavus Adolphus College worked during January term, 1993 on the invertebrate and amphibian collections and on accession of ASCS aerial photographs. The volunteer student from Hamline University worked too short a time for training on the algae. These will be analyzed under the proposed EPA funding.

The water chemistry analysis is completed. The sediment chemistry data will be delivered to MPCA early in July 1993. The sites have been flown for aerial photography and the landscape analysis based on the stereoscopic aerial photographs will be done by September 1993, both under EPA funding.

The vegetation from a least 20 of the 35 sites has been analyzed. The analysis of the vegetation from the rest of the sites, as well as the algae, will be done as part of a proposed EPA/MPCA Cooperative agreement. Vegetational composition will be important as an indicator of wetlands condition.

Most of the invertebrate analysis is done. Reference collections for some major invertebrate groups are being verified by Dr. Ralph Gunderson. The snails, caddisflies and leeches are being verified by local experts. As part of the proposal to the EPA, some of the reference collections will be vouchered to museums in 1994 as part of the QC/QA plan.

The chironomid analysis of a subset of samples is being done by Dr. Len Ferrington at the University of Kansas in Lawrence. Dr. Ferrington is a recognized national expert in the taxonomy of this important group of wetland invertebrates. The data on the chironomid community composition and densities obtained from the different sampling methods will be used to describe the reference condition and to develop protocols in the future.

This project has formed the basis for funding requests to the U.S. EPA, which is interested in using the data as part of the nationwide effort in developing biological criteria for wetlands. Once source will cover the aerial photography of the 35 reference sites and a landscape analysis of the landcover and site area and small watersheds and processing biological and the sediment data with descriptive statistics by early fall 1993.

A proposal for a Cooperative Agreement between EPA and MPCA will cover analysis of algae, analyzing additional chironomid and vegetation samples, plus an overall analysis of the biological data in relation to the chemical and landscape information using cluster and principal components analysis on the biological communities and relating these findings to the physical parameters.

Finally, the EPA may provide funding for developing metrics and protocols suitable for assessing wetlands to contribute to the effort for developing biological criteria. Most of the work on biological assessment methods has

occurred with stream riffle communities. The indicators or metrics for assessing wetlands health will differ from stream methods because the physical conditions and biological adaptations of species differ. There is a real need for guidance towards more uniform ways of analyzing the condition of our wetlands, including their biological quality.

B6. Benefits:
This project will help initiate longterm recording of key species or communities in best-condition representative aquatic habitats. The information will be used as a basis for the development of biological criteria for detecting pollution based on Minnesota's aquatic invertebrates. It will provide a framework for understanding longterm biodiversity changes, during rehabilitation and recovery, or during worsening pollution or environmental stress.

C. Develop the database as a usable resource.

C1. Narrative:
We need to program into the database the mathematical tools for analyzing invertebrate data to provide a basis for judging the biological integrity of the habitat. A version of the database that can be made available to other users will be developed, and complete documentation will be written.

C2. Procedure:
The database will be programmed with tools for the calculation of various indices for analyzing biomonitoring collections, such as ratios of sensitive taxa to total, species richness, community similarity, diversity and biotic indices that will permit us to analyze existing or new quantitative invertebrate collection data. We may develop Minnesota-specific or ecoregion-specific ratios and indices.

C3. Budget:

Amount budgeted:	$12,000
Spent by 1/1/93:	$ 2,000
Spent by 6/30/93:	$12,000

C4. Timeline for Products/Tasks:

	Jul 91	Jan 92	Jul 92	Jan 93	Jun 93
Program database for math analyses	**********	**********			
Interact with potential users	**********	**********			
Develop applications version			**********	**********	
Write documentation	**********	**********	**********	**********	**********

C5. Status:
The aquatic invertebrate database has been revised, making it easier to work with. Report functions have been developed, but more are needed. MPCA has recently purchased FOXBASE for use in the data management group in Assessment and Planning, and we expect to make more use of this program both on the Mac and IBM PC computers. Other states are using PC's for their biological assessment data rather than the mainframe computer, so a precedent has been set. The applications version of the database is the version in use by Dr. Gunderson. Because of the commitment to having the data from this project be compatible with EPA's STORET data system, used nationally and by MPCA, the BIOS coding system was used. This created problems, partly because the codes were difficult to import. We are now hand-entering codes. A serious drawback of the BIOS system is that many of the Minnesota species are not coded. Now, EPA is changing to a new coding system sometime in the near future. As work is carried out on the biological data from this project during the year to come, the database will continue to be refined. In addition, the database has not been programmed for math analysis. Instead, mathematical functions for some biological indices are already developed in EXCEL and will be used to apply to data exported from the database to EXCEL spreadsheets.

C6. Benefits:
Developing the capability to perform mathematical analyses on biomonitoring collection data is essential for interpretation of the data, and for future development of biological criteria based on reference site work. Development of an application version of the database will make it available to others doing biological assessment.

IV. Evaluation: A technical report on the data from this project will be delivered to LCMR in the fall of 1993. The project has stirred the interest of U.S. EPA and continuation of the work is possible under proposed EPA funding.

Future work to derive from this project:

1. work on landscape and descriptive data analysis (EPA July-Sept. 1993);
2. a Cooperative Agreement proposed to EPA for 1993-1994 (6 months' work) to cover additional analysis and multivariate techniques to relate the biological data to the chemical and physical data; and
3. proposed work with E{A (3 months in 1993-4) on development of metrics appropriate for wetlands assessment and protocols at different levels of information needed on wetland condition.

Reports to derive from the Aquatic Invertebrate Assessment Archive:

1. A short technical report to LCMR on the data analyzed under LCMR funding to include: the biological data, the water and sediment chemistry data and the reference site descriptions.
2. There will be a short report to EPA on the landscape analysis aspect and overview of analysis done in July-Sept. 1993.
3. A major report to EPA for the proposed Cooperative Agreement work in which analyses are completed, and multivariate techniques are applied to the biological, chemical and physical (landscape) data.
4. A paper will be submitted for publication in a scientific journal sometime in 1994, assuming the Cooperative Agreement is funded.

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VI. Context:

A. Records of Minnesota's aquatic invertebrates are widely scattered. The database under development is the only one for this state. It may be unique to the nation.

B. The database will be built upon excellent work already done by biologists and others. The reference site work will be developed in cooperation with other projects, such as the MN County Biological Survey, where appropriate. New work on assessment sampling will be done, as the project moves in the direction of biological criteria development.

C. There is no other source of funding to continue this project. The pilot aquatic invertebrate database under development now is funded by LCMR to Dr. Helgen through the Science Museum of Minnesota. To fulfill the mission of stewardship to clean water as evidenced by the invertebrates, this project will require future funding from LCMR, so we can record trends in species changes, and develop and test Minnesota-specific analytical tools for assessing changes.

D. Program title for FY 1989-1990: Aquatic Invertebrate Education and Database. Budget for current biennium: $60,000.

E. Biennial Budget System Program Title and Budget: title not available, budget shown here is tentative.

	FY 1992	FY 1993
Salary/fringes 1 FTE	40,000	40,000
Temporary assistants	3,000	3,000
Consultant, contract	3,500	3,500
Travel (instate)	500	500
Travel (outstate)	1,000	1,000
Supplies	2,000	2,000
Indirect costs	15,000	15,000
Total	65,000	65,000

VII. Qualifications:

Program Manager: Dr. Judy Helgen, Research Scientist II
Water Quality Division, Minnesota Pollution Control Agency

B.A. Zoology, Mount Holyoke College; M.A. Zoology, Columbia University; Ph.D. Zoology, University of Minnesota
Publications on invertebrates:

1991 RESEARCH PROJECT ABSTRACT
FOR THE PERIOD ENDING JUNE 30, 1991
This project was supported by the Minnesota Environment and Natural Resources Trust Fund

TITLE:	Aquatic Invertebrate Assessment Archive
PROGRAM MANAGER:	Dr. Judy Helgen
ORGANIZATION:	MN Pollution Control Agency
APPROP. AMOUNT:	$146,000

STATEMENT OF OBJECTIVES

To analyze unimpaired reference wetland sites for invertebrate and other biological communities in relation to physical and chemical features for the purpose of establishing the baseline reference condition for development of biological assessment methods and biological criteria of pollution in the future. To continue work on the aquatic invertebrate database in Minnesota.

RESULTS

Thirty-five wetlands in the Central Hardwood Forest ecoregion in 17 counties of Minnesota were analyzed for several invertebrate groups, amphibian densities and eleven water and six sediment chemistry parameters resulting in over 800 biological and 600 chemical samples. The reference sites show a broad representation of invertebrate groups and vegetation, and have indications of high water quality in the chemical data, e.g. low phosphorus and total suspended solids in most sites. The full analysis of the large data set will occur in 1993-1994 under proposed new funding from U.S. E.P.A. for aerial photography of the sites, landscape analysis and descriptive data analysis followed by principal components analysis, application of multivariate analysis techniques and evaluation of the methods used in the project.

PROJECT RESULTS USE AND DISSEMINATION

This project has formed the basis for funding requests to U.S. E.P.A., which is interested in the research as part of a nationwide effort to develop biological assessment methods and biological criteria. The proposals to E.P.A. cover the work described above plus additional work on developing appropriate protocols and metrics, or measures of biological condition, for wetlands. A technical report on the biological, water and sediment chemistry data will be delivered to LCMR in fall, 1993; reports to E.P.A. on landscape analysis and the full statistical data analysis and methods comparison will be submitted in fall, 1994. A scientific article will be submitted for publication in late 1994. In 1994 the data will be presented at conferences, such as the Society of Wetlands Scientists and the Midwest Pollution Control Biologists annual meeting.