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# A SUMMARY REPORT ON BIOLOGICAL SEDIMENT GUIDELINES FOR THE LAURENTIAN GREAT LAKES

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## Management Perspective

Almost all the Great Lakes Areas of Concern have documented sediment contamination. However these designations are often based on relatively few chemical measurements. Little systematic data are available on the concentrations of contaminants in many of the Areas of Concern and there is far less information on direct impacts of sediment associated contaminants on biota.

Current sediment guidelines are based on the comparison of chemical concentrations at a site to those which have been established as representing a perceived safe concentration on a chemical by chemical basis. However, the chemical approach has been criticized in recent years because it frequently fails to achieve its objectives or because it is so excessively rigorous that it has limited value. As an alternative, the National Water Research Institute and Ontario Region of Environment Canada have developed an approach using **biological sediment guidelines**.

There are two basic assumptions behind these biological sediment guidelines. First, that the it is the effects of sediment contamination on biological processes that are the primary concern and that therefore assessment of biological effects is paramount. Second, that the complexity of the sediment matrix makes chemical concentration a poor predictors of the biological availability of contaminants.

The biological sediment guidelines incorporate (a) the structure of benthic invertebrate communities by using predictive models that relate site habitat attributes to an expected community, and; (b) functional responses (survival, growth and reproduction) in four sediment toxicity tests (bioassays) with benthic invertebrates using ten test endpoints. For both community structure and toxicity guidelines have been established that allow determination of the community as either, unstressed, potentially stresses, stressed or severely stressed and the sediment as either non-toxic, potentially toxic or toxic.

To simplify the assessment process the BEAST software has been developed which incorporates the complex multivariate analysis required by this approach and presents the user with straightforward categories of sediment quality on a site by site basis. Designed for the BEnthic Assessment of SedimenT, the software automates the methodology and employs the RAISON Mapping and Analysis package from Environment Canada as a foundation, the BEAST combines new methods with a simple, straight-forward software user interface. The result is a powerful new tool for sediment assessment.

## Summary

Current sediment guidelines are based on the comparison of chemical concentrations at a site to those which have been established as representing a perceived safe concentration on a chemical by chemical basis. However, the chemical approach has been criticized in recent years because it frequently fails to achieve its objectives (Cairns and van der Schalie 1980, Long and Chapman 1985, Chapman 1986, Chapman 1990) or because it is so excessively rigorous that it has limited value (Painter 1992, Zarull & Reynoldson 1993). There are two basic assumptions behind the biological sediment guidelines described in this report. First, that the effects of sediment contamination operate on biological processes and that therefore assessment of biological effects is paramount. Second, that the complexity of the sediment matrix results in chemical concentrations being poor predictors of the biological availability of contaminants. Accordingly methods were used to develop sediment guidelines that use biological effects of sediment contamination directly rather than indirectly through chemical surrogates.

The difficulty with developing biological guidelines for application in the ambient environment has been the temporal and spatial variability of biological attributes that has made site specific targets difficult to establish. The development of biological guidelines for sediments, used a modification of techniques developed in the United Kingdom (Wright et al. 1984, Furse et al. 1984; Armitage et al 1987). The technique involves a multivariate statistical approach using (a) data on the structure of benthic invertebrate communities; (b) functional responses (survival, growth and reproduction) in four sediment toxicity tests (bioassays) with benthic invertebrates; and, (c) selected environmental variables. This approach results in predictive models being produced that resolve the issues of temporal and spatial variability and allows site specific numeric guidelines to be established from reference data sets.

Two pattern recognition techniques are employed in the analysis: cluster analysis and ordination. The ordination scores from reference biological data sets are correlated with environmental variables which are anticipated to be least affected by anthropogenic activities (*e.g.*, alkalinity, depth, silt, sodium, etc.). Multiple discriminant analysis (MDA) is used to relate the site groupings from the cluster analysis of the biological data to the environmental variables and to generate a model which can be used to predict community assemblages and functional responses at new sites with unknown but potential contamination. The predicted community assemblages and functional responses from the reference data sets are then compared with the benthic communities and functional responses at a tests site to asses the quality of the sediment. The need for remedial action or appropriate disposal of dredged material can then be determined.

It has been suggested that such multivariate methods are too complex, require specialised practitioners, and are difficult to convey to managers and the public (Gerritsen, 1995). Limitations associated with multivariate methods can be attributed to the lack of a comprehensive tool for application. The need for a simple, inexpensive software tool which encapsulates the requirements for multivariate analysis has led to the development of the BEAST. Designed for the **BE**nthic **A**ssessment of **S**edimen**T**, the software automates the methodology outlined in this report. Employing the RAISON Mapping and Analysis package from Environment Canada as a foundation, the BEAST combines new methods with a simple, straight-forward software user interface. The result is a powerful new tool for sediment assessment.

# 1.0 Sediment Issues and Current Guidelines

## 1.1 Sediment Issues

The aquatic ecosystem can be viewed as consisting of three physical compartments; water column, surficial sediments and deep sediments. In the water column the major processes involving sediment particles are physical, with settling and re-suspension as the major forces; chemical, with precipitation of different materials being added to the suspended sediment pool, as well as co-precipitation and adsorption of contaminants on particle surfaces; and biological, primarily grazing and fecal-pellet generation and biodegradation. These processes also affect the transport and partitioning of contaminants in particles sinking to the bottom.

Sediments play an important role in the physical movement, chemical partitioning and biological fate of metals, organics and nutrients (Allan 1984). Metals, and many of the more commonly detected organic chemicals and nutrients are often closely associated with both suspended solids and bottom sediments. Furthermore, many chlorinated organic contaminants have a low solubility in water and thus concentrations several orders of magnitude or higher are found in association with sediment particles (Golterman et al. 1983). Fine-grained sediments have the potential for collecting the highest concentrations of contaminants. These sediments accumulate in low energy areas such as nearshore embayments in lakes, near the mouths of rivers and in harbours. Many of these areas are also recipients of urban, industrial and agricultural inputs of contaminants.

Bottom sediments are the primary sink for materials in aquatic environments. However, physical resuspension and biological and geochemical processes at the sediment-water interface can substantially prolong the time during which contaminants remain bioavailable and accumulate in the food chain. In the active sediment layer, usually the upper 10 cm, a number of chemical, physical and biological processes affect sediment-associated contaminants. These include ingestion and egestion of sediment particles by the benthic fauna, chemical and physical sorption and desorption and diffusion processes through sediment pore water. The major net effects of these processes are changes in the food web through species changes and loss, the bioaccumulation of contaminants through the benthic food chain and the recontamination of the water column. In all cases the ultimate concerns are the effects produced in all organisms, including man.

Once in the deep sediments (below 10 cm) particles are often considered to be lost to the system, however, two processes can result in the physical transport of materials back into the water column. Bioturbation, resulting from the activity of benthic invertebrates, can recycle material from as deep as 40 cm to the more active surface layer and thus can keep contaminants circulating in the ecosystem much longer (Sorokin 1966; Karickhoff and Morris 1985). The second major process affecting physical movement of contaminated sediments is their periodic resuspension by major storm events, internal waves and currents.

## 1.2 Current sediment guidelines

Most management issues regarding contaminated sediments have been associated with the testing, dredging and disposal of material for navigational purposes. In the period 1980 through 1984 some 321 dredging projects were reported, in which 24,255,380 $m^3$ of material was removed and disposed of throughout the Great Lakes. The "criteria" used for assessing contaminated sediments were the dredging guidelines of the Ontario Ministry of the Environment (Persaud and Wilkins 1976) and those of the U.S. EPA (1977). Recently, the Ontario Ministry of the Environment and Energy (OMOEE) has proposed new sediment guidelines (Table 1) for use in the assessment of navigational dredging as well as remedial investigations in Areas of Concern (Persaud et al. 1990). These guidelines are based on the Screening Level Concentration Approach (SLC) developed by Neff et al (1986) in which the co-occurrence of concentrations of selected contaminants in sediments and the presence/absence of benthic infaunal species are used to devise three levels of biological effect - the No Effect Level, the Lowest Effect Level and the Severe Effect Level. Federally, the Canadian Federal Department of the Environment (Environment Canada) is in the process of developing national sediment quality guidelines using a weight of evidence approach in which biological and chemical data from numerous modelling exercises, laboratory toxicity tests and field studies performed on freshwater sediments are compiled, analyzed and matched (Smith et al. 1996). Two assessment values (a threshold effect level (TEL) and a probable effect level (PEL) have been derived using this system for 23 substances i.e., eight trace metals, six individual polycyclic aromatic hydrocarbons (PAHs), total polychlorinated biphenyls (PCBs) and eight pesticides. Again this is a process similar to that initiated by the Province of Ontario in that sediment guidelines are derived on a chemical-by-chemical basis. The U.S. EPA (1993) have developed guidelines for deriving site- specific criteria using a tiered approach which generates physical, chemical, toxicological and bioaccumulation information prior to discharge of dredged materials; however, we are not aware of its application in any published material.

The assessment of the ecological risk to biota in the Great Lakes from contaminated sediments in Areas of Concern as well as the need for remedial action have been based on the traditional methods developed for water quality assessments and often incorporate the Sediment Quality Triad (SQT) approach (Long and Chapman 1985, Canfield et al. 1996, Besser et al 1996). The SQT approach uses a combination of results from whole sediment laboratory toxicity tests (bioassays), chemical concentrations of contaminants measured in sediments and *in-situ* benthic invertebrate community composition to determine the nature and extent of sediment contamination. This approach was used extensively in the Assessment and Remediation of Contaminated Sediments Program (ARCS) used to address the contaminated sediments problem at the 42 Great Lakes Areas of Concern (Fox and Tuchman 1996; Burton et al. 1996). Again, correlations between observations of biological effects and perceived safe concentrations of chemicals are made on a chemical-by-chemical basis (Ingersoll et al. 1996). Good concordance in using the SQT approach was evident for extremely contaminated sites among measures of laboratory toxicity, concentrations of contaminants in sediments and the composition of the benthic invertebrate communities; however, in moderately contaminated samples, less concordance was observed, especially between the benthic commuinities present and either laboratory toxicity tests or sediment contaminant loading (Canfield et al 1996). Scientists involved in the ARCS study suggest that evaluations of non-contaminant factors is needed to better interpret the responses of benthic invertebrates exposed to contaminated sediments

Measuring the concentrations of various chemicals present in the sediments does not address the ultimate concern; namely, whether the contaminants present are exerting biological stress and/or are being bioaccumulated. In several cases, despite contaminants levels lower than historic background concentrations found in sediment cores taken from the nearby, open lake depositional basins, sites are identified as impacted (Reynoldson et al, 1988). A series of bioassessment techniques, along with appropriate criteria, are necessary to identify the types of stress being exerted, their severity, and the bioavailability of the contaminants present.

It is our view that an alternate approach should be used. This report describes the development of such an approach and a method for setting site-specific guidelines incorporating attributes of the sediment of concern and the array of physico-chemical interactions occurring at a site. In essence this approach assumes that the objective of sediment guidelines is the protection of aquatic ecosystem "health" from deleterious substances of anthropogenic origin associated with bottom sediments. As "health" is a description of organic state it follows that biological attributes and not chemical surrogates are the most appropriate indicators to use.

**Table 1.** Summary of Ontario Sediment Quality Guidelines (values in ug/g dry weight)

| | No effect level | Lowest effect level | Severe effect level |
|---|---|---|---|
| **METALS** | | | |
| arsenic | | 6 | 33 |
| cadmium | | 0.6 | 10 |
| chromium | | 26 | 110 |
| copper | | 16 | 110 |
| iron (%) | | 2 | 4 |
| lead | | 31 | 250 |
| manganese | | 460 | 1100 |
| mercury | | 0.2 | 2 |
| nickel | | 16 | 75 |
| zinc | | 120 | 820 |
| **NUTRIENTS** | | | |
| TOC (%) | | 1 | 10 |
| TKN | | 550 | 4800 |
| TP | | 600 | 2000 |
| **ORGANICS** | | | |
| Aldrin | | 0.002 | 8 |
| BHC | | 0.003 | 12 |
| a BHC | | 0.006 | 10 |
| b BHC | | 0.005 | 21 |
| C BHC | 0.0002 | 0.003 | 1 |
| Chlordane | 0.005 | 0.007 | 6 |
| DDT (total) | | 0.007 | 12 |
| op + pp DDT | | 0.008 | 71 |
| pp DDD | | 0.008 | 6 |
| pp DDE | | 0.005 | 19 |
| Dieldrin | 0.0006 | 0.002 | 91 |
| Endrin | 0.0005 | 0.003 | 130 |
| HCB | 0.01 | 0.02 | 24 |
| Heptachlor | 0.0003 | | |
| H epoxide | | 0.005 | 5 |
| Mirex | | 0.007 | 130 |
| PCB (total) | 0.01 | 0.07 | 530 |
| PCB 1254 | | 0.06 | 34 |
| PCB 1248 | | 0.03 | 150 |
| PCB 1016 | | 0.007 | 53 |
| PCB 1260 | | 0.005 | 24 |
| PAH (total) | | 2 | 11000 |

## 2.0 A New Approach - Biological Guidelines

An ideal sediment assessment strategy should: (1) integrate physical data along with chemical and biological data, to provide an accurate assessment of the specific problems; (2) utilize the results from each technique to reduce subsequent sampling requirements and, therefore costs; (3) provide adequate proof of the linkage between the contaminated sediments and the problem (i.e. cause-effect relationship); (4) quantify problem severity, thereby enabling inter-comparisons between and within areas of investigation; (5) consider the impacts or effects on different species and different trophic levels - since biological impairment may occur in both the water column (if resuspension occurs) and the sediments, and there is no such thing as the universal, most sensitive species (Cairns 1981, Monk 1983). A proposed strategy consists of four stages (Zarull and Reynoldson 1993):

1. *Identification* - Sediments are screened using a relatively small battery of sensitive biological toxicity tests and *in situ* methods to identify or confirm the presence of problems that may be associated with sediment contamination.

2. *Assessment* - A more comprehensive analysis using physical, chemical and biological techniques to spatially define the extent of the contamination, identify the causative factors and determine the appropriate methods and level for remediation.

3. *Remediation* - Control of active anthropogenic sources of contaminants followed by appropriate remedial action (e.g., capping, in-situ treatment, natural recovery).

4. *Monitoring* - A follow-up monitoring program will be required to determine if the remediation has been successful. The monitoring program must include measurements of those variables that failed to meet the guidelines and triggered the remedial action. The success of remediation will be determined by those guidelines being met.

Measure of "health" for setting guidelines should be based on both the resident infauna as well as laboratory tests measuring functional attributes such as survival, growth and reproduction. Furthermore, the guidelines will be expressed as the biological attributes themselves rather than as chemical surrogates. In the past there has been difficulty with setting such biological guidelines because of the inherent spatial and temporal variability of biological systems. The problem has been resolved by developing a reference database of a large number of unimpacted reference sites. The reference database establishes the range of normal communities and the response range for the functional measurements. Using this data base, a predictive model can be built that allows the prediction of the value for biological indicators at a new test site based on habitat (physical and chemical in sediment and water) attributes. Thus the biological guidelines are site-specific and tailored to the attributes of the test site. This approach is now being described as the *Reference Condition Concept* (Reynoldson et al. 1997).

## 2.1 The Reference Condition

The fundamental concept behind the reference condition approach is to establish a data base of sites that represents unimpaired conditions (reference sites) at which biological and environmental attributes are measured. The data-base is then used to develop predictive models that match a set of environmental variables to biological conditions. These predictive models then allow a set of environmental measurements to be made at a new site and used in the model to predict the expected biological condition. A comparison of the actual biological condition at the new (test) site with conditions at the reference sites to which the new site is predicted as belonging allows an assessment of the condition of the new site to be made.

Reference sites refer to locations at which data are collected for comparison with test sites. They must be carefully selected because they form the benchmark against which test sites will be compared. The condition at reference sites should represent the optimal range of minimally impaired conditions that can be achieved at sites anticipated to be ecologically similar. The determination of the reference condition from reference sites is based on the premise that sites least affected by human activity will exhibit biological conditions most similar to those at natural, pristine locations. The reference condition is described using biological attributes. Because there is no single reference condition, the appropriate reference condition for any site is selected from a set of possible reference states using a predictive model based on environmental site attributes.

Three basic characteristics exist for describing a suitable reference condition (Hughes 1995):

(1) be politically acceptable and reasonable;

(2) should represent a sufficiently large number of sites or areas of reference within waterbodies;

(3) must represent important aspects of natural conditions.

### *2.1.1 Reference Sites*

Typical reference sites should have minimal impairment from anthropogenic activities such as watershed disturbance, habitat alteration, nonpoint source runoff, point-source discharges, atmospheric deposition or angling pressure. Sites without any of these disturbances are ideal reference sites. In many regions, human land-use practices and atmospheric contamination have so altered the landscape that truly undisturbed sites are unavailable. However, a criterion of minimal impairment must be used in selection of reference sites. Therefore, co-operation at the national or international level may be necessary to acquire appropriate reference-site locations.

Reference sites will vary from region to region and for different waterbodies. A general guide to minimal impairment can be obtained from the characteristics of a reference site as modified from Hughes (1995):

(1) extensive natural riparian vegetation;

(2) appropriate diversity of substrate;

(3) a natural channel or shoreline structure;

(4) a natural hydrograph or water level, and;

(5) stable banks or shorelines.

The trend in many of the approaches to establishing reference conditions has been to use terrestrial habitat attributes based on ecoregions. Ecoregions define areas with similar geographic attributes in order to define reference sites (Hughes 1995, Omernik 1995). The assumption is then made that biological conditions within such geographically defined strata represent the reference condition. However, we suggest that this is only the first step in reference site selection and stratification; analysis and classification of biological data should be the final arbiter of an appropriate reference condition.

## 2.2 Difficulties in the New Approach

Until recently, the development of numeric biological objectives was considered too difficult due to the temporal and spatial variability inherent in biological systems. However, over the past 10 years, methods developed in the United Kingdom (Wright et al. 1984, Moss et al. 1987, Armitage et al. 1987, Ormerod and Edwards 1987) and elsewhere (Corkum and Currie 1987, Johnson and Wiederholm 1989) have demonstrated the ability to predict the community structure of benthic invertebrates in clean (or 'uncontaminated') sites using simple habitat and water quality descriptors. This approach allows appropriate site-specific biological objectives to be set for ecosystems from measured habitat characteristics, and also provides an appropriate reference for determining when degradation at a site due to anthropogenic contamination is occurring.

Major approaches to data analysis involving reference conditions include the use of biotic indices with pre-established thresholds, multimetric indices (Gerritsen 1995), and taxonomic prediction using multivariate analysis (Wright 1995). Biotic indices have the longest history; they have been widely used and codified in legislation in several European countries (Metcalfe-Smith 1994).

In practice, multimetric and multivariate approaches differ considerably in determination of whether a test site is equivalent to the reference condition. However, both methods begin from the same premise and require the same data. As commonly used, multimetric methods classify reference sites based on geographic and physical attributes, whereas the multivariate approaches classify sites using multivariate analysis of the macroinvertebrate fauna. For the multivariate methods, selection of the most appropriate group of reference sites to which test sites are compared or comparison of a test site to all the reference sites with probability weightings is based on a predictive model. This selection is generally based on the location of the site (e.g., the ecoregion) when using the multimetric approach. Finally, when comparing the test site with the reference condition (as described by the reference sites) the multimetric approach uses taxa counts and assumptions about the taxa to derive a set of metrics, whereas the multivariate approach uses only taxa counts.

In terms of multimetric indices, Gerritsen (1995) maintains that such additive indices, developed specifically for assessment and management of environmental quality, are sensitive to biological degradation and function well when developed from reference data bases. He maintains that multivariate methods are more complex, require specialised practitioners, and are difficult to convey to managers and the public. Gerritsen (1995) also suggests that a lack of consensus on which multivariate approaches are most reliable demonstrates that the use of these techniques for management of resources may be premature. Norris (1995), however, has argued that predictive models developed from multivariate analysis of reference data bases are effective in assessing water quality (e.g., Wright 1995) and the method can be incorporated into an interactive computer system for use by managers.

## 3.0 Developing Assessment Guidelines using Multivariate Methods

### 3.1 The Approach

The multivariate approach to establishing the reference makes no *a priori* assumptions about the similarity of invertebrate communities at different sites - based on physical or chemical descriptions. Rather it uses the fauna to group sites that are most similar, and thus provides an objective way of providing groups of reference sites with similar invertebrate communities. In our view the most appropriate organisms to use to assess sediment contamination are those that are in most direct contact with the sediment environment. There are four major categories of organism associated with the sediment environment, bacteria and micro-organisms, algae and plants, invertebrates and fish. For reasons of time and spatial scale, availability of methods and pragmatic reasons the invertebrate fauna are generally the most useful group of organisms for use as indicators.

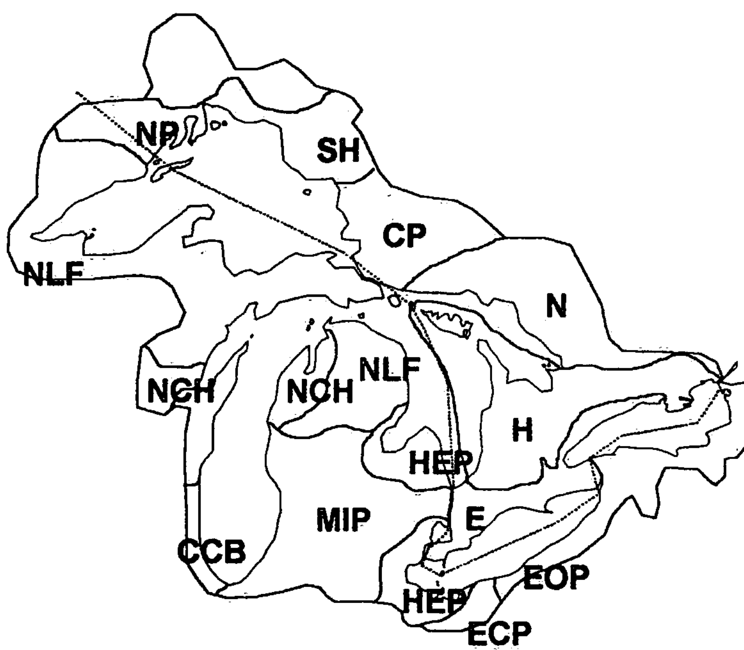
In multivariate approaches, physical and chemical data presumed to be not affected by human activity are used to match test sites with reference conditions and subsequently predict the fauna expected at the test sites (Wright 1995). A method is required to match a test site to the appropriate reference group once reference sites have been classified into groups based on the uniformity of their invertebrate fauna. Clearly, if a test site can be associated with a group of reference sites representing the *reference condition* then those reference sites can be used to predict the fauna expected at the test site in the absence of an impact.

The groups of reference sites (based on the biota) are used to describe the structure in the environmental data collected from them using discriminant function analysis (DFA). A subset of environmental variables known to be little affected by most human activity (*e.g.*, latitude, longitude, altitude, alkalinity) is chosen. Correlation analysis between the ordination matrices from biological and environmental data can be used first to ensure that variables associated with the structure present in the biological data are included, and then stepwise DFA which exploits correlations among the predictors to maximize discrimination of the groups. The final discriminant model is developed through an iterative process to ensure the lowest possible error rate. We recommend using cross-validation rather than re-substitution when testing the discriminant model. The former removes each site in turn from the data set, re-constructs the model, and tests the site against the model, whereas the latter constructs the model with all sites and then tests each site in turn.

### 3.2 Establishing Guidelines: The Great Lakes Example

A large data base has been assembled from reference sites in Lakes Ontario, Erie, Michigan, Superior and Huron and includes information on, (1) the structure of the benthic invertebrate communities, (2) measured environmental variables and (3) the responses of four species of benthic invertebrates (*Hyalella azteca, Chironomus riparius, Hexagenia* spp. and *Tubifex tubifex*) exposed in the laboratory to sediment collected from the same sites. These data have been used to develop numeric biological sediment objectives for the Great Lakes with a total data set of more than 300 reference sites sampled from all the Great Lakes over the period 1991-1993.

The study area encompassed all the lakes of the Laurentian Great Lakes. To ensure the range of habitat characteristics were adequately represented, a preliminary list of 250 sites were identified and stratified among six ecoregions described by Wickware and Rubic (1989) for the Canadian shores of the Great Lakes (Figure 1). As a result of interest by the United States EPA in expanding the data base into Lake Michigan, 53 sites in the lake were distributed through four ecoregions designated for Lake Michigan.




| | Ecoregion |
|---|---|
| E | Erie |
| H | Hurontario |
| N | Nipissing |
| CP | Chapleau Plains |
| SH | Superior Highlands |
| NP | Nipigon Plains |
| TBP | Thunder Bay Plains |
| NLF | Northern Lakes and Forests |
| NCH | North Central Hardwood Forests |
| SEW | SE Wisconsin Till Plain |
| CCB | Central Corn Belt plains |
| MIP | S. Michigan/N. Indiana Clay Plains |
| HEP | Huron/Erie Laker Plain |
| ECP | E. Corn Belt plains |
| EOP | Erie/Ontario Lake Plain |

**Figure 1:** Ecoregions of the Great Lakes.

A total of 345 site visits were made over the study period (1991-93). Initial examination of the data set based on both toxicity and community structure data resulted in 252 sites being included as potential reference sites (Figure 2). Fifty-two sites were excluded due to one or more of the following reasons: (1) the site had less than 50% survival for any test species; (2) two or more toxicity endpoints were below the acceptability criteria for the response (i.e., < the lower 5$^{th}$ percentile of the distribution); (3) no invertebrates were present at the site. This removal did not preclude a site from being re-instated as a reference site if it was found to be equivalent to reference in future testing.

A more detailed description of the methods, analyses and results is available in the technical report of this study (Reynoldson and Day 1997).

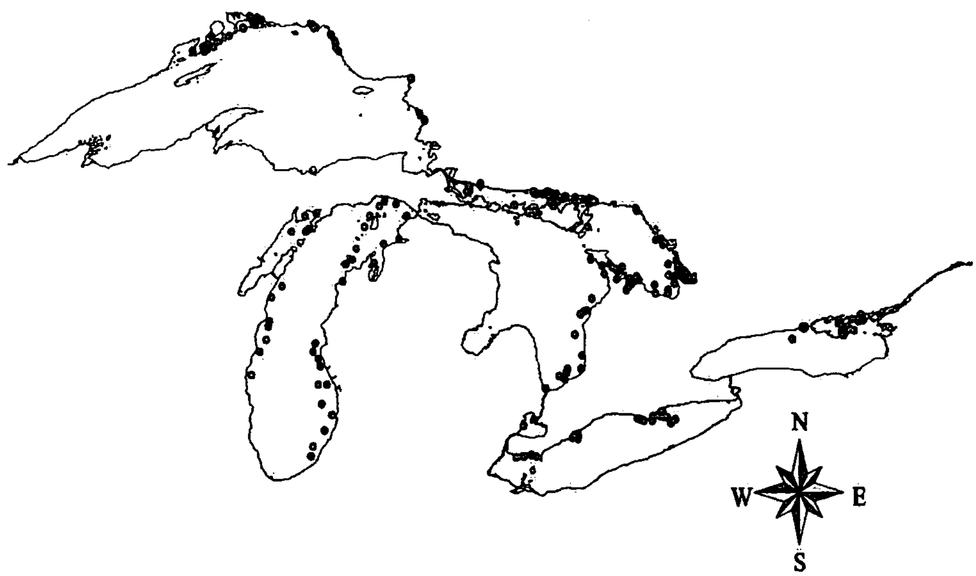



**Figure 2:** Location of Great Lakes reference sites

*3.2.1 Functional Data Analysis - The Benthic Community Data*

Pattern analysis was used to describe the biological structure of the data for the communities at the reference sites. Correlation and multiple discriminant analysis (MDA) was used to relate the observed biological structure to the environmental characteristics.

The biological structure of the data was examined using two pattern recognition techniques, cluster analysis and ordination. The mean values from the five replicates for the species abundance were used as descriptors of the benthic invertebrate community. The Bray and Curtis association measure was used as an association metric for the benthic invertebrate counts and environmental measures because it performs consistently well in a variety of tests and simulations on different types of data (Faith et al. 1987). Clustering of the reference sites was done using an agglomerative hierarchical fusion method with unweighted pair group mean averages (UPGMA). The appropriate number of groups was selected by examining the group structure and, particularly, the spatial location of the groups in ordination space.

Ordination was used to reduce the variables required to identify the structure of the data. A multi-dimensional scaling (MDS) method of ordination was used, *i.e.*, Semi-Strong- Hybrid multidimensional scaling (Belbin 1991). Multi-dimensional scaling uses metric and non-metric rank order rather than metric information and thus provides a robust relationship with ecological distance. MDS does not assume a linear relationship, an inherent assumption in some dissimilarity measures used by other ordination techniques (Faith et al. 1987). This is of particular value when relating ordination scores to environmental characteristics.

**Table 2:** Measured environmental variables at Reference Sites.

| **Measured Variable** | **Use as Potential predictor** | **Rationale** |
|---|---|---|
| **Geographic** (5 variables) | | |
| latitude<br>longitude<br>lake basin<br>ecodistrict<br>date | yes<br>yes<br>no - non-continuous<br>no - non quantitative<br>no - temporal effects examined separately | geographic descriptors provide a synthesis of the effects of spatial processes on animal distribution |
| **Limnological** (8 variables) | | |
| water depth<br><br>dissolved oxygen | yes<br><br>no - modified by seasonal processes | integrates effects of temperature and oxygen on organisms<br>critical for most aerobic organisms |
| pH<br>temperature<br><br>alkalinity<br>total phosphorus<br><br>kjeldahl nitrogen<br><br>nitrate-nitrite nitrogen | yes<br>no - requires temporal integration<br>yes<br>no - modified by anthropogenic inputs<br>no - modified by anthropogenic inputs<br>no - modified by anthropogenic inputs | modifies chemical interactions<br>effects growth and reproductive processes<br>summarizes dissolved materials<br>effects nutrient status and primary producers<br>effects primary producers<br><br>effects primary producers |
| **Sediment** (31 variables) | | |
| Particle Size - 7 variables (% gravel, sand, silt clay, mean, $75^{th}$, $25^{th}$ %le)<br><br>Major elements - 11 variables (oxides of Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P)<br><br>Nutrients - 4 variables (TP, TN, loss on ignition, TOC)<br><br>Metals - 9 variables (Totals for V, Cr, Co, Ni, Cu, Zn, As, Cd, Pb) | Yes<br><br>Yes<br><br>Yes<br><br>no - modified by anthropogenic inputs | Effects burrowing organisms, modifies bioavailability of materials.<br><br>Provide a good descriptor of overall sediment conditions, provides a regional signal.<br><br>Provide an indicator of food availability<br><br>Provide a descriptor of anthropogenic inputs and general contaminant levels, allow verification of reference status |

Of the 44 environmental variables measured in this study (Table 2), 26 were examined for their relationship with the biological structure of the data. We excluded those variables most likely to be influenced by anthropogenic activity, particularly those associated with sediment contamination. Thus, all the metals were excluded from consideration as potential predictor variables. The variables used were general descriptors of sediment type such as the major elements, particle size and organic material as a potential indicator of nutritive quality. These together with physical attributes such as water depth and general water chemistry were considered as the most appropriate general habitat descriptors that will not be subject to modification from human activity.

The relationship with the biological data was examined in three separate ways:

1) Principal axis correlation is a multiple-linear regression method to determine how well a set of attributes (environmental data) fit ordination space (the species matrix). The method takes each environmental attribute and determines the location of the best-fitted vector in ordination space. These can be represented as an axis on an ordination plot and a correlation of the axis with the ordination is provided. A Monte Carlo simulation can be performed to establish the statistical significance of the correlations.

2) An ANOVA was conducted using the site groups from the benthic data as the class variable. ANOVA was used to establish those environmental attributes that differed significantly ($P < 0.0001$ and $P < 0.05$) between biological site groupings.

3) Stepwise discriminant analysis was used to establish which variables best described the ~~biological groupings of the environmental~~ data set.

Based on the results from these three analyses, environmental variables were selected for use in multiple discriminant analysis (MDA) to relate the biological site groupings to the environmental characteristics of the sites. MDA was used with raw environmental data to generate discriminant scores, and to predict the probability of group membership. The more rigorous cross-validation method was used to verify the accuracy of the predictions from the discriminant model. Using this method, each of the sites is in turn removed from the data set and a model is generated without that site. The site group can then be predicted. The predicted groupings and actual groupings can then be compared to provide a group and total error rate.

Selection of the optimal predictor variable data set was done by iteration. Various combinations of predictor variables were selected from the stepwise discriminant analyses and principle axis correlation. The optimal set was defined as that with the lowest error rate from cross-validation in discriminant analysis.

### *3.2.2 Functional Data Analysis - Whole-Sediment Laboratory Toxicity Test Data*

The three-year data set for the laboratory bioassays with benthic invertebrates consisted of 212 sites. In addition, because feeding of *Hexagenia* spp. and *T. tubifex* was not conducted in 1991 but was added to the standard operating procedures in 1992, analyses of the data for these two species included only sediments collected in year 2 and year 3 of the study. Thus, the number of reference sites used in the data set for each species was as follows: *C. riparius* (212); *H. azteca* (212); *Hexagenia* spp. (167); and *T. tubifex* (167).

Frequency distributions of the data for each species and end point were plotted as histograms to present a graphical picture of the responses of each organism to a variety of reference sediments collected throughout the Great Lakes. In addition, the descriptive statistics of mean, median, standard error, standard deviation, maximum and minimum values and range were determined for each endpoint. The data were tested for normality and homogeneity of variance using SigmaplotR V.1.02 (Jandel Scientific). For purposes of analysis, the data pertaining to percent survival were transformed using the arcsine square root transformation (USEPA 1994). For comparative purposes, the responses of the four species to repeated bioassays with the quality control sediment from a marsh near Long Point, Lake Erie, were similarly plotted and the descriptive statistics tabulated.

Both univariate (regression analysis with single variables) and multivariate statistics were used to determine if the range in any given response for a particular species in clean sediments could be correlated with specific characteristics of sediments. No statistical significance with single variables could be demonstrated, and the range in response for each endpoint in a variety of sediments was similar to the same endpoint in only one reference sediment (Section 4.3). It was therefore concluded that the range in each endpoint noted for the reference sediment data set represents the natural range in the responses of each organisms in repeated laboratory bioassays using clean sediment. In addition, benthic invertebrates may respond to a combination of environmental factors in any given sediment or to variables that were not measured in this particular study. Based on these results, a decision was made to treat each response for a species as a continuum of data points with a range rather than to artificially separate the responses into groups using multivariate analyses or a lake-by-lake comparison.

As the purpose of a toxicity test with whole sediment(s) is to determine if the biological response(s) of a cohort of organisms exposed to potentially contaminated sediment differ from the response(s) of a similar cohort of organisms exposed to a negative control or reference sediment, the data from the reference sites was used to establish three categories of responses to test sediments. The three categories were - non-toxic, potential toxicity and toxic. The delineations for the three categories were developed from the standard statistical parameters of population mean and standard deviation (mean ± S.D.) of an endpoint measured in all reference sediments. For each endpoint, the non-toxic category was set at two standard deviations (S.D.) below the mean for the reference data set; this represents the 95% confidence limit for that response. At the 95% confidence level, 1 in 20 results (5%) would be expected to fall outside of the limits by chance alone. The toxic category was set at three S.D. below the mean of an endpoint which represents the 99.7% confidence limit. At this confidence level, the probability of data falling outside of the limits by chance alone is only 0.3% (one out of every 333 tests). The range of responses between two and three times the S.D. represents the warning level of potential toxicity and indicates sediment(s) which have some detrimental effects. Additional weigh-of-evidence such as impaired benthic invertebrate communities at sites which fall in the category of potential toxicity would emphasise the need for further study or remedial action.

For comparative purposes to the rather simplistic use of twice and three times the standard deviation of the mean for responses in a large number of reference sediments in order to set criteria of toxicity, a formula which incorporates the probability of Type I and Type II errors was also utilized based on Becker et al.(1995) and Kubitz et al. (1996). A minimum detectable difference (MDD) which represents the smallest difference between two means that can be discriminated statistically using a specified sample size per treatment (n), a significance level ($\alpha$), statistical power (1-ß) and population variance was calculated for each endpoint. The MDD is expressed as a percentage change from the mean control response or response in reference sediment(s). The selection of the à and á levels for the test is a function of the costs associated with making Type I and Type II statistical errors (Fairweather 1991). Kubitz et al. (1996) argues that Type I ($\alpha$) and Type II (ß) errors of 0.10 are suitable because the costs of either re-mediation of a non-contaminated sediment or no remediation of contaminated sediments would be equal from both an environmental or a financial viewpoint. The MDDs for the end points studied in this project were thus determined using following equation:

$$MDD = \sqrt{2\sigma^2/n}\,(t_{\alpha,v} + t_{\beta,v})$$

where

$\sigma$= the true population variance
$n$ = the number of replicates for a site (5)
$t$ = critical value of $t$ for a two-tailed test
$v$ = degrees of freedom 2 ($n$-1)
$\alpha$ = 0.1; $\beta$ = 0.1; power = 0.9 or 90%

The true population variance of each end point was estimated by the variance determined from the data set for the 166 to 212 reference sites used in bioassays with each species. Comparison of the MDDs calculated versus the criterion determined using twice and three times the standard deviation of the mean showed little difference and the more conservative estimate of toxicity was used in setting the biological criterion for each toxicity end point.

## 4.0 Guidelines Established Based on the Benthic Assessment of Sediment

### 4.1 Classification of community assemblages - Species Level

The large volume of data (162 taxa at 252 sites) and the need to examine response patterns at the community level resulted in the employment of multivariate approaches for describing community structure. Multidimensional scaling ordination of the 252 site by 162 taxa data matrix produced a solution with three dimensions (new variables) explaining the variation between the sites (stress = 0.1905). This solution for the 252 sites is shown as Dimension 1 v Dimension 2 and Dimension 1 v Dimension 3 plots; the six group solution from cluster analysis representing the 252 sites shows each of the six groups with different symbols (Figure 3). Two major points are noteworthy from the results of this ordination. First, the groups formed by cluster analysis have spatial integrity and bounds; they are not distributed throughout the entire ordination space. Second, there is considerable overlap between some of the groups in ordination space, suggesting that the communities constructed by this type of analysis represent centroids along a continuum of species distributions.

#### *4.1.1 Group Memberships and Associated Species*

The 162 taxa have also been plotted in ordination space (Figure 4) and the direction of the arrow shows the contribution of the taxa to a sites location in ordination space. For the sake of clarity, the individual sites are not represented (as in Figure 3);

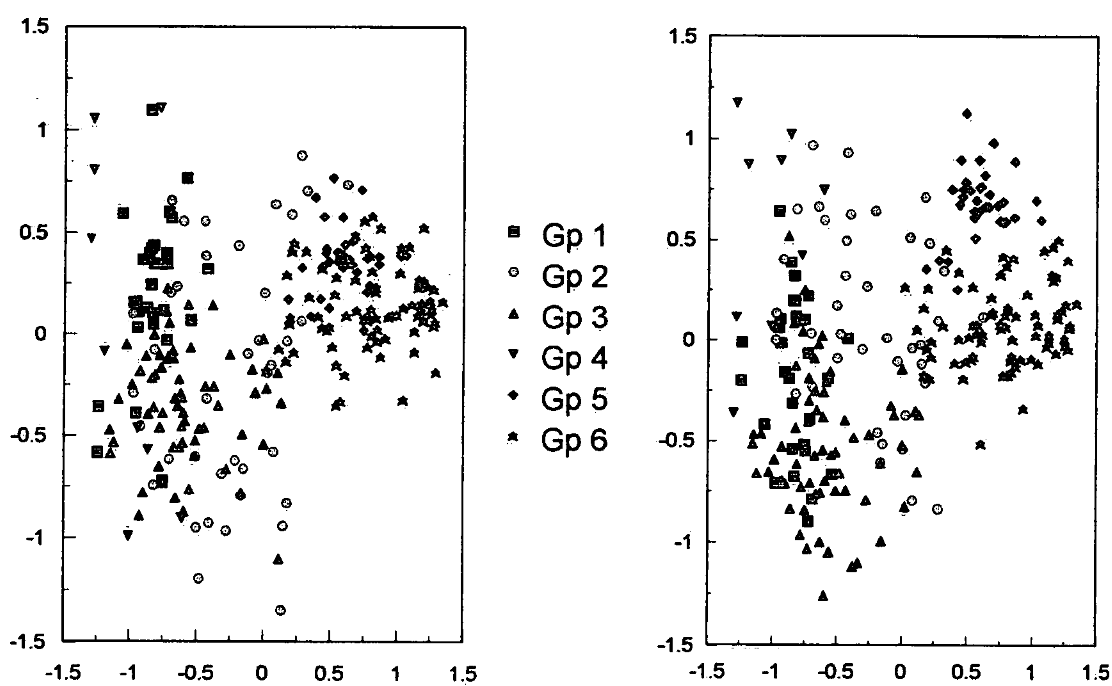



**Figure 3:** Multi dimensional scaling (HMDS) ordination of 163 taxa at 252 reference sites in the Great Lakes, identified to six groups of sites formed from cluster analysis.

instead, the 90% confidence limit around the community centroid is shown. From Figure 4 , sites representing communities 5 and 6 are most strongly influenced by the presence of the amphipod *Diaporeia hoyi* (DIA HOY), the oligochaete worm *Stylodrilus heringianus* (STY HER) and the midge *Heterotrissocladius spp.*(HET SP), also in community 6. Sites in community 4 are strongly associated with the oligochaete *Aulodrilus pigueti* (AUL PIG) on the first dimension and the molluscs *Pisidium casernatum* (PIS CAS), *Dreissena polymorpha* (DRE POL) and *Valvata tricarinata* (VAL TRI) on the third dimension. Community 2 is not strongly influenced by any particular species and therefore likely represents a more even community. Communities 1 and 3 are most influenced by chironomid midges, *Chironomus* spp. (CHI SP) in the case of community 1 and *Procladius* spp. (PRO SP) in community 3. The numbers of the principle taxa are shown for each of the six communities in Figure 5 to illustrate the differences between the communities.

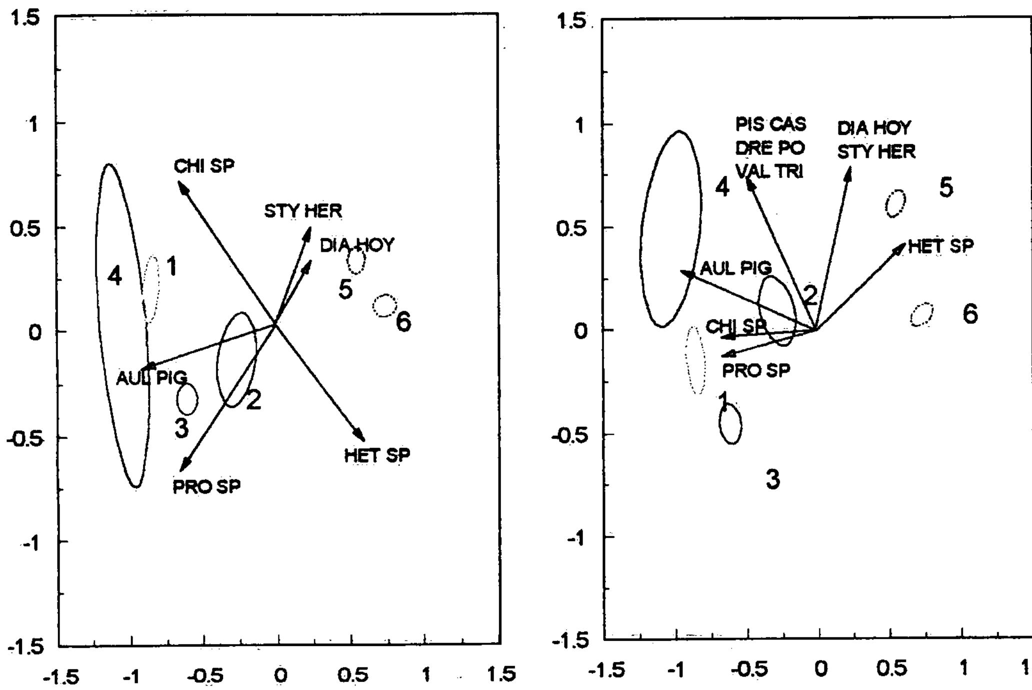



**Figure 4:** Vectors for the major taxa shown in HMDS ordination space with 90% confidence ellipses for site group centroids.

Community 1 is characterised by *Chironomus* spp. and *Dreissena*; the other common chironomid, *Procladius* spp., is also abundant in this community as is the sphaerid clam, *P. casertanum*. Numbers of *Chironomus* in community 1 are significantly greater than in the other 5 communities and the leech *Helobdella stagnalis* (HEL STA) is also characteristic of this community. This community group contains 29 sites, the majority located in western and central Lake Erie.

Community 2 is characterised by the fingernail clam *P. casernatum* and the amphipod, *D. hoyi*, which is indicative of a more oligotrophic community. This is also indicated by the location of these communities in ordination space. The first dimension appears to represent a trophic and geographic gradient where communities to the left tend to be more mesotrophic, lower lake communities, and communities to the right more oligotrophic representing the upper lakes. Community 2 is also more diverse with more taxa (Figure 5) than the other community groups but is also the least spatially defined. While the majority of sites in this group are located in Georgian Bay, it also includes sites from Lakes Erie (eastern basin?), Ontario, Huron, Michigan and the North Channel.

Community 3 is characterised by the predatory midge *Procladius* spp. and the fingernail clam, *P. casernatum*; however, total abundance in this group is generally low at these sites. Half the sites in this community are from Georgian Bay, together with sites from Lake Erie (eastern basin) and the North Channel.

Community 4 consists of only 9 sites and these are dominated by very high numbers of the exotic species *Dreissena polymorpha* and *D. quagga* (DRE QUA); however, this community is similar to community 1 with regard to the other taxa present and as indicated by the location of the sites in Lake Erie and the group centroid in ordination space (Figure 4). Both these communities are typical of the more mesotrophic Lake Erie.

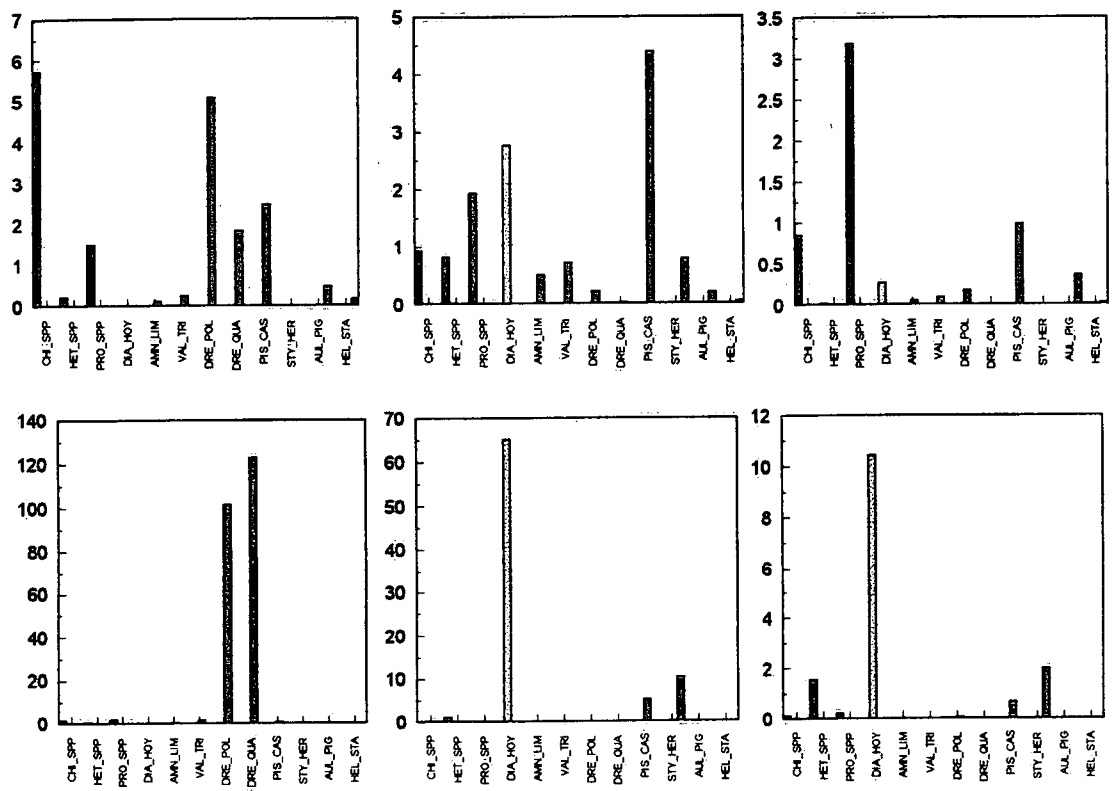



**Figure 5:** Taxa characterising site groups formed by cluster analysis from 252 Great Lakes reference sites.

The last two communities, 5 and 6, both represent a *Diaporeia hoyi/Stylodrilus heringianus* assemblage. The major differences between the two communities is in the abundance of the two species and the presence of the oligotrophic chironomid

*Heterotrissocladius* spp. Community 5 has higher abundance of both *Diporeia* and *Stylodrilus* and *Heterotrissocladius* is less numerically important. This community is primarily found in Lake Michigan.

Community 6 is composed of the largest assemblage of sites (77) and includes more than 90% of the Lake Superior sites together with a large number of Georgian Bay and the North Channel sites.

## 4.2 Classification of community assemblages - Family Level

There has been ongoing discussion in the scientific literature regarding the level of taxonomic detail required for bioassessment. Freshwater biologists have generally tended to argue that identification to genus or species level is desirable. The argument has been presented that there is considerable variation in response to environmental stress at this level, especially between different species of a genus, and, Resh and Unzinger (1975) synthesised this view. However, in the rapid Bioassessment Protocols developed in the United States (Plafkin et al 1989), family level identifications are usually recommended. Additionally, actual data analysis and comparison of the effects of taxonomic level on identification of environmental stress have been documented in several papers in the marine benthic literature (add references).

In two papers, Warwick (1988) and Warwick and Clarke (1993) have shown that identification at the family, and even phyla, level were as effective as species level in identifying pollution gradients. Warwick (1988) presents the argument that this is because anthropogenic effects modify communities at higher taxonomic levels than natural environmental variables; the latter tends to influence fauna by species replacement. In part, this is because multivariate methods of analysis use all the organisms present in the invertebrate community and thus are more sensitive than other univariate or graphical analysis methods (Warwick and Clarke 1993). If family level identification is acceptable for identification of stressed invertebrate communities, this is of considerable importance to agencies or organizations required to conduct bioassessment or biomonitoring. The cost saving will be considerable. For the Great Lakes data base, we examined the performance of family level classification for identifying site groups and in model development.

### *4.2.1 Distribution and Abundance*

A total of 39 invertebrate families have been recorded, and of these, three families are very common (> 80% occurrence): the Chironomidae (midge larvae), Tubificidae (worms) and Sphaeridae (fingernail clams). A further three families are slightly less common (>50% occurrence): the Naididae (worms), Pontoporeiidae (shrimps) and Spongillidae (sponges). Almost half the families (18), are considered rare and occur at less than 10% of the sites. The most abundant family are the freshwater sponges (Spongillidae) representing over 80% of the organisms found. However, this group are frequently excluded from invertebrate enumerations, since they are colonial animals and therefore difficult to compare with other organisms. The other most abundant families are the Tubificidae and Pontoporeiidae , representing >20% of total animals found (excluding sponges). Two other families were abundant (>10% of the total): the Chironomidae and the recent invaders, the Dreissenidae (zebra and quagga mussels). The majority of families (27 of 39) were not abundant (<1% of total).

*4.2.2 Classification and Ordination*

Sites have again been classified using cluster analysis and a final number of groups established by examination of both the tree structure and the distribution of sites in ordination space. We have selected five groups of sites as further groups consisted of small groups of sites. For example, the sixth group was formed by the splitting of group 5 and at the seventh split (8 groups), a group of 13 sites and then two more groups of three and nine sites were formed. Similar to the genus level, there is a strong spatial component to the site groups.

Group 1 is characterized by lower numbers of animals (Figure 6) and the dominant organisms are chironomids. However, the chironomids are a widespread family and are found at most sites. This assemblage of families is characteristic of south western Georgian Bay and much of the North Channel.

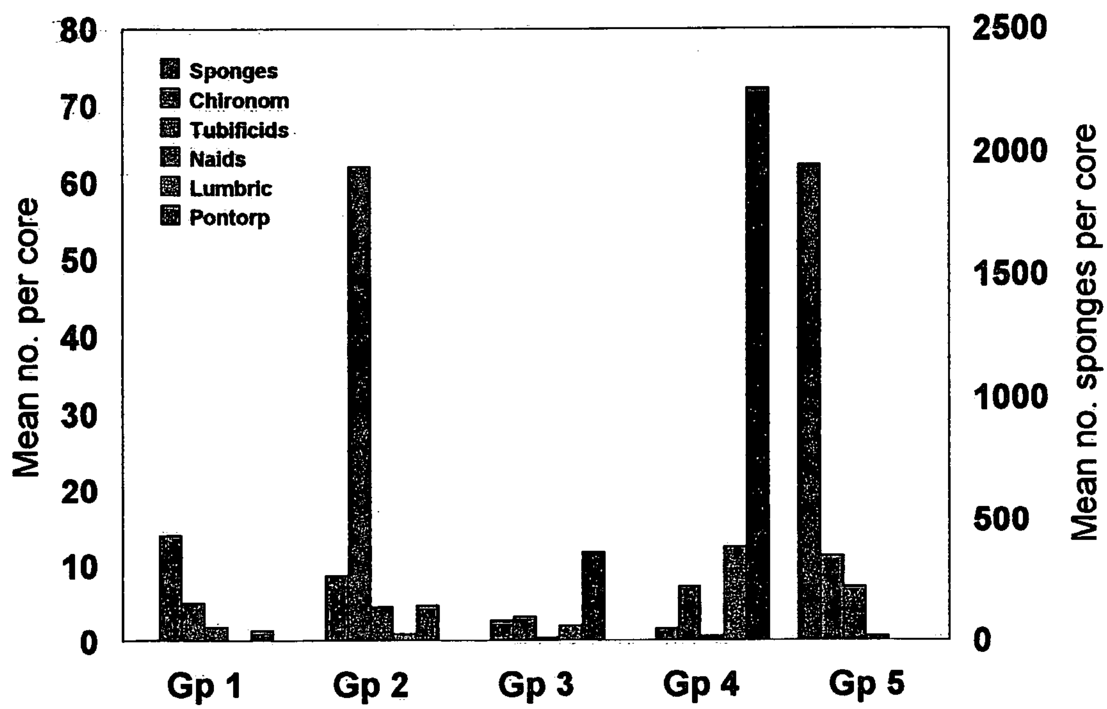



**Figure 6:** Benthic invertebrate families characterising site groups formed by cluster analysis from 252 Great Lakes reference sites.

Group 2 is dominated by tubificid and naid oligochaetes (Figure 6). Both families occur in significantly higher numbers ($P < 0.05$) than in other groups. Almost 60% of these sites are located in Lake Erie with the rest scattered through all the other lakes. The families composing this group, and the spatial distribution of the sites, suggest that it is characteristic of more mesotrophic habitats. This group also tends to be associated with water with a higher alkalinity, which is likely a surrogate for dissolved minerals, including nutrients (Figure 7).

Group 3 is characterized by sites where the Pontoporeiidae are dominant (Figure 10) and occur in significantly greater numbers than at Groups 1 and 5 (Figure 6). The Chironomidae are the second most abundant family in this group of sites. This assemblage of organisms is characteristic of Lake Superior (93% of sites) and many more-exposed Georgian Bay sites (28%) together with the remaining North Channel sites. These sites are associated with deeper water and less organic material in the sediment (Figure 7).

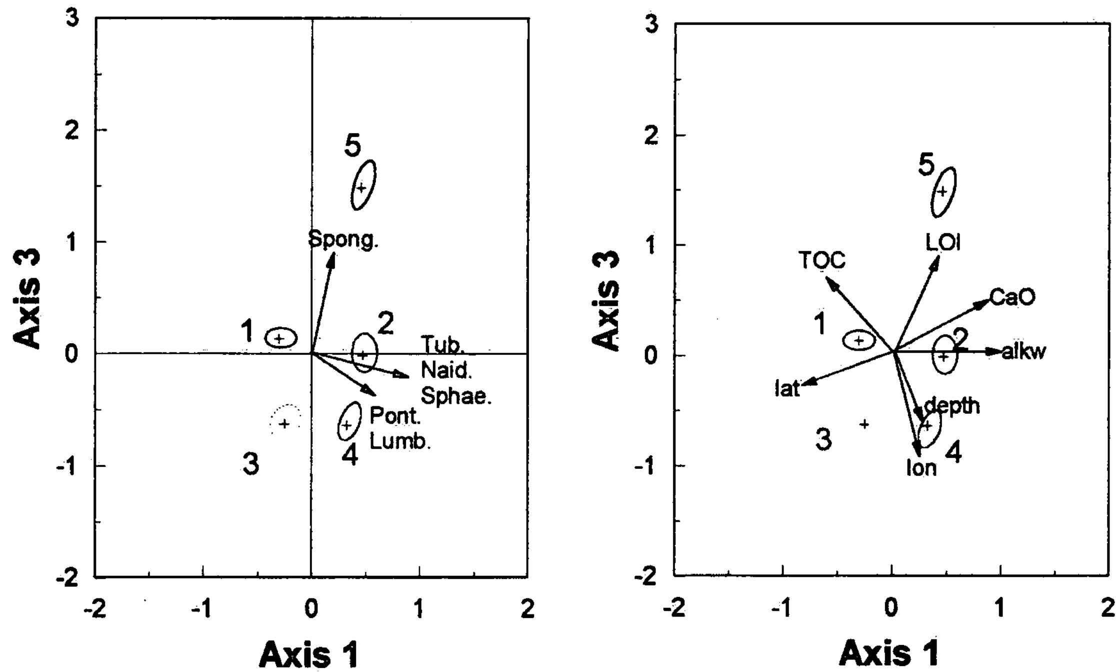



**Figure 7:** Vectors for the major taxa and environmental variables shown in HMDS ordination space and site group centroids with 90% confidence ellipses.

Twenty of the 28 (71%) Group 4 sites are in Lake Michigan. This group is dominated by two oligotrophic families, the Pontoporeiidae and Lumbriculiidae (Figures 6 & 7). These families occur in significantly greater ($P < 0.05$) numbers than in other groups. These sites represent a deeper water assemblage of organisms (Figure 7).

Finally, Group 5 is unique in that it is dominated by sponges. The sites within this group are characteristically in sheltered areas such as Long Point Bay, Lake Erie, the Bay of Quinte and Presque Isle Bay in Lake Ontario; some sites in Severn Sound, Georgian Bay, are also found in this group. Chironomids are also abundant in Group 5. These sites are associated with sediment with a high organic content (Figure 7).

### 4.3 Bio-Assay Toxicity Endpoints and Target Values

The ten measured end points for the responses of four species of benthic invertebrates in whole sediment toxicity tests can be divided into two categories: acute (four measurements of percent survival) and chronic (six sublethal measurements of either growth or reproduction). Several statistical analyses were conducted to try to correlate the responses for each end point and each species with sediment characteristics such as particle size distribution, TOC, LOI, MgO, SiO2,TP, TN, etc., for the reference sites. Both univariate (regression analysis with single variables) and multivariate statistics were used to determine if the range in any given response for a particular species in clean sediments could be correlated with specific characteristics of sediments. Although some trends were noted, especially with regard to growth and % silt or total organic matter in sediment, statistical significance with a single parameter could not be demonstrated. It was therefore concluded that the range in each endpoint noted for the reference sediment dataset represents the natural range in the responses of each organisms in laboratory bioassays. Based on these results, a decision was made to treat each response for a species as a continuum of data points with a range rather than to separate the responses into groups using multivariate analyses.

Mean percent survival of *C. riparius* in 208 reference sediments was 86.0 with a range of 53.3 to 100% and a CV of 10.2%. Only 4.7% of the reference sediments collected over the three-year period from all five of the Great Lakes caused mortality of *C. riparius* to be greater than 30%. USEPA (1994) and ASTM (1995) have set a minimum acceptable criterion of 70% for survival of *Chironomus* spp. in uncontaminated sediments used in toxicity tests. Our results show that this criterion is achievable in the majority of sediments collected from reference areas in the Great Lakes. The few reference sediments for which % survival was <70% was well within the 1 in 20 results which would fall outside the 95% confidence limits for any given test.

**Table 3:** Growth and survivorship in the midge *Chironomus riparius* at reference sites and from a single location.

| C. riparius | Reference sites (n=208) | | Long Point (n=46, over 3 years) | |
|---|---|---|---|---|
| | % Survival | Growth mg d.w./ larvae | % Survival | Growth mg d.w./ larvae |
| Mean | 86.0 | 0.35 | 87.4 | 0.37 |
| Median | 88.0 | 0.33 | 89.4 | 0.36 |
| S.E. | 0.2 | 0.02 | 1.2 | 0.01 |
| S.D. | 8.7 | 0.07 | 8.3 | 0.07 |
| Maximum | 100 | 0.60 | 98.7 | 0.55 |
| Minimum | 53.3 | 0.19 | 62.2 | 0.26 |
| Range | 46.7 | 0.41 | 36.5 | 0.29 |
| CV | 10.2 | 21.3 | 9.5 | 17.7 |

Growth of larval chironomids in a variety of reference sediments with a range of physico-chemical characteristics was variable with dry weight of individual 4th instar larvae at test termination (10-d) ranging from 0.19 to 0.60 mg with a mean of 0.35 and a CV of 21.3%. All attempts to correlate this variability in growth to sediment characteristics were negative although some parameters such as TOC, % sand, % clay, total nitrogen, total phophorus and concentrations of lead, zinc, and copper in the reference sediments were implicated in both single parameter regressions and multivariate analyses.

As with midge larvae, survival of juvenile *H. azteca* in 208 reference sediments was good with a range of 50.0 to 100%, a mean of 86.9% and a CV of 11.4%. However, in 18.4% of the sediments tested, survival was below the minimum acceptable criterion of 80 % which has been set for *H. azteca* in control sediments in a 10-d lethality test by USEPA (1994) and ASTM (1995).

**Table 4:** Growth and survivorship in the scud *Hyalella azteca* at reference sites and from a single location.

| **H. azteca** | Reference sites (n=208) | | Long Point (n=46, over 3 years) | |
|---|---|---|---|---|
| | % Survival | Growth mg d.w./ juvenile | % Survival | Growth mg d.w./ nymph |
| Mean | 86.9 | 0.50 | 91.7 | 0.59 |
| Median | 90.7 | 0.50 | 93.3 | 0.58 |
| S.E. | 0.2 | 0.03 | 1.0 | 0.02 |
| S.D. | 9.9 | 0.13 | 7.2 | 0.14 |
| Maximum | 100 | 0.80 | 100 | 0.85 |
| Minimum | 50.0 | 0.12 | 61.3 | 0.17 |
| Range | 50.0 | 0.68 | 38.7 | 0.69 |
| CV | 11.4 | 26.5 | 7.7 | 23.5 |

The growth of 3 to 9 day-old *H. azteca* in reference sediments with a variety of physico-chemical characteristics over a 28-d exposure to sediments was more variable than growth in the midge bioassay and ranged from 0.12 to 0.80 mg dry wt. per juvenile with a CV of 26.5%. A negative correlation with % clay in the sediments was noted.

Percent survival of the mayfly nymph *Hexagenia* spp. was excellent in all types of sediment (166 sites) and ranged from 66.0 to 100% with a mean of 95.9% and a CV of 5.5%.

Growth of mayfly nymphs during the 21-d test was more variable than survival and ranged from 0.5 to 6.4 mg dry weight per individual with a CV of 34.4%. Strong positive correlations with LOI, TOC, TN, TP and $SiO2$ as well as negative correlations with % sand and % silt were noted in regressions.

**Table 5:** Growth and survivorship in the mayfly *Hexagenia spp*. at reference sites and from a single location.

| Hexagenia spp | Reference sites (n=166) | | Long Point (n=46, over 3 years) | |
|---|---|---|---|---|
| | % Survival | Growth mg d.w./ larvae | % Survival | Growth mg d.w./ nymph |
| Mean | 95.9 | 2.98 | 97.1 | 5.00 |
| Median | 98.0 | 2.86 | 98.0 | 4.75 |
| S.E. | 0.2 | 0.08 | 0.6 | 0.15 |
| S.D. | 5.3 | 1.02 | 4.1 | 0.99 |
| Maximum | 100 | 6.40 | 100 | 7.5 |
| Minimum | 66.0 | 0.50 | 80 | 3.4 |
| Range | 34.0 | 5.90 | 20 | 4.1 |
| CV | 5.5 | 34.4 | 4.2 | 20.8 |

Percent survival of adult *T. tubifex* was usually 100% in all bioassays with reference sediments (166 sites); only 3.6% of sediments tested recorded mortality between 10 and 20%. Based on these results, the acceptability criterion for % survival of adult worms in nontoxic sediments can be set quite high, i.e., >90%. Percent hatch of cocoons was also fairly high and constant with a mean of 58.8 ± 10% and a CV of 15.7%. The acceptability criterion for % hatch of cocoons is thus set at >35%. The number of cocoons produced per adult worm was consistent with a range of 4.8 to 14.4, a mean of 9.8 and a CV of 13.2% being recorded.

**Table 6:** Survival and reproduction in the worm *Tubifex tubifex* at reference sites and from a single location.

| T. tubifex | Reference sites (n=166) | | | | Long Point (n=46, over 3 years) | | | |
|---|---|---|---|---|---|---|---|---|
| | % Surv. | % Hatch | No. Coc./ Adult | No. Young/ Adult | % Surv. | % Hatch | No. Coc./ Adult | No. Young/ Adult |
| Mean | 98.2 | 58.8 | 9.8 | 28.8 | 98.9 | 56.7 | 11.1 | 36 |
| Median | 100 | 60.0 | 10.0 | 30.0 | 100 | 57.7 | 11.0 | 37 |
| S.E. | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.9 | 0.1 | 1.3 |
| S.D. | 4.7 | 9.2 | 1.3 | 8.4 | 0.7 | 5.8 | 0.8 | 8.7 |
| Maximum | 100 | 91.0 | 14.4 | 48.9 | 100 | 63 | 12 | 52 |
| Minimum | 60 | 19.4 | 4.8 | 1.0 | 95 | 33 | 9.0 | 22 |
| Range | 40 | 71.6 | 9.7 | 47.8 | 5 | 30 | 3.0 | 30 |
| CV | 4.8 | 15.7 | 13.2 | 29.2 | 7.1 | 10.2 | 7.3 | 24.2 |

# 5.0 Applying The Guidelines - The BEAST Software

## 5.1 Introduction to the BEAST

Employing the reference condition approach for the benthic assessment of sediment has the potential to provide an alternative to current environmental guidelines and criteria. It has been suggested that multivariate methods such as those developed in this report are too complex, require specialized practitioners, and are difficult to convey to managers and the public (Gerritsen, 1995). Limitations associated with multivariate methods, however, can be attributed to the lack of a comprehensive tool for application. To date, someone wishing to employ multivariate methods for sediment analysis has required several expensive, cumbersome software packages to achieve their goals.

The need for a simple, inexpensive software tool which encapsulates the requirements for multivariate analysis has led to the development of the BEAST. Designed exclusively for the **BE**nthic **A**ssessment of **S**edimen**T**, the software automates the methodology outlined in this report. Employing the RAISON Mapping and Analysis package from Environment Canada as a foundation, the BEAST combines new methods with a simple, straight-forward software user interface. The result is a powerful new tool for sediment analysis.

## 5.2 Software Design

The core of the BEAST system is the reference condition data base, which provides comparative environmental and community structure data for uncontaminated sites (Figure 8). Seven individual modules surround the main core of information. The first module is responsible for the entry of data to the system which is to be compared to the Reference State. Data entered by the user is referred to as Test data. Once the data for a single project has been entered, the next two modules predict the membership of each test site using physical characteristics, and then combine each Test site with the appropriate group of Reference data. Analysis of each site's benthic community structure is computed in the fourth module. The final three modules are responsible for graphic comparison of the model's output, employing both traditional graphs, as well as spatial mapping capabilities to aid in the analysis of a site's relative level of impact.

One of the fundamental design parameters for the BEAST system was flexibility with respect to data storage and maintenance. In order to facilitate this, the maintenance of information within the BEAST was designed to allow users to add or remove data sets with a minimum of difficulty. The BEAST employs the file format of Microsoft™ Access for information storage and retrieval. A commercially available Relational Data Base Management System (RDBMS), Access files are designed to accommodate the kind of large, complex data sets common to benthic analysis.

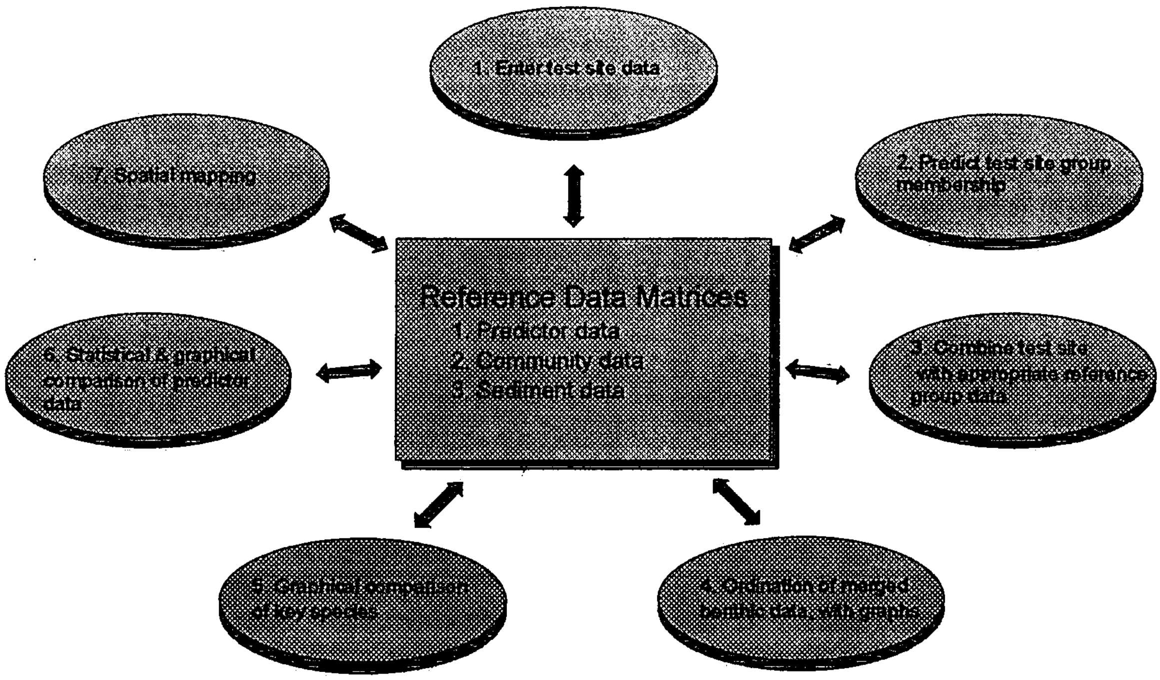



**Figure 8:** Conceptual design of the components of the BEAST software.

*5.2.1 Test Data Entry*

Incorporated into the BEAST is a Benthic Data Information System (BDIS). BDIS is an automated data entry/management tool for Test data, which employs a simple graphic interface. It was designed to reduce the errors associated with data entry, and eliminate the need for users to manually generate complex input files for analysis. Once users have entered all of the data associated with a specific project, a data base file is generated which contains all of the information required by the BEAST, in the proper format for successful analysis.

*5.2.2 Reference Data Entry*

Research which helped to develop the techniques used by the BEAST also generated a large reference data base for the Laurentian Great Lakes, and current research is working towards the development of a similar data base for the Fraser River in British Columbia. The BEAST, however, is designed to maintain any number of reference data sets, without the need for continual updating of the software itself. A new reference data base must be generated using the same format as is used for the existing reference sets. The resulting Access file can simply be placed in the same location as other reference data base files within the BEAST file structure. Once there, it is automatically available for analysis in the BEAST

*5.2.3 Resulting Analysis Files*

When an analysis is first undertaken for a particular project within the BEAST, a unique *Project* name must be assigned. When a *Project* is created, the user must select a Reference data base and a Test data base, to be used during the analysis. Any number of projects can be maintained within the BEAST at any one time, and can be deleted when they are no longer needed. Once the project has been established, the user must develop a *scenario* to be used for the analysis. A scenario represents a variation on the multivariate model, based on the availability of data within the test data set. Since it is vital that a reference set encompass the entire range of variability from which test sites may be selected, the range of variables sampled during the development of a Reference data base can be quite large. In the case test data, however, cases may occur where some variable(s) has not been sampled for various reasons. Scenarios store the results from each analysis within a single project, permitting users to make comparisons after processing is complete.

**5.3 Field Data Requirements**

The value of environmental variables collected at each test site are used to establish the appropriate reference condition (group) for comparison to individual test sites. Therefore, selection and measurement of appropriate variables plays a key role in the successful application of the BEAST. As discussed in the Great Lakes example in section three, a sub-set of the environmental variables sampled for a particular reference set is established, based on those variables least likely to suffer from anthropogenic impact. Each reference data base the BEAST maintains a list of these optimums for the user to examine.

**Table 7:** Predictor Variables in the Great Lakes Reference Data Base

| Data Type | Data Required |
|---|---|
| **Location** | Latitude (d,m,s) |
| | Longitude (d,m,s) |
| **Physical Data** | Water Depth |
| **Chemistry** | Water pH |
| | K2O |
| | TN |
| | TOC |
| | MnO |
| | MgO |
| | CaO |
| | Sio2 |

Currently, the only completed reference data set within the BEAST is for the Laurentian Great Lakes. While all variables collected during the production of this reference set are included in the data file, users are not required to provide all of these for analysis. However, users currently must provide at least the optimum variables, if analysis is to be successful. Table 7 identifies those variables currently required by the BEAST for the Laurentian Great Lakes reference set.

## 6.0 Meeting or Exceeding the Guidelines

### 6.1 Whole Sediment Toxicity Tests

There is very little information in the scientific literature which quantifies a threshold for an increase in mortality (30% or greater) or a reduction in growth and reproduction of a species before the population suffers irreversible damage and elimination from an ecosystem. Kubitz et al. (1995) suggest that a reduction in growth of the amphipod, *H. azteca*, of approximately 50 % during a 14-d sediment toxicity test corresponds with significant mortality. Borgmann et al. (1989) observed that a 46% weight reduction in this same species results in a 90 % reduction in the production of young. Two studies which investigated the size versus fecundity relationship of populations of *H. azteca* collected from field sites in several lakes throughout North America, found that a 25% inhibition of growth of this amphipod would translate to a 36 to 57% reduction in the fecundity of the species (Cooper 1965).

Sibley et al. (1997) evaluated the relationship between growth and reproduction of the chironomid, *C. tentans*, to assess whether stress-induced reductions in growth can be used to predict changes at the population level. These authors concluded that there is a minimum dry weight that must be obtained by the larvae before pupation and emergence is possible and a reduction in growth was also associated with a proportional decline in reproductive output of adult females of this species. The reduced size of larvae might also mean a reduction in biomass (food) available to organisms such as fish at higher trophic levels. Giesy et al. (1988) also found that a reduction of 30% in growth of *C. tentans* larvae in laboratory tests corresponded to restricted colonization and benthic community structure including the absence of member of the genus, *Chironomus*, in contaminanted sediments from the Detroit River.

Thus, a 25-50 % reduction in growth of a species of benthic invertebrate may be indicative of ecologically relevant effects. Greater than 50 % mortality has been the standard for acute toxicity in the laboratory for the past three decades.

Three categories of toxicity were developed for near shore sediments in the Great Lakes based on the results from the 167-212 reference sediments. The categories are: (1) non-toxic; (2) potential toxicity; (3) and toxicity. The delineations for each of the categories for each species and endpoint in whole sediment toxicity tests are presented in Table 8. An upper limit is provided in the non-toxic category for growth based on twice the standard deviation of the mean. Toxicological effects on sublethal responses are usually considered to be negative, i.e., growth or reproduction is reduced in comparison to a control. However, in areas of eutrophication or high nutrient impact, sublethality may manifest itself as an increase in biomass or production of young. This effect could have a negative impact on the structure and function of benthic invertebrate communities in aquatic ecosystems. Therefore, an upper limit for growth of *C. riparius*, *H. azteca* and *Hexagenia* and reproduction by *T. tubifex* has been set for the non-toxic category in this study. Although increased levels for growth and reproduction are not considered indications of toxicity, their presence should be noted in any managerial decisions made regarding the remediation of sediments.

**Table 8:** Limits derived from Great Lakes reference sites for determining toxicity of 10 test endpoints.

| **Species** | **Non toxic (Class 1)** | **Potentially toxic (Class 2)** | **Toxic (Class 3)** |
|---|---|---|---|
| *C. riparius* | | | |
| survival (%) | > 69 | 60 - 68.9 | < 60 |
| growth (mg d.w.) | 0.21 - 0.49 | 0.14 - 0.20 | <0.14 |
| *H. azteca* | | | |
| survival (%) | > 68 | 58 - 67.9 | <58 |
| growth (mg d.w.) | 0.24 - 0.76 | 0.11 - 0.23 | <0.11 |
| *H. limbata* | | | |
| survival (%) | > 85 | 80 - 84.9 | <80 |
| growth (mg d. w.) | 1.0 - 5.0 | 0 - 0.9 | - |
| *T. tubifex* | | | |
| survival (%) | >88 | 84 - 87.9 | <84 |
| hatch (%) | 40 - 78 | 30.8 - 39.9 | <30.8 |
| cocoon/ad. (no.) | 7.2 - 12.3 | 5.9 - 7.1 | <5.9 |
| young/ad. (no.) | 12.0 - 45.6 | 3.6 - 11.9 | <3.6 |

The use of two and three times the standard deviation about the mean for each endpoint has been chosen to separate these response categories because it is considered to be a more conservative delineation of toxicity than the *Minimum Detectable Differences* (MDD's) determined in this study. The MDD's calculated for all endpoints in the data set ranged from 11.5 to 19.3% for the lethality endpoint (% survival) and 15.9 to 29.0% for the sublethal responses such as growth and reproduction (with the exception of the data for *Hexagenia* growth in which a MDD could not calculated). It is not known at this time whether a 30% or less change in an endpoint is ecologically relevant.

**Table 9:** Assigning toxicity scores from multiple test endpoints.

| | |
|---|---|
| Assign scores<br>Class 1 - 1 point<br>Class 2 - 2 points<br>Class 3 - 3 points | |
| <u>Acute toxicity</u>:<br>CrSu + HaSu + HlSu + TtSu; | = 4 then sediment is acceptable,<br>≥6 then not acceptable. |
| <u>Chronic toxicity</u>:<br>Crgw + Ha gw + Hlgw + Tthtch + Ttcc/id + Ttyg/id | = 6 then sediment is acceptable;<br>≥ 8 then not acceptable |

Each end point has the potential of scoring (1 point) non-toxic; (2 points) potential toxicity; or (3 points) toxic. The responses of the four species in sediment collected from a potentially toxic site can therefore be graded as follows: (A) the percent survival of each species is within one S.D. of the mean for the reference site data base; score one point for each species for a total of four points. If one or more species registers a percent survival value of less than two S.D. below the reference data base mean, a score of 2 or more will be given. Therefore, a total score of >5 in the acute category will trigger a more extensive review of the potential toxicity at a site. Similarly, (B) if growth of *H. azteca*, *C. riparius* and *Hexagenia* and % hatch, number of cocoons per adult worm and number of young per adult worm are within one S.D. of the mean for each species at all reference sites, a total of score of 6 will be registered. However, if one or more sublethal end point is scored at 2 or 3, the total score for chronic end points at a site will be >7. A score of >7 for six chronic end points will therefore trigger a more extensive review of all data at a site (Table 9).

## 6.2 Invertebrate community structure

While there are a number of possible approaches to match reference and test site(s), our approach is again a multivariate method in which the entire community assemblage can be used. In this method the reference and matching test site(s) are ordinated and plotted in ordination space. The distribution of the reference sites provides the range of variation in unimpaired communities. The community at the test site can then be compared to this normal variability with a given probability of belonging to the reference group using probability ellipses built around the reference sites. The greater the departure from reference state as measured in ordination then the greater the impact, however, determining actual degree of impact and what departure from the reference state defines an unacceptable impact is ultimately a subjective decision.

A large river water quality survey conducted in the UK in 1990 provided the impetus for the development of methods to circumscribe the continuum of responses into a series of bands that represented grades of biological quality (Wright et al 1995). This is a simplification of what is a continuum of responses in sites ranging from good to poor biological quality. Despite the simplification, it was seen as an appropriate mechanism for obtaining a simple statement of biological quality allowing broad comparisons in either space or time that would be useful for management purposes.

We have adopted a similar approach for defining degrees of impact using a multivariate approach, using three probability ellipses (Figure 10). Sites inside the smallest ellipse (90% probability) would be considered *unstressed*; sites between the smallest and next ellipse (99% probability) would be considered *potentially stressed*; sites between the 99% probability and the largest ellipse (99.9% probability) would be considered *stressed*, and; sites located outside the 99.9% ellipse would be designated as *severely stressed*.

The process of determining whether an invertebrate community is impaired involves:

(1) sampling the community and measuring the predictor variables at the site of interest;

(2) running the discriminant model with the reference data base and test site(s) to predict the expected community at the test site(s);
(3) comparing test site(s) to the reference community sites, from the group to which the test site(s) were predicted.

Once a site is predicted to have a specific community structure, the actual structure is compared to the communities at the equivalent reference sites. A data file is constructed that includes the species counts for the appropriate reference sites. This is ordinated so that a matrix is calculated for both reference and test sites. The sites can then be plotted in ordination space showing the three ordination dimensions that synthesise the biological attributes of the sites (Figure 9). Probability ellipses are calculated for the reference sites ONLY. The location of the tests sites relative to the reference sites can then be determined. The overall assessment of stress is based on site locations on the first versus second and first versus third axes, and the overall assessment based on its worst position.

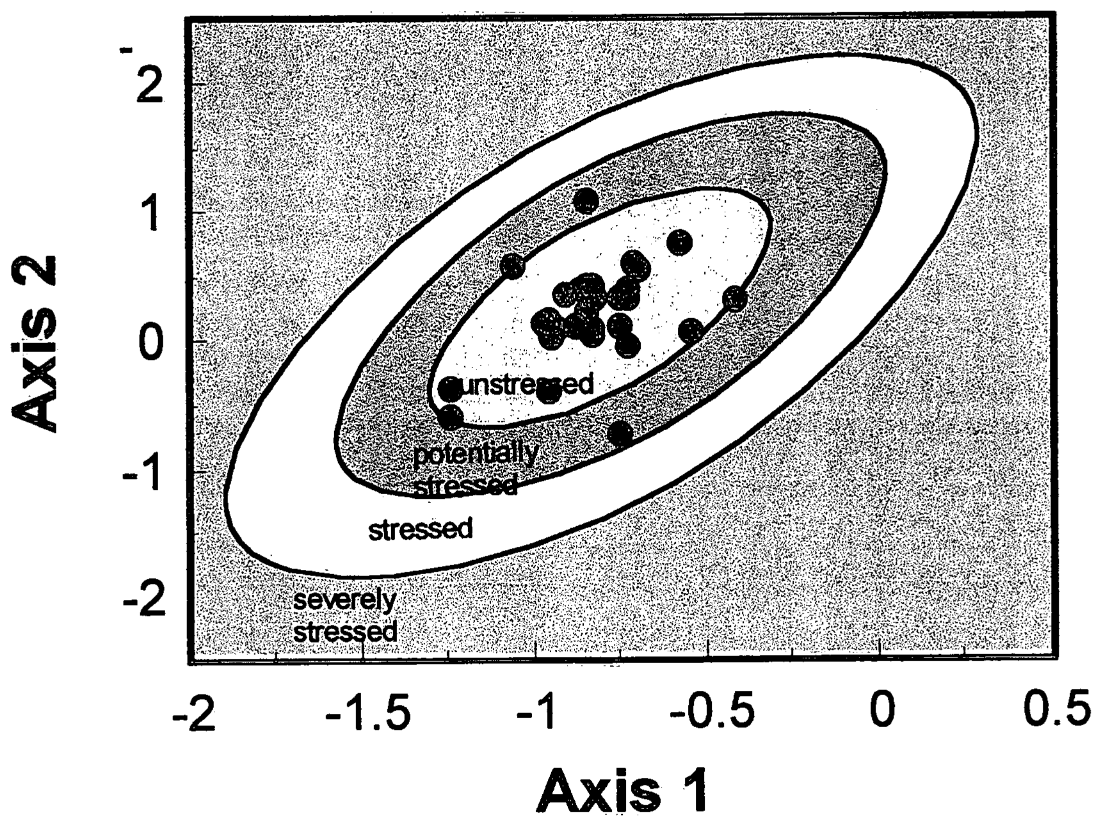



**Figure 9:** Probability ellipses calculated for reference sites to indicate: potential stress (90%), stress (99%) and severe stress (99.9%) in the invertebrate community of test sites.

The scale of response and the actual species that have been lost determine the degree of stress. It is also possible to assess which species may be responsible for the change in biological state and which environmental variables are associated with the biological response by examining the species and environmental vectors. The results of principle axis correlation with the species matrix provides an improved interpretation of the biological response. Similarly performing principle axis correlation between all the environmental variables, not just predictor variables provides some indication of the causation in the biological response.

## 7.0 Conclusions

The process of assessing whether a sediment is contaminated is based on two components. Response of the in situ invertebrate community and laboratory response of four invertebrate species.

Determination of invertebrate community impairment involves:

1. Sampling the community and measuring the predictor variables at the site of interest;

2. Running the discriminant model with the reference data base and test site(s) to predict the expected community at the test site(s);

3. Comparing test site(s) to the reference community sites, from the group to which the test site(s) were predicted.

Once a site is predicted to have a specific community structure, the actual structure is compared to the communities at the equivalent reference sites. A data file is constructed that includes the species counts for the appropriate reference sites. This is ordinated so that a matrix is calculated for both reference and test sites. The sites can then be plotted in ordination space showing the ordination dimensions that synthesize the biological attributes of the sites. Probability ellipses are calculated for the reference sites ONLY. The location of the tests sites relative to the reference sites can then be determined. The overall assessment of stress is based on site locations all the ordination vectors, and the overall assessment based on its worst position.

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Determination of sediment toxicity is made by comparison of individual test responses with criteria established from reference sites. A summary score is determined for both acute and chronic endpoints and a determination of the degree of toxicity made.

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