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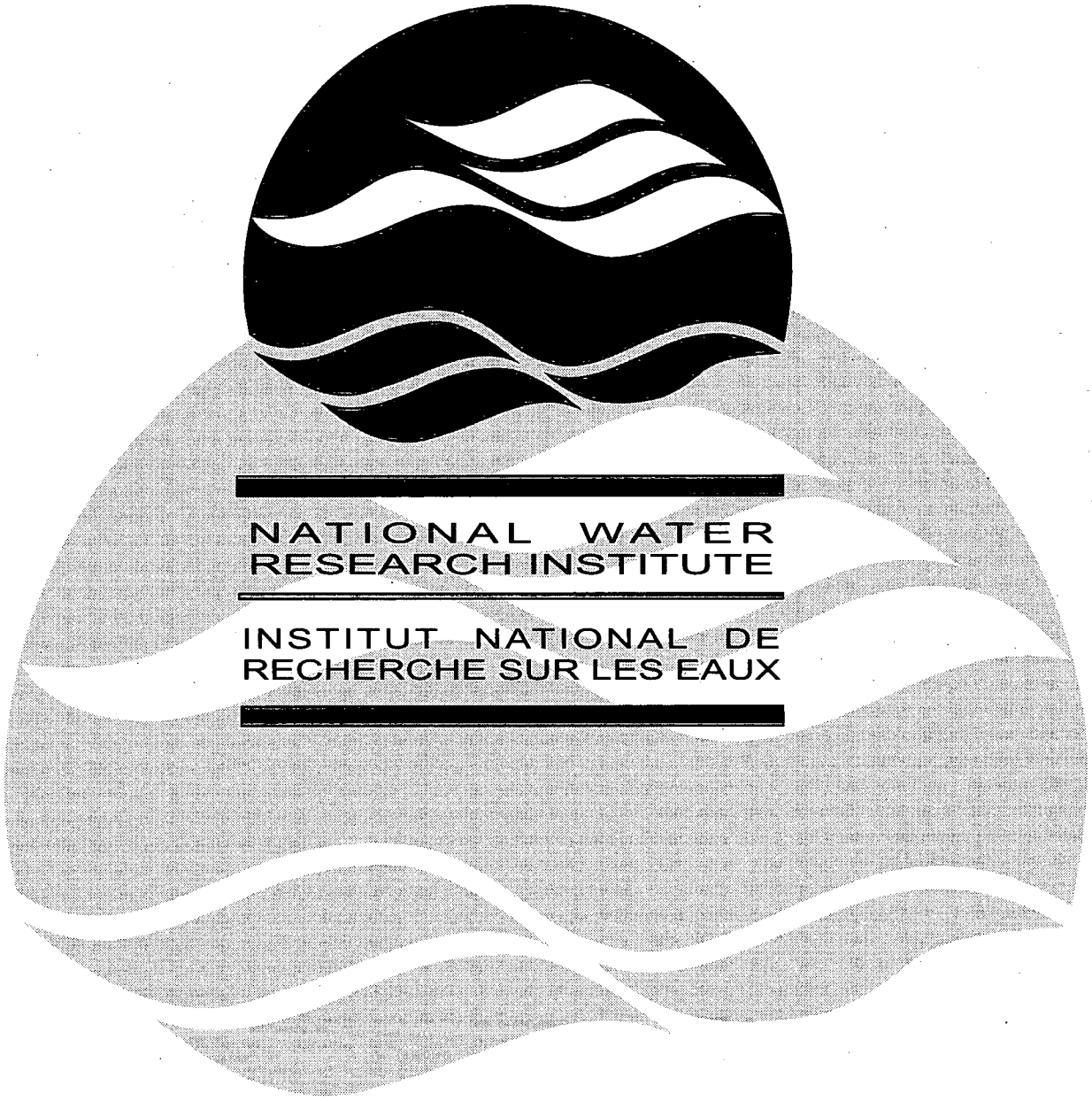

BIOLOGICAL GUIDELINES FOR THE ASSESSMENT OF SEDIMENT QUALITY IN THE LAURENTIAN GREAT LAKES

T.B. Reynoldson and K.E. Day

NWRI Contribution Number 98-232

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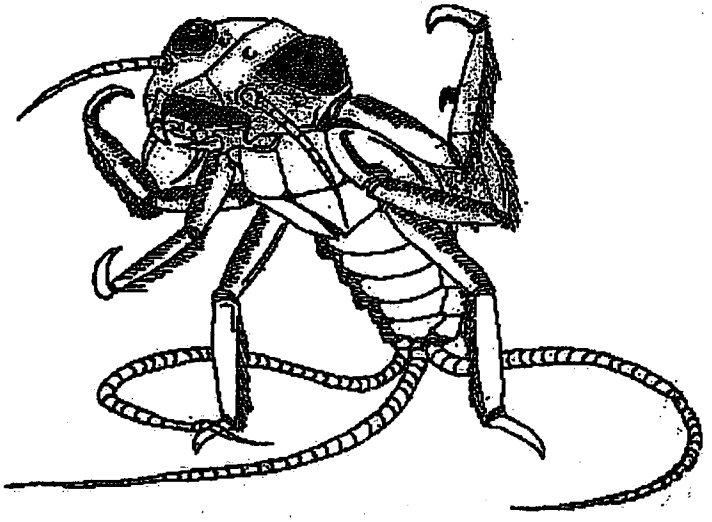

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MANAGEMENT PERSPECTIVE

Development of sediment guidelines for the Great Lakes (GL2000)

Almost all the Great Lakes Areas of Concern have documented sediment contamination. 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**. This technical report is the provides the scientific basis for a new approach.

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.

Because of the difference in this biological approach the technical report has undergone detailed scientific review by a 6 member review panel. The report and approach will then be forwarded to headquarters and the Province of Ontario.

Discussion will begin with the Province to incorporate these guidelines as a component in sediment assessment for the Great Lakes and for use in all sediment related projects, this should also be adopted as the COA standard approach for sediment assessment in the Great Lakes.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Élaboration de lignes directrices visant les sédiments pour les Grands Lacs (GL 2000)

On observe des cas documentés de contamination des sédiments dans presque tous les secteurs préoccupants des Grands Lacs. Les lignes directrices actuelles visant les sédiments sont basées sur la comparaison de concentrations de substances chimiques d'un site à celles qui sont considérées comme des concentrations sans danger d'après leurs propriétés chimiques. Toutefois, au cours des dernières années, on a critiqué l'approche chimique parce souvent, elle n'atteint pas ses objectifs ou parce qu'elle est si rigoureuse que son utilité s'en trouve limitée. Comme approche de remplacement, l'Institut national de recherche sur les eaux et la Région de l'Ontario d'Environnement Canada ont élaboré une approche utilisant des **lignes directrices biologiques visant les sédiments**. Ce rapport technique doit présenter les fondements scientifiques d'une nouvelle approche.

Ces lignes directrices biologiques visant les sédiments sont basées sur deux hypothèses fondamentales. Premièrement, ce sont les effets de la contamination des sédiments sur les processus biologiques qui constituent la principale préoccupation et, par conséquent, l'évaluation de ces effets est essentielle. Deuxièmement, à cause de la complexité des matrices de sédiments, les concentrations de produits chimiques sont de mauvais indicateurs de la disponibilité biologique des contaminants.

Les lignes directrices biologiques visant les sédiments prennent en compte a) la structure des communautés d'invertébrés benthiques en utilisant des modèles de prévision reliant les attributs de l'habitat d'un site à une communauté prévue et b) les réponses fonctionnelles (survie, croissance et reproduction) obtenues avec quatre tests de toxicité des sédiments (épreuves biologiques) effectués avec des invertébrés benthiques et utilisant dix résultats expérimentaux. En effet, on a établi la structure de

la communauté et les lignes directrices de toxicité afin de pouvoir déterminer si une communauté est non stressée, potentiellement stressée, stressée ou fortement stressée, et si les sédiments sont non toxiques, potentiellement toxiques ou toxiques.

Afin de simplifier le processus d'évaluation, on a développé le logiciel BEAST, qui intègre l'analyse multivariable complexe requise par cette approche et qui présente à l'usager des catégories simples de qualité des sédiments propres à chaque site. Conçu pour l'évaluation des sédiments benthiques, le logiciel BEAST (BEnthic Assessment of SedimenT) qui automatise la méthodologie est fondé sur le progiciel d'analyse et de cartographie RAISON d'Environnement Canada. De plus, il combine des nouvelles méthodes à une interface utilisateur simple et directe. Pour ces raisons, le logiciel BEAST est un nouvel outil très performant pour l'évaluation des sédiments.

À cause de la différence de cette approche biologique, un comité d'examen de six membres a effectué un examen scientifique détaillé du rapport technique. Le rapport et l'approche seront transmis ensuite au bureau chef et à la province de l'Ontario.

On doit entreprendre des discussions avec cette province afin d'incorporer ces lignes directrices au processus d'évaluation des sédiments des Grands Lacs et pour leur utilisation dans tous les projets touchant les sédiments; de plus, on doit aussi les adopter comme approche standard pour l'évaluation des sédiments des SP de la région des Grands Lacs.

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1. ACKNOWLEDGEMENTS

The project was supported financially and conceptually by Environment Canada, Ontario Region, the Great Lakes Cleanup Fund and the National Water Research Institute. Numerous individuals, and in particular, Mr Griff Sherbin, Mr. S. Lewelyn, Ms. Susan Humphrey, Ms Alena Mudroch and Dr Rod Allen were critical to its success and patient in its delivery.

The design of the project was significantly enhanced through two technical workshops, held prior to the project for input into the study design and to review progress after the first year of the study. All of those participants (Appendix 8.1) are thanked for their input into this project.

Special thanks goes to those involved in the field collection of samples, the crew and technical staff of the CSS Limnos, the Technical Operations Division of NWRI and, in particular, Mr. Barry Moore.

The toxicity component of the study could not have been completed without the support and enthusiasm of Cheryl Clarke, Scott Kirby and Danielle Milani who frequently worked weekends and extra hours to deal with the vagaries and frequent mis-timing of culture animals. We also wish to thank Katherine Stephenson and Patti Gillis who also assisted in the Sediment Toxicity Laboratory.

The sometimes tedious process of sorting, picking and identifying benthic samples was conducted by Scott Hughson and supervised by Craig Logan. We particularly wish to express our gratitude to Craig who has probably contributed as much as anyone to all aspects of this project, including the organisation, collection and analysis of the samples. Also, Sherri Thompson was frequently asked to drop other work and provide invaluable support, as well as maintaining the laboratory during periods of some stress.

We also wish to make particular mention of some external advisors, Drs. Richard Norris and Robert Bailey for their advice on matters pertaining to multivariate statistics. Finally, we wish to thank the reviewers of the report, Drs D. Barton, R.O. Brinkhurst, G. Allen Burton, C. Ingersoll, D. Jackson and R. H. Green, whose comments improved the report substantially.

2. INTRODUCTION

2.1 Sediment Issues

Sediments play an important role in the physical movement, chemical partitioning and biological fate of metals, organics and nutrients (Allan 1986). Such contaminants 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 associated with particles of sediment are often several orders of magnitude higher than those in water (Golterman *et al.* 1983). The highest concentrations of contaminants are associated with fine-grained sediments. These sediments accumulate in areas of low energy such as nearshore embayments of lakes, river mouths, 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 biogeochemical materials in aquatic environments. However, physical resuspension via 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 topmost 2-3 cm), a number of chemical, physical and biological processes affect the association of contaminants with sediment. These include ingestion and egestion of sediment particles by the benthic fauna, chemical and physical sorption and desorption to the particles, and, diffusion of soluble contaminants throughout the sediment pore water. The major concerns involving these processes are the bioaccumulation of contaminants through the benthic food chain to higher trophic levels, the re-contamination of the water column with subsequent bioaccumulation by other organisms and toxic effects. In all cases, the ultimate concerns are the detrimental effects produced in all organisms, including man.

Particles are often considered as lost to the ecosystem once they have been incorporated into the deeper sediments; however, two processes can result in the physical transport of materials back into the water column. Bioturbation which results from the

activity of the 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 water column 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.

2.2 Current Conditions in the Great Lakes

While information is available on the distribution of sediments and their geochemical composition in some nearshore and harbour areas of the Great Lakes, more information is available for the open lake. The open lake data base is primarily a result of a series of surveys conducted by the National Water Research Institute, Canada Centre for Inland Waters, beginning in 1968 in Lake Ontario and finishing in 1975 in Lake Michigan (Thomas 1981; Rosa 1985). The results of these surveys have been used for mapping the distribution of trace elements and selected organic contaminants, particularly polychlorinated biphenyls (PCBs), in surficial sediments in the Great Lakes (Oliver *et al.* 1982). However, we have very little information on the biogeochemical processes that affect the release, toxicity and food chain impacts of these contaminants (Allan and Ball 1990).

From a survey of the distribution of trace elements and PCBs in the surficial sediments, Allan (1986) concluded that there were two basic distribution patterns for these compounds in the Great Lakes. The first is associated with metals, particularly chromium, nickel and cobalt, and relates to the natural mineralization of these elements. The highest concentrations of these metals occur in the upper Great Lakes, particularly Lake Superior and Georgian Bay. This distribution is due to the geochemical composition of the bedrock in the upper part of the basin which is underlain by the igneous and metamorphic rocks of the Canadian Shield. The other pattern found in the sediment of the open waters of the Great Lakes is associated with both major and trace elements and organic contaminants originating from urban, industrial and agricultural developments. In this case, the greatest concentrations are in the lower lakes, particularly near major urban areas. For example, elevated concentrations of mercury are associated with sediments in the western basin of

Lake Erie, Lake St. Clair, the Detroit River and the depositional basins of Lake Ontario - particularly the Niagara Basin (Thomas 1981). The distribution of the pesticide, Mirex, in Lake Ontario sediments demonstrates the large-scale spatial redistribution that can result from the spread of localised contamination. Samples taken in 1968 indicated two non-atmospheric sources of Mirex, the Niagara and Oswego Rivers. The major source was from process manufacturing losses at the Hooker Chemical Plant in Niagara Falls. The Oswego River contamination resulted from an upstream, short-term loading to the river in the early 1960's. Mirex, associated with sediment, was gradually transported down the 14 km stretch of the Oswego River to Lake Ontario. A 1977 survey indicated a great extension in the area of contamination and increased concentrations of mirex in the surficial sediments (Van Hove Holdrinet *et al.* 1978). The subsequent closure of the Lake Ontario fishery was, in part, due to the lake-wide distribution of mirex. The ultimate fate of this material is likely the far-field contamination of the St. Lawrence River.

Table 2.1 Summary of the nature and extent of sediment contamination in Areas of Concern, based on bulk sediment chemistry (adapted from Painter 1992).

	Severe Effect Level*		Lowest Effect Level*		Contaminants exceeding SEL
Area of Concern	Km^2	% of AOC	Km^2	% % of AOC	
Hamilton Harbour	7.9	44.2	20.0	94.5	Zn, Cr, Cd, Pb, Cu, PAH's
Bay of Quinte	4.5	0.2	132.7		Cu
St Lawrence River	0.2	0.7	6.8	22.7	Hg, Zn, Cu, Pb
Spanish Harbour	66.0	12.4	262.7	51.0	Ni, Cu
Toronto Harbour	1.4	1.8	28.5	35.9	Cr, Cu, Pb, Cd
Peninsula Harbour	0.5	2.7	5.3	27.4	Hg
St Marys River	0.9	0.3	80.9	17.4	As, Cr, Cu, Pb, PAH's
Nipigon	0.0	0.0	55.2	27.2	
Jackfish Bay	0.0	0.0	0.6	7.2	
Collingwood Harbour	0.0	0.0	0.1	11.0	
Wheatley Harbour	0.0	0.0	< 0.1	54.6	

* Severe and Lowest Effect Levels as defined in Persaud *et al.* 1992

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

Table 2.2 Summary of Ontario Sediment Quality Guidelines (values in ug/g^{-1} dry weight) from Persaud et al 1992.

	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

sediment associated contaminants on biota. The data presented in Table 2.1 represent areal summaries of sediment contamination for some Areas of Concern according to the three effect levels using chemical concentration criteria (Painter 1992). These data demonstrate that in many cases large areas exceed the severe category and all areas have

zones of considerable size that exceed the lowest safe level. The large areas of sediments designated as contaminated using such chemical criteria makes many remediation methods impractical. Furthermore, it has been found that in many apparently unperturbed systems sediment levels exceed both lower and upper criteria for several metals (Painter 1992).

2.3 Current sediment guidelines

Most management issues regarding contaminated sediments in the Laurentian Great Lakes 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 (IJC 1991). The only existing "criteria" for assessing the quality of sediments at this time were the Open Water Disposal Guidelines of the Ontario Ministry of the Environment (Persaud and Wilkins, 1976) and the dredging guidelines of the U.S. EPA (IJC 1982). Recently, the Ontario Ministry of the Environment and Energy (OMOEE) has proposed new sediment guidelines (Table 2.2) for use in the assessment of navigational dredging as well as remedial investigations in Areas of Concern (Persaud *et al.* 1992). 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 (derived from partition coefficients or background levels), the Lowest Effect Level (based on the concentration of 5th percentile of the distribution for all species for which data are available) and the Severe Effect Level (based on the concentration of the 95th percentile of the distribution for all species for which data are available).

Federally, the Canadian Federal Department of the Environment (Environment Canada, 1996) 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, analysed 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. The TEL represents the concentration below which adverse biological effects are expected to occur rarely and is based upon the geometric mean of the lower 15th percentile concentration of the effects data set and the 50th percentile of the no-effect data set. The PEL defines the level above which adverse effects are expected to occur frequently, the PEL is calculated as the geometric mean of the 50th percentile concentration of the effect data set and the 85th percentile of the no-effect data set. 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 conducted on mixtures of chemicals from field-collected samples and based on biological (laboratory test) variables. The U.S. EPA (USEPA 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.

At present decisions as to whether or not a sediment is contaminated is based on chemical concentration. However, current assessments of the ecological risk associated with contaminated sediments in Areas of Concern, and the need for remedial action, are using biological information in addition to data on chemical concentration. The methods most frequently used derive from the Sediment Quality Triad (SQT) approach (Chapman *et al* 1992; Canfield *et al.* 1996; Besser *et al* 1996; Chapman 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) to address the contaminated sediments problem in the American Great Lakes Areas of Concern (Fox and Tuchman 1996; Burton *et al.* 1996). The SQT approach yielded good concordance among measures of laboratory

toxicity, concentrations of contaminants in sediments and the composition of the benthic invertebrate communities for extremely contaminated sites. However, in moderately contaminated sites, less concordance was observed, especially between the benthic communities present and either laboratory toxicity tests or sediment contaminant loading (Canfield *et al* 1996). Scientists involved in the ARCS study suggested that evaluation of non-contaminant factors and understanding of the normal variability of the biological endpoints used are needed to better interpret the responses of benthic invertebrates exposed to contaminated sediments.

As discussed above, environmental managers and regulatory decision-makers have traditionally set water and sediment quality guidelines based on concentrations of selected contaminants within environmental matrices. The primary advantage of a chemical approach is the apparent ease of simple numerical comparisons of concentrations of chemicals found in environmental samples with levels of these compounds known to cause toxic responses in biota. However, the chemical approach has been criticised 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 conservative (Table 2.1) that it has limited value (Painter 1992, Zarull & Reynoldson 1993).

As the purpose of environmental assessment and management is ultimately the maintenance of biological integrity, it is our view that the setting of water and sediment quality objectives should include biological targets together with chemical surrogates. This approach is the basis of the sediment quality triad proposed by Chapman and co-workers and strongly endorsed in two International Joint Commission (1987, 1988) reviews of assessing sediment problems in the most contaminated areas of the Great Lakes. Both the sediment quality triad and the IJC promote the incorporation of laboratory and field biological assessment in identifying contaminated sediment. In both cases the use of invertebrate assemblage structure is suggested as the appropriate field component and toxicity testing as the laboratory component. This report describes the development of numeric target values for these biological measures.

2.4 Reference condition concept

Until recently, the development of numeric biological targets was considered too difficult due to the temporal and spatial variability inherent in biological systems. However, over the past 15 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 biological response in clean (or 'uncontaminated') sites using simple habitat and water quality parameters. In all these studies the biological attributes of choice have been invertebrate assemblages. 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. The acceptance by regulatory agencies of biological water and sediment quality objectives has been slow but is now being given serious consideration as shown by current work in Canada (Reynoldson and Zarull 1993, Reynoldson *et al.* 1995), the USA (Hunsaker and Carpenter 1990), the United Kingdom (the RIVPACS method; Wright *et al.* 1984) and Australia (Parsons and Norris 1996).

This report describes the development of biological guidelines for sediments in nearshore fine-grained habitats in the Laurentian Great Lakes. These guidelines have been developed for invertebrate assemblages and benthic invertebrate laboratory tests using a modification of the technique developed in the UK (Wright *et al.* 1984, Furse *et al.* 1984; Armitage *et al* 1987) and now described as the *reference condition concept* (for more detail, see Reynoldson *et al.* 1997). The choice of invertebrate assemblages was made on the basis of the fact that these organisms are in direct contact with the contaminants associated with the sediment and are therefore most likely to exhibit effects. The use of laboratory tests was supported to confirm that any responses observed in the field are due to sediment and not other environmental stressors. In selecting the test organisms and endpoints it was the view that, again, infaunal invertebrate species would be most appropriate, and that ecologically relevant (growth and reproduction) chronic as well as acute endpoints should be used.

Fundamental to the scientific method is the use of controls or control conditions against which results obtained under test conditions can be compared. In field comparisons, attempts are made to choose test and control sites that are as similar as possible. The variable of interest can then be manipulated but uncontrolled variables are assumed to fluctuate. The actual choice of separate sites in the field that are similar in all aspects, and, that can be divided into control and experimental sites, is difficult. Traditionally, this problem has been solved in aquatic studies by choosing adjacent sites in streams (i.e., upstream and downstream comparisons; Norris *et al.* 1982), dividing lakes into halves (Schindler 1974), using artificial enclosures or mesocosms (Graney *et al.* 1994) or by locating sites thought to be similar at an appropriate distance from any source of contamination. Such approaches have several problems (Cooper and Barmuta 1993) especially the problem of "pseudoreplication" (Hurlbert 1984). In the *reference condition approach*, a wide range of minimally disturbed sites are sampled and organized by selected physical, chemical and biological characteristics to form one or more 'reference conditions'. These reference conditions then serve as the control(s) against which individual test sites can be compared. The notion of a *reference condition* is therefore really a description of *best available condition.*

Using the reference condition approach in developing biological guidelines for the Great Lakes involves the following steps (Figure 2.1):

Data collection

Collection of data on invertebrate assemblages, sediment toxicity tests and habitat descriptors from reference sites that describe the broadest range of natural variation in fine grained sediments from the nearshore of the Great Lakes.

Site classification and model building

Reference sites are organized into groups with similar biological attributes based either on the composition of their invertebrate fauna or the response in the laboratory test endpoints. The characteristics of these community groups and the test endpoint ranges form the bases for the guidelines.

Predictive models are developed that relate a set of habitat attributes to the groups of sites formed from the biological data. The models are used to determine the probability of a test site belonging to individual reference site groups.

The data collection, reference site classification and model building required to develop the guidelines and are a substantial one time effort. However, the models can be refined and periodically upgraded as further data are collected.

The following steps are used in the assessment of sediment quality using the biological sediment guidelines.

Selection of reference sites for comparison

A statistical technique, discriminant function analysis (DFA), with physio-chemical variables is used to determine the probability of a test site belonging to one or more of the reference groups.

Test site assessment

This is the step that defines whether the biological response at a test site meets expectation, and compares the biological attributes of the test site with the normal range observed at the appropriately matching reference sites.

In this study, a large data base was assembled from 271 sites in Lakes Ontario, Erie, Michigan, Superior and Huron and analysed to establish reference conditions. Information from each site included, (1) the responses of four species of benthic invertebrates (*Hyalella azteca, Chironomus riparius, Hexagenia* spp. and *Tubifex tubifex*) exposed in the laboratory; (2) the structure of the benthic invertebrate community; and(3) selected environmental variables from the same site.

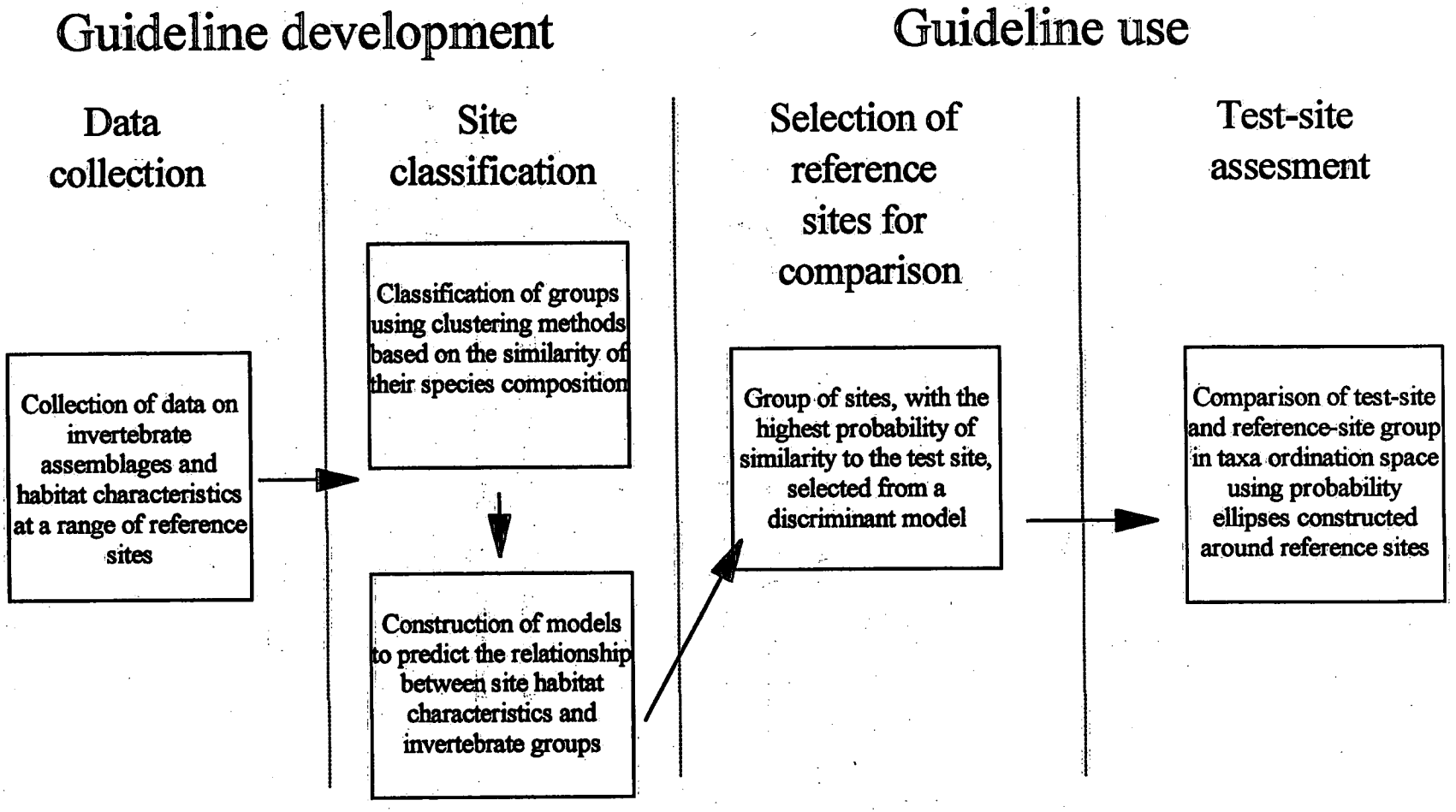


Figure 2.1. Stages in the development and application of biological sediment guidelines.

3. SAMPLING METHODS

3.1 Selecting Reference Sites

Reference sites refer to locations at which data are collected for comparison with test sites. One of the critical limitations of this approach is that the predictive models that are developed cannot extrapolate beyond the range of variability contained within the reference data set, that is a reference data set developed for the Great Lakes cannot be applied to lakes in northern Ontario. Furthermore, it is vital that the reference sites encompass the entire range of variability from the geographic area from which test sites will be examined. A process must be used to select reference sites as these form the benchmark against which test sites will be compared. The condition at reference sites should represent the normal range of minimally impaired conditions that can be achieved in soft sediments in the Great Lakes. 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 approach that we recommend bases reference condition on the biological attributes of a site where selection of the appropriate reference condition from a set of possible reference states is determined by a predictive model based on environmental attributes of the site.

Reference sites should have minimal impairment from anthropogenic activities such as watershed disturbance, habitat alteration, non-point source runoff, point-source discharges, atmospheric deposition or fishing pressure. Sites without any of these disturbances are ideal reference sites. However, in many regions human land-use practices and atmospheric contamination have so altered the landscape that truly undisturbed sites are unavailable. Therefore, a criterion of *minimal impairment* must be used to determine the selection of reference sites.

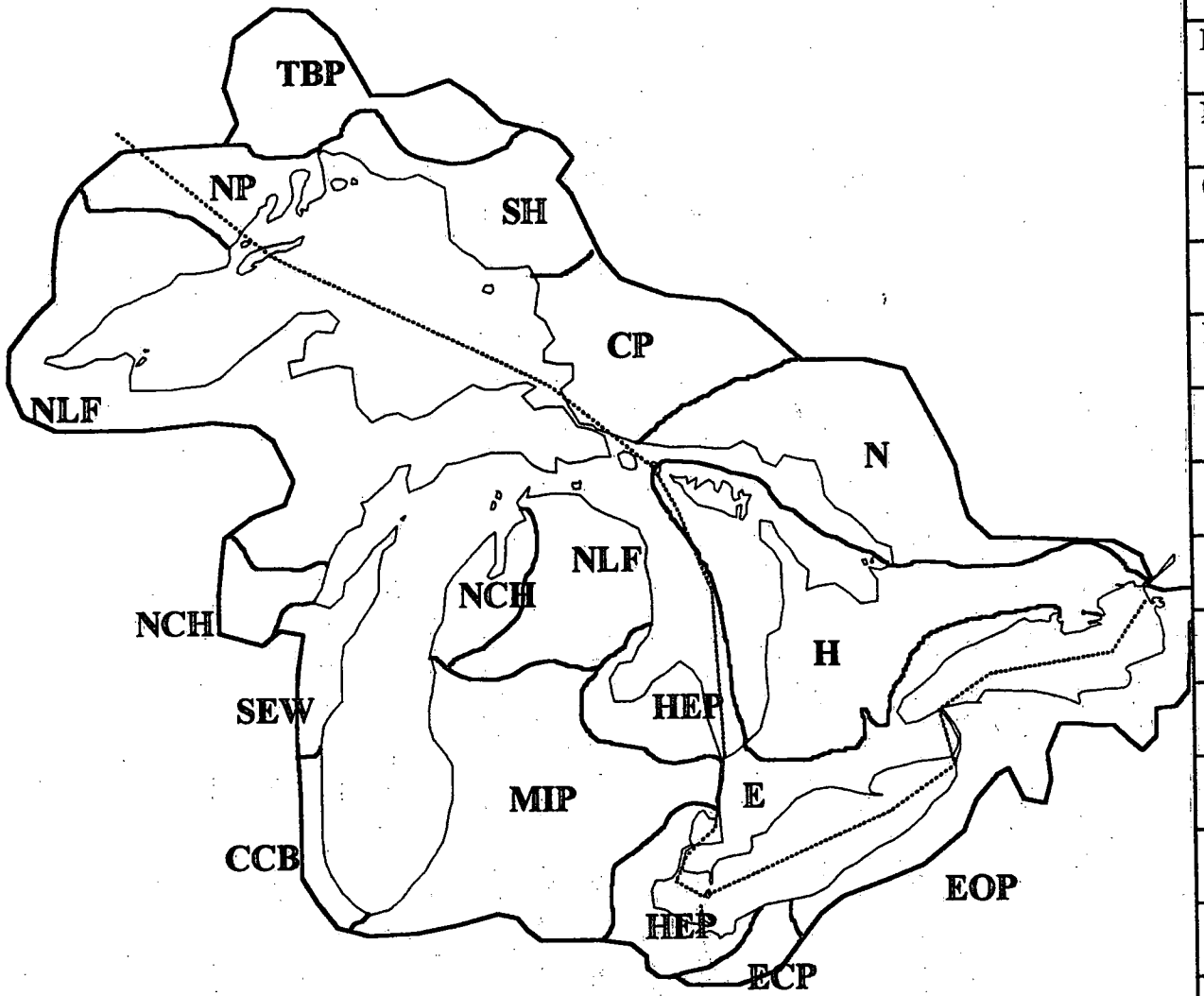
Several requirements were considered necessary prior to the location of the sites for sampling:

1. The selected sites had to capture as much of biological variability in the benthic invertebrate communities of the Great Lakes as practicable;

2. The sites should be located in depositional areas because contaminants are primarily associated with fine grained material;
3. The sites should represent the nearshore environment as sediment remediation is considered impractical in offshore or deeper waters, primary application of the guidelines is expected to be in the International Joint Commission "Areas of Concern", all of which are nearshore, and;
4. The sites should be unimpacted or clean.

Capturing the range of biological variability

The trend in many of the approaches to establishing reference conditions, particularly in the United States has been to use terrestrial habitat attributes to define reference sites (Hughes 1995, Omernik 1995). This has usually been based on ecoregions, which define areas with similar geographic attributes. Ecoregions are helpful in capturing the potential range of reference conditions that may exist, however, we do not support basing aquatic reference conditions solely on ecoregions or any other physical classification. Rather, we suggest that ecoregions are the first step in reference site selection and stratification as there is little evidence that invertebrate communities show high levels of uniformity within such regions (Corkum 1990, 1991, Richards et al. 1993). Hughes (1995) provides an outline of the steps involved in selecting regional reference sites, which can be usefully used when using a multivariate approach to predicting reference conditions. Lake systems are normally classified based on their trophic state; however, in the Great Lakes, the majority of the basins are naturally oligotrophic, with the exception of mesotrophic western and central Lake Erie. As aquatic systems are reflections of the surrounding terrestrial environment, it was considered reasonable to use a terrestrial land classification system to stratify the Great Lakes nearshore environment. Since early 1980, an ecological land classification system has been developed in Canada using an interdisciplinary, ecological approach with a standardised terminology. This has become known as the *Ecological Land Survey*, in which the landscape is conceived as large and small ecosystems, nested within one another in a spatial hierarchy (Rowe and Sheard 1981; Rubec and Wiken 1983). This classification process includes the description, comparison and synthesis of data related to the biological and physical



	Ecoregion	Number of Ecodistricts sampled
E	Erie	01, 02, 03, 04
H	Hurontario	15, 06, 13
N	Nipissing	14, 15, 16
CP	Chapleau Plains	24
SH	Superior Highlands	25, 26
NP	Nipigon Plains	50
TBP	Thunder Bay Plains	51
NLF	Northern Lakes and Forests	none defined
NCH	North Central Hardwood Forests	none defined
SEW	SE Wisconsin Till Plain	none defined
CCB	Central Corn Belt plains	none defined
MIP	S. Michigan/N. Indiana Clay Plains	none defined
HEP	Huron/Erie Laker Plain	not sampled
ECP	E. Corn Belt plains	not sampled
EOP	Erie/Ontario Lake Plain	not sampled

Figure 3.1. Ecoregions and ecodistricts of the Great Lakes basin.

characteristics of the land, including parent material, landform, hydrology, vegetation, climate and wildlife. Seventeen ecoregions have been identified in Ontario (Figure 3.1) and, of these, seven intersect the shoreline of the Great Lakes (Figure 3.1). Each of the ecoregions is further subdivided into ecodistricts (Wickware and Rubec 1989) and it was this level of stratification that was used to select the reference sites along the Canadian shoreline. In Lake Michigan the ecodistrict level boundaries are not established and stratification was at the level of the ecoregion.

Identifying fine grained habitat

Within each ecodistrict, hydrographic charts were used to identify areas having fine-grained sediment. Boundaries were drawn around those areas indicated on the hydrographic charts as having either a silt or mud substratum. These were identified as regions where sites could be potentially located.

Defining nearshore

As the focus of the study was on the nearshore environment, a depth criteria was used to limit site location. The initial decision was to restrict sites to a 30 m water depth limit and within 2 km of shore. However, the absence of fine-grained material within this depth stratum in some geographic areas did require the inclusion of some deeper sites.

Defining minimal impairment

Finally, the objective was to sample sites that were minimally impaired. Optimal reference sites should represent pristine conditions; however, such an objective is unrealistic in many areas. The primary source of information on point source discharges is the Canadian Great Lakes Basin Intake-Outfall Atlas (Ontario Environment 1990), an eight volume set describing water intakes and industrial and municipal discharges to the lakes. Areas within 10 km of a point source were therefore excluded as potential reference site locations. Avoidance of areas likely to be affected by non-point sources was achieved by using topographic maps to select areas that had minimal agricultural or urban shoreline development.

Once the above steps had been taken to identify potential sites the final decision on a site being sampled was its suitability in the field in terms of depth and substrate.

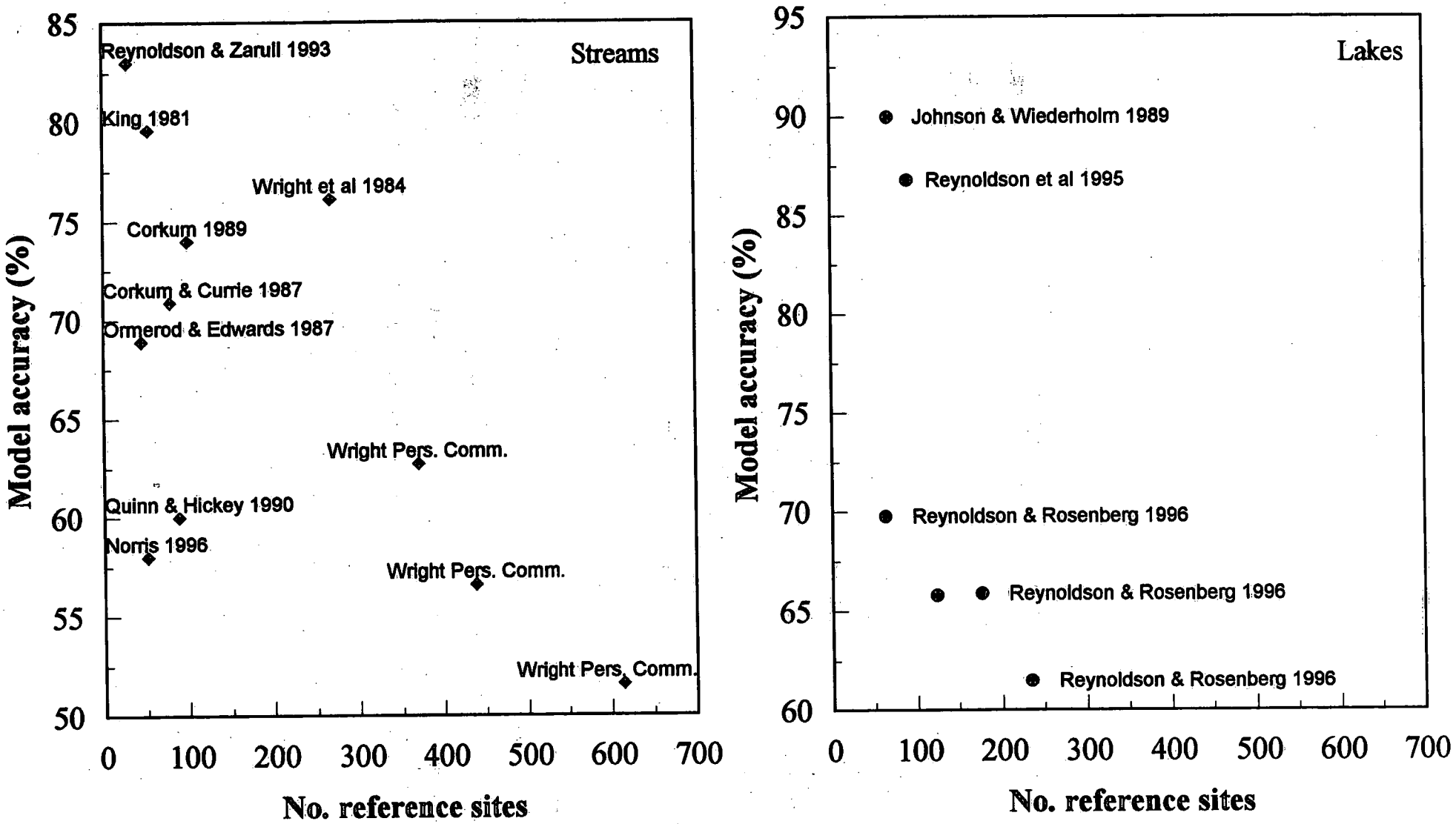


Figure3.2 Effect of number of sites on predictive capability.

To estimate the number of sampling sites required to build a predictive model we examined the relationship between the accuracy of predictive models in correctly classifying sites and number of reference sites used to build the models from published data for both stream and lake data sets. Counter intuitively there seems to be a negative relationship between the number of sites and the quality of the predictive models (Figure 3.2). The large data sets developed by the RIVPACS group in the United Kingdom have found that as the number of reference sites increases the percentage of correct predictions decreases (Wright pers. comm.). However, this is largely because that the greater the number of sites then the progressively broader the spectrum of sites within the area of study. As the number of sites increases the classification groups represent finer divisions than in classifications with fewer sites. Thus if a site is incorrectly predicted the correct group is usually very similar.

The specific number of sites to be distributed among the sampling divisions is often largely determined by available budgets, the number of sampling divisions and the desired level of accuracy in the predictive model. While there appears to be a negative relationship between predictive accuracy and total site number (Figure 3.2) based on literature reported studies, more importantly increasing the number of sites allows increased ability to discriminate impacts (Reynoldson 1996). Wright (1995) has suggested that individual site groups formed from a reference site data base should have a minimum of five and preferably 10 sites per group, thus allowing a reasonable estimate of variance to be established for a given reference group. While there was no a priori way of determining the number of reference sites required we aimed for a target of between 250-300 reference sites.

A total of 349 samples were taken over the 1991-93 study period (Table 3.1) from 271 different site locations (Figure 3.3). Most sites (306) were sampled in late summer or early fall over a three-year period. The sampling period ranged from mid July to the end of October, the median sampling date was September 12th, and 80% of the sites were sampled in August and September

In addition, some sites were sampled in each of the three field years or in two of the field years and four sites were sampled monthly over two years (1992-93). These data allowed a determination of the effects of both annual and seasonal variation. The seasonal samples

comprised 43 seasonal visits to four sites (303, 1213, 1307, 1601) visited monthly in 1992-93 (Figure 3.3), the annual samples repeat visits to 16 sites in each of the three years and repeat visits to a further 13 sites for two of three years (Table 3.1).

Table 3.1 Summary of sites sampled to develop a reference database for the Great Lakes (1991-93).

	1991	1992	1993	Total
Fall Samples	50	147	109	306
Sites re-visited (1991-93)	16	16	16	
Sites re-visited (1991-92)	10	10		
Sites re-visited (1992-93)		3	3	
Seasonal samples	0	21	22	43

Sediment, water and pore-water samples were collected at each site for chemical and physical analysis. In addition, samples were collected for the determination of the community structure of benthic macroinvertebrates and for laboratory sediment bioassays with selected species of benthic invertebrates.

3.2 Invertebrate Community Structure

Samples for the identification and enumeration of benthic invertebrates were collected from either a large box corer (50 cm x 50 cm) or a mini-box corer (40 cm x 40 cm) operated from either the CSS Limnos or the 'P' class vessels. Box corers are gravity-operated devices designed for collecting large, square, undisturbed sediment cores for scientific examination and sub-sampling. A complete description of box corers and other sediment sampling devices is available in Mudroch and MacKnight (1994). The large box corer used in the first year of this study requires both a winch and a crane, and can only be used from a large vessel such as the Limnos. The mini-box corer used in years 2 and 3 only requires a winch and can be used on smaller vessels (e.g., the 'P'class vessel).

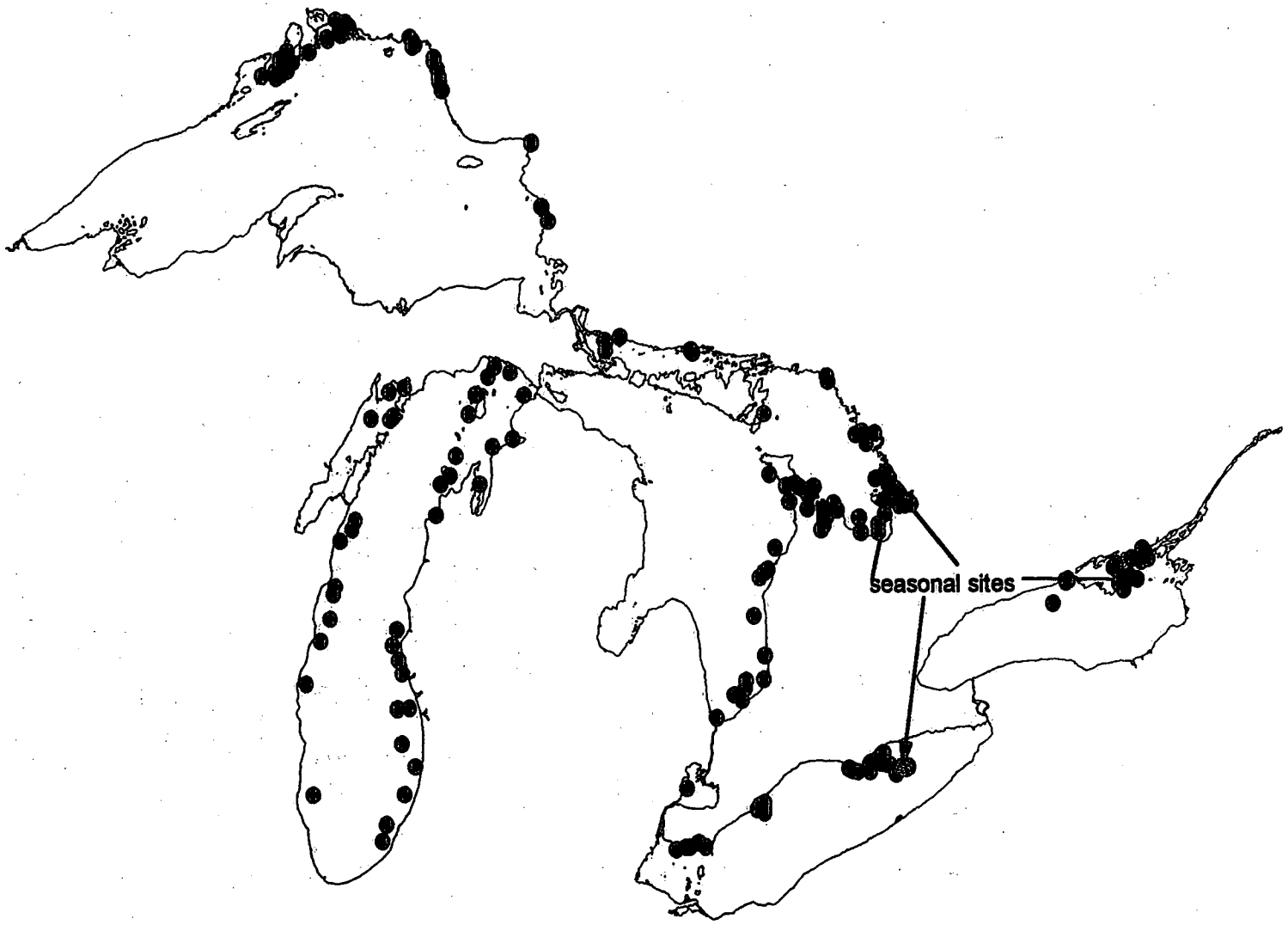


Figure 3.3. Location of Great Lakes reference sites.

Table 3.2. Comparison of mean abundance and mean richness of paired samples (5 replicates / sample) taken on three separate occasions with a box corer and mini-box corer from sites sites in L. Erie (east basin -23, central basin - 84, west basin - 357, 358). (Samples showing a significant difference using a paired t-test ($P < 0.05$) are identified in bold).

Site	Box corer organisms / core	Mini-box corer organisms / core	Box corer taxa/core	Mini-box corer taxa/core
23 (Oct '91)	307.2	350.8	10.6	10.8
23 (Jul '92)	225.4	184.6	**10.4***	**8.6***
23 (Sep '92)	359.2	324.8	9.0	10.8
84 (Oct '91)	**35.2***	**75.0***	**8.0***	**10.4***
84 (Jul '92)	63.8	47.2	7.6	7.4
84 (Sep '92)	66.0	66.6	10.8	10.6
357 (Oct '91)	35.6	34.8	11.4	9.6
357 (Jul '92)	39.8	35.0	8.0	7.2
357 (Sep '92)	35.6	77.8	9.0	11.6
358 (Oct '91)	32.4	25.8	10.6	8.6
358 (Jul '92)	41.2	21.8	6.0	5.4
358 (Sep '92)	**31.4***	**16.6***	**8.4***	**5.0***

Comparison of paired samples using the two box corers showed no differences in estimates of invertebrate community structure. Twelve sets of paired samples were taken from four sites in L. Erie over the period October 1991 to October 1992. Both a box corer and mini-box corer were used on each occasion at each site, 5 replicated core tubes were taken from each corer and the mean abundance per core tube for each box corer compared (Table 3.2). The results show that in two of the 12 paired samples there were significant differences between the number of organisms collected by the corers. On three of 12 occasions the differences between the number of taxa collected were different. However, there was no trend in the performance of either sampler to collecting lower abundance or number of taxa. While these differences are more than expected due to chance (5%), i.e., 17% for abundance and 25% for richness, we do not

consider that they are sufficient, given the small number of comparisons (12) to suspect that there are true sampler differences as opposed to small scale patchiness.

Each box corer sample was treated as an intact section of sediment that has simply been translocated to the surface. It was sampled by completely inserting five 10 cm long plexiglass tubes (i.d. 6.5 cm, enclosed area 34.2 cm^2) into the sediment in the box corer. Each core tube was considered a replicate sample unit. The appropriate number of replicates to sample from an individual box corer was determined by an examination of data on total abundance of invertebrates collected from 19 box corers taken in Lake Erie. From each of the 19 box corers, 10 replicate core tubes were collected. Based on total number of invertebrates, five replicate samples produced a coefficient of variation (CV) of 30%; the addition of another five replicates (one-by-one) only reduced the CV by 0.5% (Figure 3.4). Thus, five replicate tube cores were considered adequate.

The contents of each core tube were removed, placed into a plastic bag and kept cool until sieved. Sieving in the field was conducted through 250 µm mesh within 24h of sampling. If sieving could not be done in the field, 4% formalin was added to the bag and the samples were stored at 4 °C; sieving was conducted as soon as possible thereafter. After sieving, the samples were placed in plastic vials (50 mL) and preserved with 4% formalin. Replicates with large amounts of organic material were placed in larger containers and again preserved with 4% formalin. After 24 h, the formalin was replaced by ethanol.

Samples were sorted with a low power stereo microscope and identified to species or genus, whenever possible. As required, slide mounts were made for higher power microscopic identification (e.g., Chironomidae and Oligochaeta). Appropriate identification guides were used and voucher specimens of all identified specimens were submitted to experts for confirmation; Oligochaeta: R.O. Brinkhurst, D.R. Spencer; Chironomidae: B. Bilyj and D. Oliver; Mollusca; G. Mackie, Other taxa: B. Bilyj. The confirmed voucher specimens are being maintained as a reference collection at the National Water Research Institute.

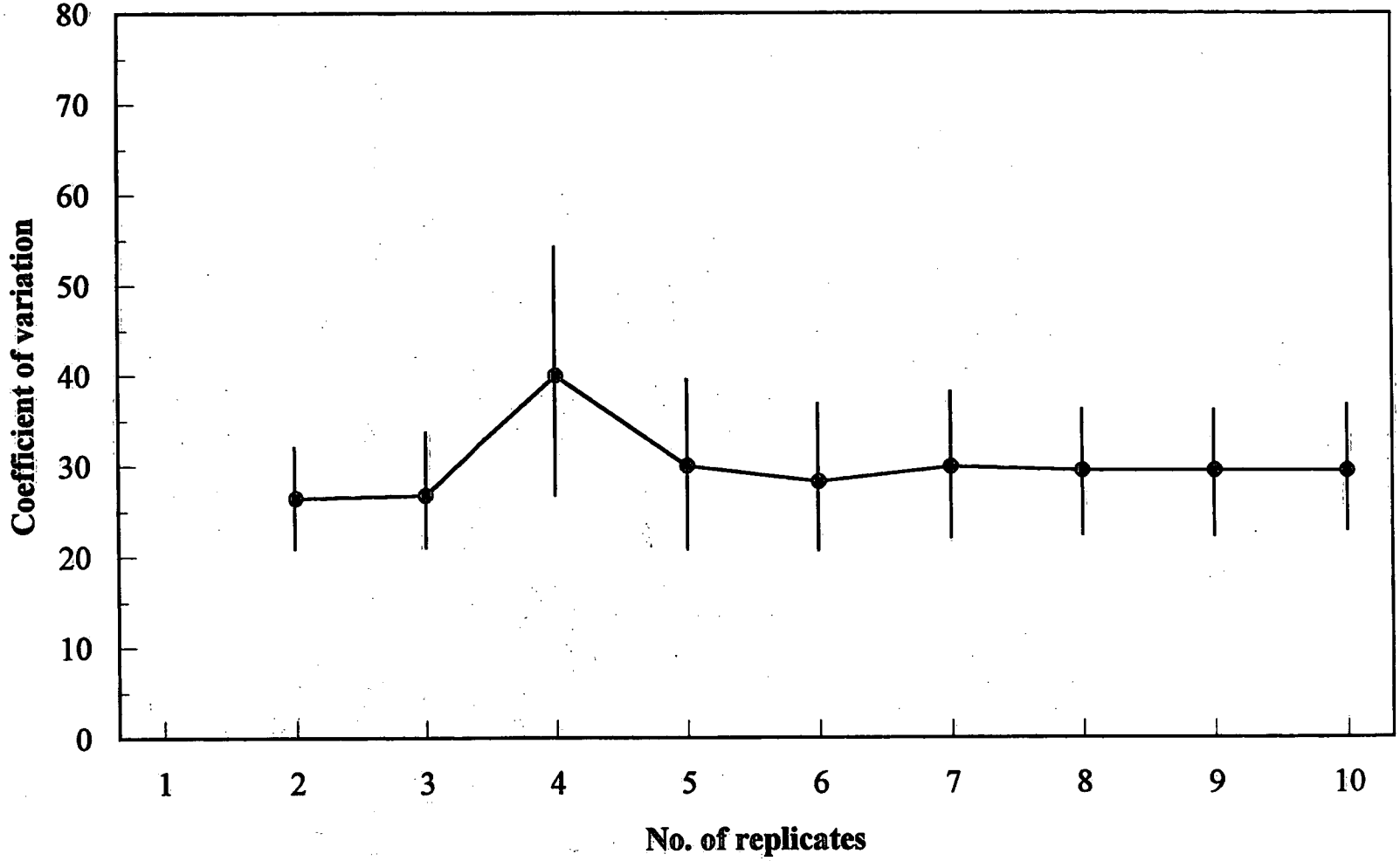


Figure 3.4. Average effect of increasing replication on estimates of total invertebrate abundance.

Table 3.3. Measured environmental variables at reference sites, selection criteria and consideration as a predictor variable.

Measured Variable	Rationale	Use as Potential predictor
Geographic (5 variables)	geographic descriptors provide a	
latitude	synthesis of the effects of spatial	yes
longitude	processes on animal distribution	yes
lake basin		no - non-continuous
ecodistrict		no - non quantitative
date		no - temporal effects examined separately
Limnological (8 variables)		
water depth	integrates effects of temperature and oxygen on organisms	yes
dissolved oxygen	critical for most aerobic organisms	no - modified by seasonal processes
pH	modifies chemical interactions	yes
temperature	effects growth and reproductive processes	no - requires temporal integration
alkalinity	summarizes dissolved materials	yes
total phosphorus	effects nutrient status and primary producers	no - modified by anthropogenic inputs
kjeldahl nitrogen	effects primary producers	no - modified by anthropogenic inputs
nitrate-nitrite nitrogen	effects primary producers	no - modified by anthropogenic inputs
Sediment (28 variables)		
Particle Size - 7 variables (% gravel, sand, silt clay, mean, 75^{th}, 25^{th} %le)	Effects burrowing organisms, modifies bioavailability of materials.	Yes
Major elements - 12 variables (oxides of Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P, TP, TN)	Provide a good descriptor of overall sediment conditions, provides a regional signal.	Yes
Nutrients - 2 variables (loss on ignition (LOI), TOC)	Provide an indicator of food availability	Yes
Metals - 9 variables (Totals for V, Cr, Co, Ni, Cu, Zn, As, Cd, Pb)	Provide a descriptor of anthropogenic inputs and general contaminant levels, allow verification of reference status	no - modified by anthropogenic inputs

3.3 Environmental Variables

In the development of predictive models, there is no *a priori* certainty as to which variables will be appropriate predictors; therefore, both scientific and pragmatic decisions were

necessary for the selection of the environmental variables to be measured in this study. Both information from previous studies (Wright *et al.* 1984; Faith and Norris 1989; Johnson and Wiederholm 1989; Reynoldson *et al.* 1995; Wright 1995) as well as understanding of lentic ecology were used in selecting potential predictors. In addition, there was a need to measure other variables (e.g., metals) that could support the characterisation of a site as reference, or, if a site was an outlier, provide supportive information as to the possibility of it being impaired. Three categories of environmental variables were measured: (1) large spatial scale variables categorised as geographical descriptors; (2) physico-chemical descriptors of sediment that would relate to small scale interactions between the organisms and their surrounding environment; and, (3) limnological descriptors which relate the sediment to overlying water column processes. The actual variables measured and the rationale for their inclusion are identified in Table 4. Fourty three environmental variables were measured; from these, 26 were considered as potential predictor variables (Table 3.3).

The location of each site was established in the field using either Loran C or a hand-held Geographical Positioning System (GPS). Latitude and longitude data were stored as both degree, minute second and decimal degrees.

Sediment and sediment pore-water were characterized from samples collected from either the large box corer (50 cm x 50 cm) or the mini-box corer (40 cm x 40 cm). Samples of sediment for geochemical analysis were collected from the surface (top 2 cm) of the box corer. Each sample was homogenized in a glass dish with a nalgene spoon and divided as follows:

A sub-sample of sediment for chemical analyses of organic contaminants was placed into a glass bottle pre-washed with hexane and covered with hexane-rinsed aluminum foil. Samples were sealed, held at 4 °C in the field until return to the laboratory and subsequently frozen for freeze-drying and storage. Due to the cost of chemical analyses, these samples were not analyzed for contaminants but were archived in the event of a site being suspected as being contaminated.

A sub-sample of sediment for the determination of particle size distribution was placed into a plastic pill jar and stored at ambient temperature in the field. Upon return to the laboratory, samples were lypholysized and analyzed following the method described by Duncan and LaHaie (1979). Large particles (> 63μ) were removed from the sediment sample prior to analysis.

The remaining sediment was stored in a 500 mL plastic container at 4°C in the field and shipped to Bondar Clegg & Co., Ltd., Ottawa, Canada for analyses of major elements, total phosphorous, total organic carbon (TOC), loss on ignition (LOI), and total Kjeldahl nitrogen using standard techniques outlined by the EPA (1981). Concentrations of metals were determined by acid digestion followed by ICP-AES analysis (multi-channel Jarrell-Ash AtomComp 1100) using the methods of McLaren (1981).

Samples of water for chemical analyses were collected using a Van Dorn sampler from 0.5 m above the sediment-water interface. A one litre sample was stored at 4 °C prior to analysis of total phosphorus, Kjeldahl nitrogen, nitrate-nitrite and alkalinity at the National Water Research Laboratory in Burlington, Ontario, Canada. Dissolved oxygen, pH and temperature were measured in the field.

For both sediment and water samples the standard procedure was to take a single sample for analyses. However, at 40 sites triplicate samples were taken to provide an estimate of the variability associated with the chemical measurements.

3.4 Whole Sediment Toxicity Tests with Reference Sediments

A mini-ponar sampler was used at each site to collect five separate samples of sediment for use in laboratory bioassays with each species of benthic invertebrate. Care was taken at each site during the sampling process to obtain sediment that was not disrupted by a previous sample collection method. The contents of each mini-ponar were placed in a food grade quality plastic bag and the bag was tightly tied with a plastic tie. All samples of sediment were placed in a cooler on ice until they were returned to the laboratory. In the laboratory, the bags of sediment were placed in plastic pails with lids and refrigerated at 4 °C in the dark until bioassays could be conducted using the sediment.

Six to seven sediments were run concurrently on a weekly basis for each species over a period of approximately six months following the collection of sediments for any given year. Storage of sediment for this period has been shown to not have an effect on toxicity data (Defoe and Ankley 1998). A clean control sediment from the Canadian Wildlife Bird Sanctuary, Long

Point marsh, Lake Erie, was also run with each set of weekly samples and each species to provide biological quality assurance. The culture of the chironomid, *Chironomus riparius*, and the oligochaete worm, *Tubifex tubifex*, are described in Reynoldson *et al.* (1991), Day *et al.* (1994), and Reynoldson *et al.* (1995). The culture of *H. azteca* was maintained according to the procedure described in Borgmann *et al.* (1989). Eggs of the mayfly, *Hexagenia* spp. (both *H. limbata* and *H. rigida*), were collected during late June and July in each year of the study (1991-1993) according to the method of Hanes and Ciborowski (1992) and organisms were raised to a suitable age for use in bioassays following a procedure based on Bedard *et al.* (1992) but modified by the addition twice weekly of a diet of a yeast:Cerophyll:NutrafinR (YCT) dissolved in deioinized water.

Tests with *H. azteca*, *C. riparius* and *T. tubifex* were conducted in 250 mL glass beakers containing 60 to 100 mL of sieved (500 µm mesh), homogenized sediment with approximately 100 to 140 mL of overlying carbon-filtered, dechlorinated and aerated Lake Ontario water (pH 7.8 to 8.3; conductivity 439 to 578 $\mu s.cm^{-1}$; hardness 119 to 137 mg/L). Tests with the mayfly, *Hexagenia* were conducted in 1 L glass jars with 150 mL of test sediment and 850 mL overlying water. The sediment was allowed to settle for 24 h prior to addition of the test organisms. Tests were initiated with the random addition of 15 organisms per beaker for *H. azteca* (mean dry weight = 0.022 mg) and *C. riparius*, 10 organisms per jar for *Hexagenia* spp. and 4 organisms per beaker for *T. tubifex*. Juvenile *H. azteca* were 3 to 10 d old at test initiation; *C. riparius* larvae were in first instar and within 48h of hatching; *Hexagenia* nymphs were 1.5 to 2 months old (approximately 5 to 8 mg wet weight) and *T. tubifex* adults were 8 to 9 weeks old. Tests were conducted at 23 ± 1° C with a 16L:8D photoperiod except for the test with *T. tubifex* which was conducted in the dark. The test system was static with the periodic addition of distilled water to replace water lost by evaporation. Each beaker was covered with a plastic petri dish with a central hole for aeration using a Pasteur pipette and air line. Dissolved oxygen, temperature, pH and conductivity were measured at the beginning, middle and end of each exposure period. In 1993, total ammonia wis also measured at test termination. Tests were terminated after 10 d for *C. riparius*, 21 d for *Hexagenia* and 28 d for *H. azteca* and *T. tubifex* by sieving the sediment samples through 250 µm mesh. Sediment from the *T. tubifex* test was sieved through 500 µm

mesh plus an additional 250 μm mesh at test completion. Endpoints measured in the tests were percent survival and increase in weight (growth measured as dry weight/individual) at test termination for *C. riparius*, *Hexagenia* spp. and *H. azteca*. Initial weights of *Hyalella* and *Chironomus* were considered to be zero. End points measured in the *T. tubifex* bioassay were percent survival, percent hatch of cocoons, number of cocoons produced per adult worm and number of live young produced per adult worm. Mean dry weights of *H. azteca*, *C. riparius* and *Hexagenia* spp. were determined after drying the surviving animals from each replicate as a group to a constant weight in a drying oven (60°C).

For bioassays conducted in 1991, chambers containing *Hexagenia* spp. and *T. tubifex* did not receive additional food during the course of the exposure to whole sediment. However, larvae of *C. riparius* and juvenile *H. azteca* received a food ration of 8 mg moistened Nutrafin^{R} fish food flakes added as a slurry twice per week to each beaker over the course of the exposure period. During this preliminary year of the study, it was noted that both growth and\or reproduction of *Hexagenia* and *T. tubifex* was quite variable, particularly in samples with low organic carbon content. Therefore, the standard operating procedures for the 1992 and 1993 bioassays were modified to include a ration of food for both species as follows: chambers containing *Hexagenia* received 50 mg of YCT twice per week; 80 mg of a Nutrafin^{R} slurry was mixed into the sediments at the onset of the *T. tubifex* tests and no other feeding was carried out during the worm exposures.

3.5 Data Analysis

3.5.1 Community Analysis of Benthic Invertebrates

Classification and ordination were used to describe the biological structure of the data at the reference sites; correlation and discriminant function analyses (DFA) were 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 of abundance counts for each taxon from the five replicates for each box corer were used as descriptors of the benthic invertebrate

community. The Bray-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 using different types of data (Faith *et al.* 1987, Jackson 1993). 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 explain the variability observed among the large number of taxa by a reduced number of new variables (ordination axes). A hybrid multi-dimensional scaling (HMDS) method of ordination was used, *i.e.*, Semi- Strong- Hybrid multidimensional scaling (Belbin 1991). Multi-dimensional scaling methods can use either metric or non-metric rank order information. We have used a hybrid technique that incorporates both metric and non-metric scaling (Faith *et al.* 1987). Metric scaling methods assume that the dissimilarity measure chosen has a linear relationship with ecological distance, non-metric sacling assumes only monotonicity and the distances between sample pairs are only maintained in rank order with their dissimilarities. The hybrid method described by Faith *et al* (1987) differs from these two approaches in using a prescribed dissimilarity measure that has a robust metric (linear) relationship with distance only over a certain range. A monotonic regression serves as the only direct constraint on larger dissimilarities. This hybrid attribute is of particular value when relating ordination scores to environmental characteristics. All clustering and ordination was done using PATN, a pattern analysis software package developed by CSIRO in Australia (Belbin 1993).

Of the 43 environmental variables measured in this study (Table 3.3), 26 were examined for their relationship with the biological structure of the data. Variables were excluded if they were likely to be influenced by anthropogenic activity, particularly those associated with sediment contamination. This is because the predictive models being developed are to be used to establish what community would occur at a test site if it were not affected by human activity. Thus, all the values describing concentrations of major and trace metals were excluded from consideration as potential predictor variables. The variables included were general descriptors of sediment type, such as particle size and organic matter (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 as subject to modification from human activity. The relationship with the biological data was examined in three separate ways:

(1) Principal axis correlation from PATN which determines how well a set of attributes (environmental data) can be fitted to ordination space (species matrix). This multiple-linear regression method takes each environmental attribute and determines the location of the vector with the best fit 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 randomization model was used 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. Procedure ANOVA in SAS was used to establish those environmental attributes that differed significantly ($P < 0.0001$ and $P < 0.05$) between biological site groupings.

(3) Stepwise discriminant function analysis (Procedure STEPDIS in SAS) was used to establish which environmental variables "best" separate cases into the predefined groups formed from the biological data set. Stepwise selection of variables was used and the significance level for variable entry and retention was 0.05.

Based on the results from these analyses, environmental variables were used in discriminant function analysis (DFA) to establish functions of the variables that "best" separate cases into the predefined biological groups. The SAS version of DFA 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, a model is generated without that site and then the site group 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 principal axis correlation. The optimal set was defined as that with the lowest error rate from cross-validation in discriminant analysis

3.5.2 Whole-Sediment Laboratory Toxicity Tests

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 Sigmaplot^{R} 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 Long Point marsh were similarly examined and the descriptive statistics tabulated.

A formula which incorporates the probability of Type I and Type II errors was also used 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 (α), statistical power ($1-\beta$) 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 α and β 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 (α) and Type II (β) errors of 0.10 are suitable because the costs of either remediating a non-contaminated sediment or not remediating a contaminated sediment 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:

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

where

σ = the true population variance

n = the number of replicates for a site (5)

t = critical value of t for a two-tailed test

υ= degrees of freedom 2 (n-1)

α= 0.1; β = 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 reference sites used in bioassays with each species.

4.0 RESULTS

The study area encompassed all five of the Laurentian Great Lakes. To ensure that the range of habitat characteristics were adequately represented, a preliminary list of sites was identified and stratified among 17 ecoregions described by Wickware and Rubic (1989) for the Canadian shores of the Great Lakes. Additional funding by the U.S. Environmental Protection Agency allowed the expansion of the data base into Lake Michigan and 53 sites in this lake were distributed throughout the five ecoregions designated for the terrestrial shoreline of Lake Michigan (Figure 3.3).

The data set of 306 fall samples was examined for outliers. Inclusion of sites in the reference site database was based on examination of both the community structure and toxicity data. As we had no a priori method of determining whether the selected sites were minimally impaired we excluded sites from the reference data base if either invertebrates were absent in the sample or the site had less than 50% survival for any test species. Of the 306 samples in the data set 18 were excluded because of poor laboratory survival, and one site because of community structure. A further 35 sites were removed at random for validation of the community models resulting in 252 being included in the reference database.

4.1 Environmental attributes of sites

The range of environmental conditions found at the 252 reference sites is summarised in Table 4.1. The median sampling depth was 15 m and 80% of the sites were between 5 and 63 m. the reference data base included a broad range of physical substrates, including sites with between 0% to > 90% of each of sand, silt and clay. Total organic carbon ranged from 0.01 - 12.85%, with a median of 2.16% and the mineralogical range of the sediment is summarised in Table 4.1.

Metal levels varied considerably, the median levels of several metals, chromium, nickel, copper, cadmium and zinc exceeding the Ontario lowest effect level (Table 2.2). Three metals had values that exceeded the severe effect level (Table 2.2), chromium, nickel and arsenic, and 10% of the sites exceeded the severe effect level for nickel.

Table 4.1. Summary statistics for environmental variables at 252 Great Lakes reference sites.

	Average	Median	Maximum	Minimum	10th percentile	90th percentile
Water Depth (m)	24.8	15	102	1	5	63
Gravel (%)	0.6	0	36.2	0	0	0.7
Sand (%)	37.0	18.8	99.8	0	1.2	95.4
Silt (%)	31.6	32.3	95.7	0	0	65.9
Clay (%)	30.7	29.9	91.1	0.1	2.0	63.8
SiO_2 (%)	60.10	59.07	93.30	19.84	46.06	76.79
TiO_2 (%)	0.52	0.51	1.92	0.05	0.22	0.76
Al_2O_3 (%)	10.06	10.48	15.02	2.39	5.46	13.59
Fe_2O_3 (%)	4.46	4.28	21.70	0.44	1.77	7.28
MnO (%)	0.16	0.10	3.29	0.01	0.04	0.23
MgO (%)	2.71	2.30	8.35	0.28	1.10	4.69
CaO (%)	5.92	3.38	32.15	0.49	1.56	12.64
Na_2O (%)	1.62	1.58	3.53	0.12	0.68	2.75
K_2O (%)	2.35	2.36	4.18	0.74	1.51	3.10
P_2O_5 (%)	0.17	0.16	0.56	0.02	0.07	0.29
Total Nitrogen (µg/g)	2199.8	1460.5	12528.0	0.0	421.6	5202.7
Total Phosphorus (µg/g)	684.5	563.5	7180.0	20.0	174.5	1176.5
Loss On Ignition (%)	11.39	10.52	38.72	0.59	3.17	20.12
Total Organic Carbon (%)	2.16	1.75	12.85	0.01	0.39	4.88
V (µg/g)	47	38	159	4	14	88
Cr (µg/g)	45	40	123	4	12	83
Co (µg/g)	12	11	49	1	3	21
Ni (µg/g)	45	33	348	1	10	79
Cu (µg/g)	26	25	91	0	5	51
Zn (µg/g)	112	96	482	5	27	207
As (µg/g)	10	5	115	3	3	24
Cd (µg/g)	1	1	4	0	0	2
Pb (µg/g)	42	35	153	1	16	77
Water pH	8.0	8.0	11.9	6.3	7.3	8.7
Dissolved Oxygen (mg/l)	9.3	9.1	15.0	4.9	6.4	12.4
Alkalinity (mg/l)	78.3	75.7	118.0	38.1	45.0	108.9
Total Phosphorus (mg/l)	0.0126	0.0096	0.1094	0.0014	0.0045	0.0216
Total Kjeldahl Nitrogen (mg/l)	0.186	0.145	1.480	0.031	0.075	0.324
Nitrate-nitrite (mg/l)	0.221	0.249	0.416	0.001	0.019	0.349

These summary data provide an indication of the range of environmental conditions captured by the 252 reference sites. They also set the bounds of applicability for which the biological targets being set for community structure and toxicity endpoints can be applied. Test sites within this range can be assessed with confidence by the targets described below. Any site outside the range should be compared to the reference data base with caution.

4.2 Community Structure throughout the Great Lakes Basin

4.2.1 Taxonomic Composition

The 252 sample reference database includes 162 taxa, a complete taxa list is presented in the appendices (Appendix 8.2). The majority of taxa were not abundant and only 16 taxa contributed more than 1% each of the total number collected (Figure 4.1). In fact, the 10 most abundant taxa (Figure 4.1) comprised more than 70% of all the organisms found. The most diverse groups of organisms identified were the Chironomidae (midge larvae) with 44 genera, the Oligochaeta (worms) with 40 species identified (19 Tubificidae, 18 Naididae and 3 Lumbriculidae) and the Mollusca (snails and clams) with a total of 38 species identified (20 Gastropoda, 18 Bivalvia). The most common taxa, occurring at more than 50 % of the sites, were the chironomid, *Procladius* spp., the sphaerid clam, *Pisidium casertanum,* and the amphipod, *Diporeia hoyi*. The chironomids, *Heterotrissocladius* spp., *Chironomus* spp and *Tanytarsus* spp., occurred at more than 40% of the sites. The more abundant (density) and common (frequency of occurrence) species are shown in Table 4.2, ordered by abundance; the contribution of each taxon to the overall structure of the community described by ordination (section 4.2.2) is indicated by the correlation co-efficient from principal axis correlation. The most interesting feature of the distribution of the most abundant species, is that of the ten most abundant, only half are the most common (e.g., *Diporeia hoyi, Pisidium casertanum, Procladius* spp., *Tanytarsus* spp. and *Chironomus* spp.). The distribution of the three most common oligochaete species, *Stylodrilus heringianus, Potamothrix vejdovskyi* and *Spirosperma ferox,* was slightly more restricted with each species found at 30, 20 and 26.6% of the sampled sites, respectively. Two other oligochaete species were more widely distributed, the tubificid worm, *Limnodrilus hoffmeisteri* (34.9% of the sites), and the naidid worm, *Vejdovskyella intermedia* (31.7% of sites). However, most notable is that the second and third most abundant species, the recent exotic invaders *Dreissena polymorpha* and *D. bugensis,*, are the 23rd and 88th most common species, respectively. This reflects their very recent arrival in the lakes and suggests that their distributional patterns are not yet final; this can be attributed to dispersal processes more than local environmental conditions.

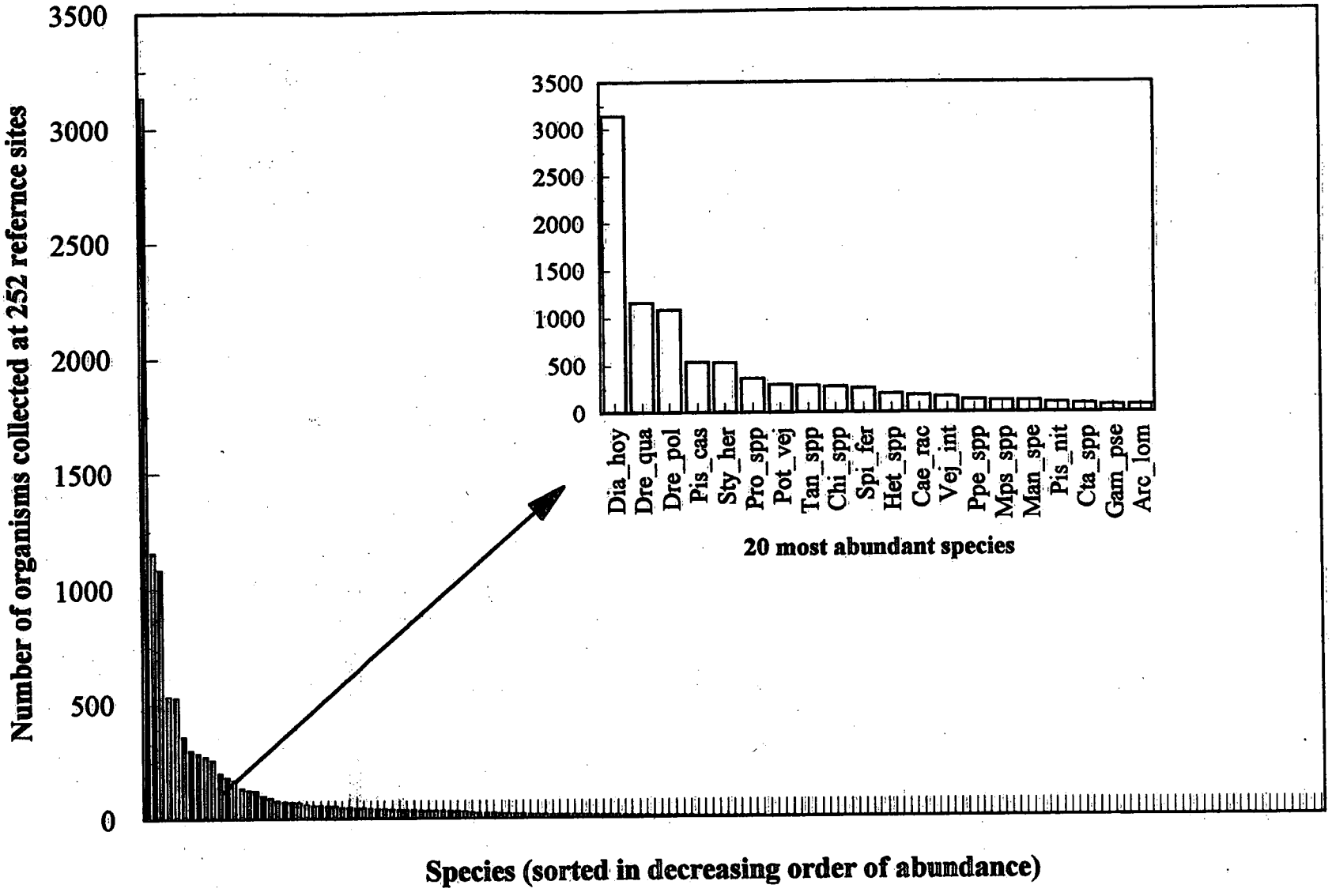
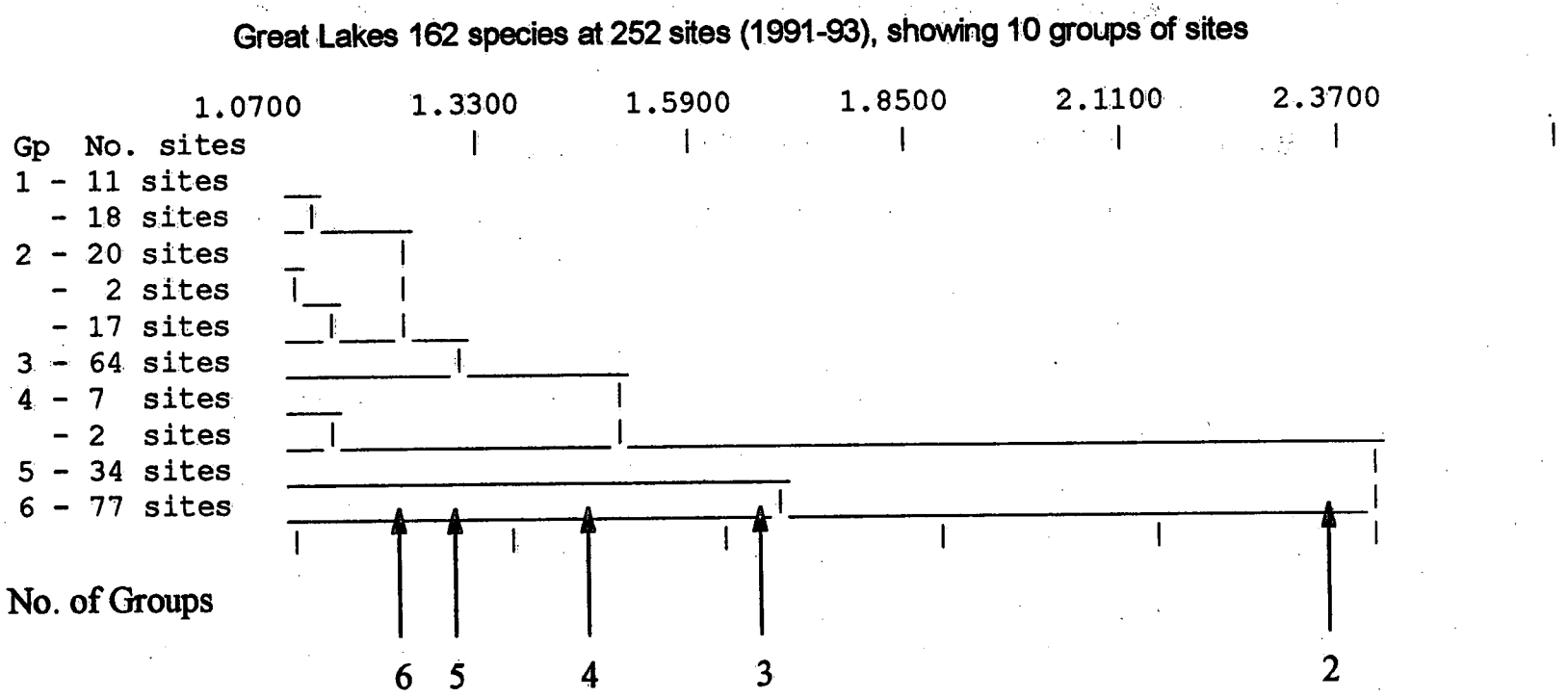


Figure 4.1. Abundance of taxa collected at 252 reference sites (see Table 4.2 for list of abbreviations)



Number of Sites

2 groups	141				111	
3 groups	141				34	77
4 groups	132			9	34	77
5 groups	68		64	9	34	77
6 groups	29	39	64	9	34	77

Figure 4.2. Dendrogram of species groups formed from cluster analysis of 252 Great Lakes reference sites, with a table of site occurrence at different group levels

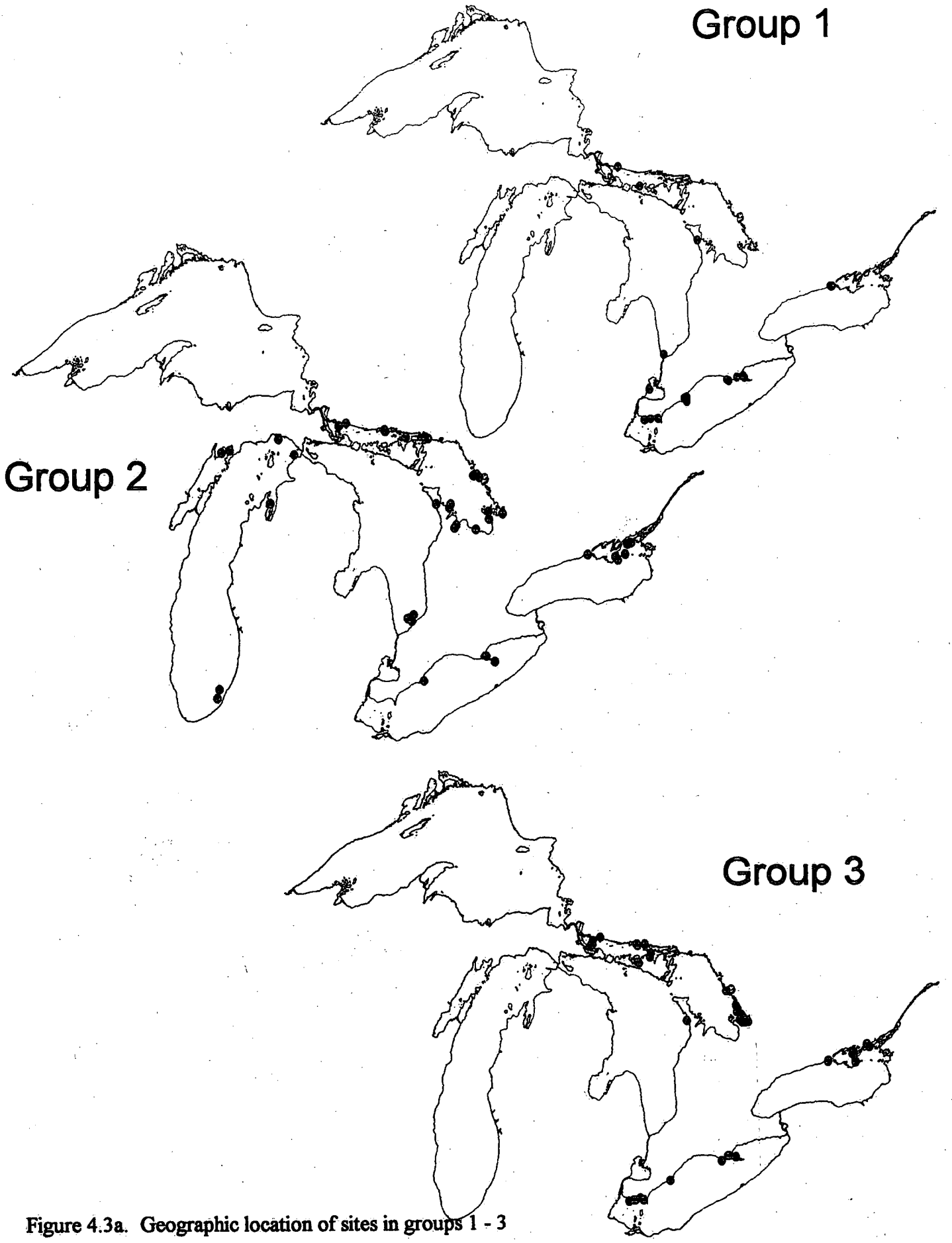


Figure 4.3a. Geographic location of sites in groups 1 - 3

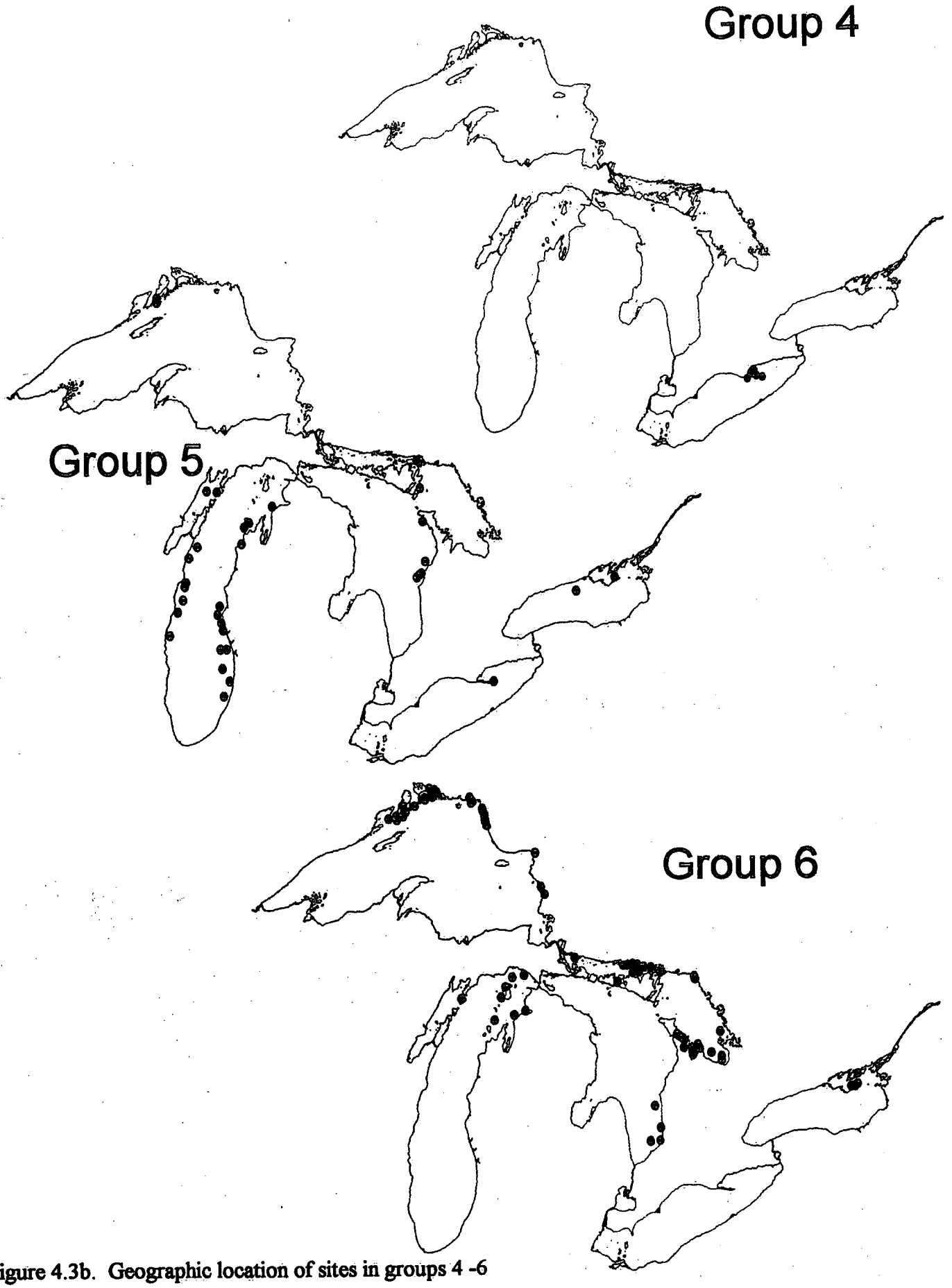


Figure 4.3b. Geographic location of sites in groups 4 -6

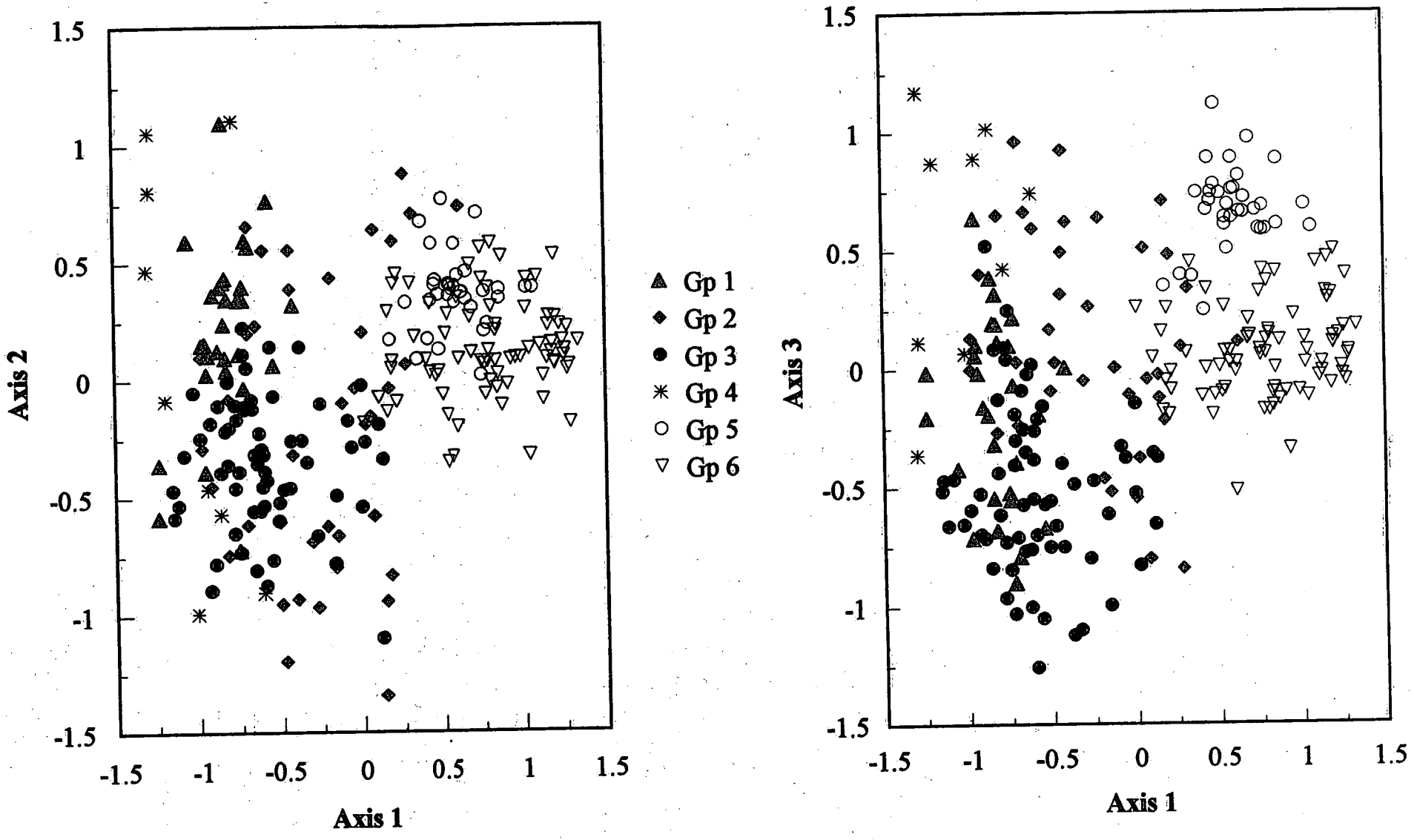


Figure 4.4. Multii dimensioinal scaling ordination of species level data in 3 dimensions of 252 Great Lakes reference sites (stress = 0.1905). Sites are identified as belonging to 1 - 6 groups formed by cluster analysis.

Table 4.2. Ranking of more abundant and common taxa (with abbreviations) at 252 Great Lakes reference sites and contribution of taxa to ordination structure as indicated by the correlation co-efficient (r) from principal axis correlation.

Taxa	Abundance ranking	Occurrence ranking	Correlation 'r' with ordination structure
Diporeia hovi (Dia hov)	1	3	0.607
Dreissena bugensis (Dre qua)	2	88	0.358
Dreissena polymorpha (Dre pol)	3	23	0.372
Pisidium casertanum (Pis cas)	4	2	0.414
Stylodriulus heringianus (Sty he)r	5	11	0.534
Procladius spp. (Pro spp)	6	1	0.583
Potamothrix vejdovskyi (Pot vej)	7	16	0.267
Tanytarsus spp (Tan spp)	8	6	0.288
Chironomus spp. (Chi spp)	9	5	0.424
Spirosperma ferox (Spi fer)	10	13	0.247
Heterotrissocladius spp (Het spp)	11	4	0.405
Caecidota racovitzai (Cae rac)	12	28	0.306
Vejdovskyella intermedia (Vej int)	13	10	0.253
Polypedium spp (Ppe spp)	14	8	0.276
Micropsectra spp (Mps spp)	15	36	0.063
Manayunkia speciosa (Man spe)	16	14	0.322
Pisidium nitidum (Pis nit)	17	26	0.271
Cladotanytarsus spp (Cta spp)	18	20	0.213
Gammarus pseudolimnaeus (Gam pse)	19	27	0.290
Arcteonais lomondi (Arc lom)	20	12	0.167
Valvata tricarinata (Val tri)	27	18	0.354
Aulodrilus pigueti (Aul pig)	31	15	0.391
Amnicola limosa (Amn lim)	44	31	0.362
Helobdella stagnalis (Hel sta)	67	30	0.379

4.2.2. Classification of community assemblages

The amount of data (162 taxa at 252 sites) made multivariate analysis most appropriate for describing patterns at the community level. Two methods of pattern analysis were used to describe structure in the data. First, cluster analysis was used to explore similarity of sites with regard to the taxa present and to establish which groups of sites represented different community assemblages of organisms. From this analysis, a dendrogram was produced for all 252 sites (Appendix 8.3); however, in the interest of clarity, we have provided a dendrogram showing only 10 groups of sites (Figure 4.2).

The reference sites first split into two groups: a group of 111 sites (Gps 5 and 6) which

represent the upper Great Lakes (Superior, Michigan, the North Channel and parts of Georgian Bay) and a second group (Gps 1 -4) of 141 sites representing the lower Great Lakes (Erie, Ontario) and parts of Georgian Bay. Sites in the upper Great Lakes group further divide into two large groups: 34 sites found in Lake Michigan (Gp 5) and 77 sites (Gp 6) which include all but two of the Lake Superior sites, a third of the Georgian Bay sites and almost half of the North Channel sites. Further groups of sites are formed by separation of the lower Great Lakes sites into Groups 1 - 4. We used these six groups (Figure 4.2), the divisions beyond this were into two small groups of two sites each (in Gp 2 and Gp 4 - Figure 4.2) suggesting that there is no further underlying structure in the data. The geographic distribution of sites in the six groups is shown in Table 4.3 and Figure 4.3.

Table 4.3. The number of reference sites present from different lake basins in each of six community groups formed from cluster analysis.

Lake	Number of reference sites in each cluster group						
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Total
Erie	17	3	11	9	1	0	41
Ontario	7	6	9	0	2	4	28
St Clair	1	0	0	0	0	0	1
Huron	2	4	1	0	6	4	17
Georgian Bay	0	11	32	0	0	18	61
North Channel	2	8	11	0	1	14	36
Michigan	0	7	0	0	22	8	37
Superior	0	0	0	0	2	29	31
Total	29	39	64	9	34	77	

Ordination techniques are a class of methods for examining similarity between sites based on species composition. Ordination is a method of analysis that can be used to reduce the 162 taxa variables to a reduced number of new axes (usually two or three) and allows sites to be examined in ordination space. This analytical approach provides: a visual representation of the differences between sites (i.e., sites with greater similarity in number and type of taxa are located closer together); an assessment of the groups formed by cluster analysis (i.e., if the groups are truly different they will be distinguishable in ordination space); a determination of the taxa contributing

most to the community structure, including a statistically measurable correlation; and, a determination of the correlation of a second environmental data matrix with the species matrix to provide an explanation of the relationship between community and environmental structure.

Ordination of the data matrix (252 sites x 162 taxa) using hybrid multidimensional scaling ordination 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 vs. Dimension 2 and Dimension 1 vs. Dimension 3 plots (Figure 4.4) in which the six group solution from cluster analysis is diagramatically represented. Two major points are noteworthy from the results of this ordination. First, the groups formed by cluster analysis have spatial integrity and boundaries; they are not distributed throughout the entire ordination space. Secondly, there is considerable overlap between some of the groups in ordination space. This suggests that while the communities can be characterised based on their species composition, there is also some similarity among the various community types. We believe that the communities constructed by this type of analysis represent centroids along a continuum of species distributions and are more appropriately described as assemblages of taxa rather than distinct communities. Use of principal axis correlation shows that 51 of the taxa contributing to the community groups are significantly related ($P < 0.01$) with the ordination structure (using 100 random iterations). We have shown the relationship of the 12 taxa contributing the most to the ordination axes ($r > 0.35$ from principal axis correlation - Table 4.4) of the reference sites (Figure 4.5), the direction of the arrow shows the contribution of the taxa to the location of sites in ordination space. For the sake of clarity the individual sites are not represented (as in Figure 4.4); instead, the 90% confidence limit around the community centroid is shown. In addition we have shown the abundance of these 12 taxa in each of the six groups (Table 4.4).

Group 1 is characterised by *Chironomus* spp., *Dreissena* spp., the other common chironomid, *Procladius* spp., the sphaerid clam, *P. casertanum*, and the leech *Helobdella stagnalis*. Numbers of *Chironomus* spp. in group 1 are significantly greater ($P < 0.05$) than in the other five types of community. This community group contains 29 sites, most located in western and central Lake Erie.

Group 2 is characterised by the fingernail clam *P. casertanum* and the amphipod , *D. hoyi*; this community type is indicative of a more oligotrophic lake environment. The first ordination space dimension (Axis 1) appears to represent a trophic and geographic gradient. The communities located more to the left in ordination space (Gps 1 and 4) tend to belong to the more mesotrophic lake environment of the lower Great Lakes; and groups to the right (Gps 5 and 6) represent the more oligotrophic upper Great Lakes. Group 2 is also more diverse than the other community groups but is the least spatially defined. While the majority of sites in this group are located in Georgian Bay, the group also includes sites from Lakes Erie (eastern basin), Ontario, Huron, Michigan and the North Channel.

Table 4.4. Mean and SD (in parentheses) of 12 taxa in six community groups formed from cluster analysis of 252 reference sites. Taxa are those most correlated ($r > 0.35$) with the ordination structure of 252 reference sites (No. core - 34.2cm^2).

Taxa	Gp 1 29 sites	Gp 2 39 sites	Gp 3 64 sites	Gp 4 9 sites	Gp 5 34 sites	Gp 6 77 sites
Chironomus spp	5.7 (5.8)	0.9 (3.1)	0.8 (1.3)	1.3 (1.8)	0.0 (0.0)	0.1 (0.4)
Heterotrissocladius spp	0.2 (1.1)	0.8 (2.5)	0.0 (0.0)	0.4 (0.7)	1.2 (1.7)	1.6 (1.8)
Procladius spp	1.5 (1.9)	1.9 (2.3)	3.2 (2.7)	2.0 (1.4)	0.1 (0.3)	0.2 (0.6)
Diaporeia hoyi	0.0 (0.1)	2.8 (6.2)	0.5 (2.1)	0.0 (0.0)	65.1 (41.8)	10.4 (5.1)
Amnicola limosa	0.1 (0.3)	0.5 (1.2)	0.0 (0.2)	0.6 (0.7)	0.0 (0.0)	0.0 (0.2)
Valvata tricarinata	0.3 (0.4)	0.7 (1.9)	0.1 (0.2)	1.7 (2.0)	0.0 (0.0)	0.0 (0.0)
Dreissena polymorpha	1.8 (7.2)	0.0 (0.1)	0.0 (0.0)	122.8 (181.2)	0.0 (0.0)	0.0 (0.0)
Dreissena bugensis	5.1 (7.7)	0.2 (1.0)	0.2 (0.6)	101.3 (78.1)	0.0 (0.1)	0.1 (0.7)
Pisidium casertanum	2.5 (2.8)	4.4 (8.8)	1.0 (1.8)	0.8 (0.8)	5.0 (8.4)	0.7 (1.1)
Stylodrilus heringianus	0.0 (0.0)	0.8 (1.8)	0.0 (0.0)	0.0 (0.0)	10.2 (8.9)	2.0 (3.8)
Aulodrilus pigueti	0.5 (0.7)	0.2 (0.6)	0.4 (0.8)	0.2 (0.4)	0.0 (0.0)	0.0 (0.0)
Helobdella stagnalis	0.2 (0.3)	0.0 (0.2)	0.0 (0.2)	0.3 (0.3)	0.0 (0.0)	0.0 (0.0)

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

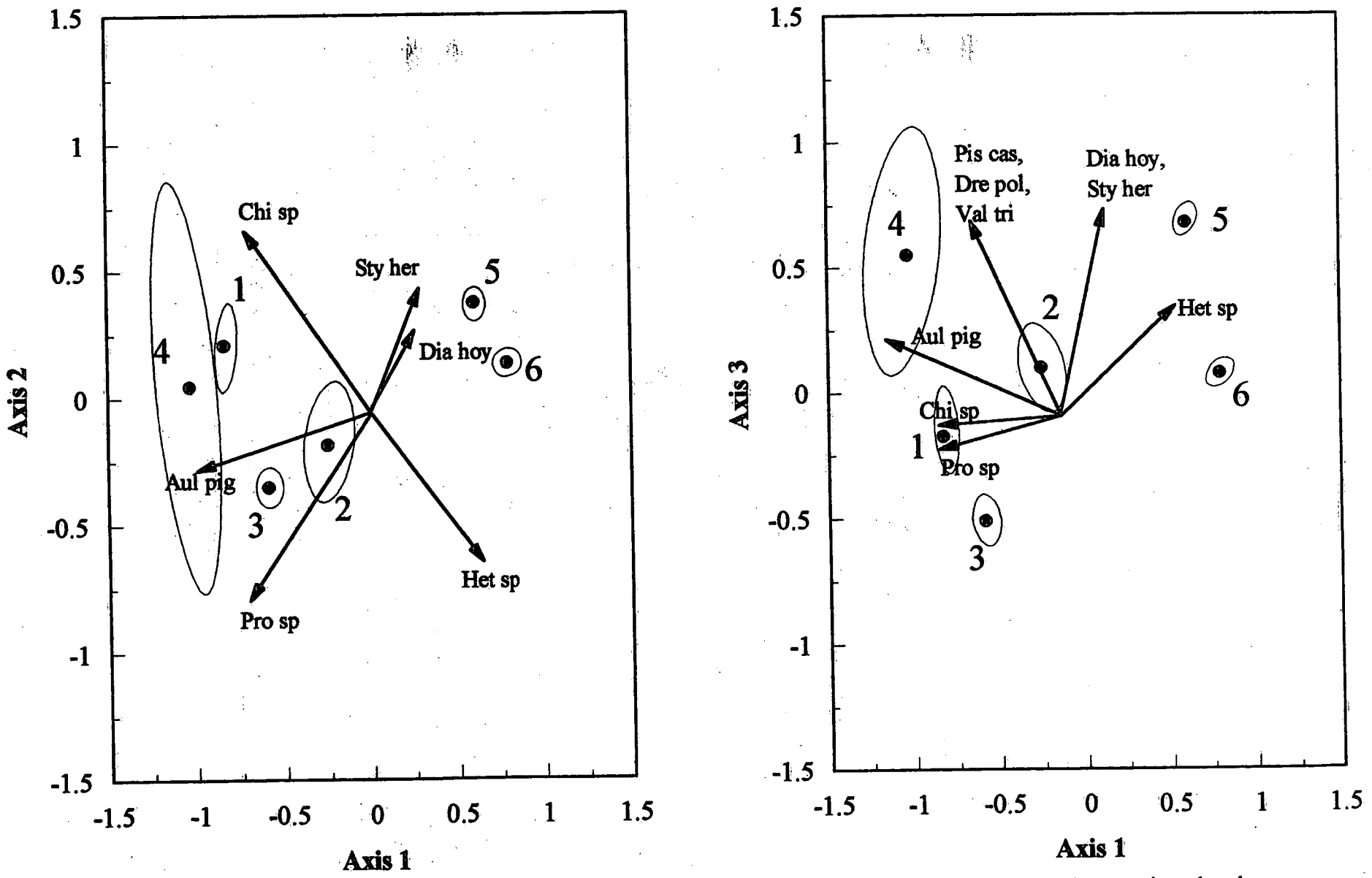


Figure 4.5. Multi-dimensional scaling ordination of species level data in three dimensions of 252 Great Lakes reference sites showing group centroids from cluster analysis and 90% probability ellipses for each group of sites (stress = 0.19048). The contribution of individual taxa and the axis are illustrated.

Group 4 consists of only 9 sites in the eastern basin of Lake Erie dominated by very high numbers of the exotic species *D. polymorpha* and *D. bugensis*. However, this group is similar to group 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 4.5), both groups 1 and 4 are typical of the more mesotrophic Lake Erie (Table 4.3).

The last two groups, 5 and 6, both represent a *Diporeia hoyi/Stylodrilus heringianus* assemblage. The major differences between the two groups are the abundances of these two species and the presence of the oligotrophic chironomid, *Heterotrissocladius spp*. Group 5 has a higher abundance of both *Diporeia* and *Stylodrilus* and *Heterotrissocladius* is less numerically important. This community is primarily found in Lake Michigan. Group 6 contains 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 North Channel sites (Table 4.3, Figure 4.3).

These six groups of sites with their different relative abundances of benthic invertebrate taxa form the six different reference states for the Great Lakes. Selection of which of these states is most appropriate for any test site requires the understanding of factors that determine the distribution of benthic organisms.

4.2.3 Relationship with environmental variables

Only 26 environmental variables, those less modified by anthropogenic activity, were examined as potential predictor variables even though 43 variables were measured at each site (Table 3.3). To establish the relationship between the biological structure in the data set (i.e., the species ordination matrix) and the 26 environmental variables, we used principal axis correlation. Eighteen of the 26 variables were significantly correlated ($P < 0.01$) with the species ordination matrix (Table 4.5) and we have plotted the 10 variables with an "r" value > 0.35 (Figure 4.6) in species ordination space showing the vectors for the environmental variables. The first dimension in species' ordination space is clearly related to depth and geographic location (latitude and longitude) and the organic matter in the sediment. The second and third dimensions relate more to sediment and water characteristics. For example, when the vectors for taxa (Figure 10) and habitat variables (Figure 12) are compared, the occurrence of molluscs is strongly related to high

alkalinity and calcium. The presence of *Procladius* appears to relate to high TOC and total nitrogen values whereas the mesotrophic worm, *A. pigueti,* occurs wherever TOC, silt and LOI are high. Finally, *D. hoyi* and *S. heringianus* are strongly related to deeper sites.

Table 4.5. Relationship between environmental variables and invertebrate fauna as indicated by either principal axis correlation (PCC) or stepwise discriminant function analysis (stepwise).

Variable	r (PCC)	Partial r^2 (stepwise)	Prob. (stepwise)
Depth - water	0.7458	0.519	0.0001
Latitude (Lat)	0.6496	0.405	0.0001
Longitude (Lon)	0.5639	0.085	0.0007
Alkalinity (Alk)- water	0.5183	0.196	0.0001
Calcium oxide (CaO) - sediment	0.4650	0.064	0.0066
Total nitrogen (TN) - sediment	0.4131	0.216	0.0001
Total organic carbon (TOC) - sediment	0.4126		
Loss on ignition (LOI) - sediment	0.3982		
Aluminium oxide (Al_2O_3) - sediment	0.3919		
Percent silt (Sil) - sediment	0.3766		
Silica oxide (SiO_2) - sediment	0.3449	0.056	0.0170
Percent sand (San) - sediment	0.3298		
Potassium dioxide (K_2O) - sediment	0.3138	0.098	0.0001
Total phosphorus (TP) - sediment	0.2557		
Magnesium oxide (MgO) - sediment	0.2436	0.089	0.0004
Sodium dioxide (Na_2O) - sediment	0.2333		
Percent clay (Cly) - sediment	0.2282		
Particle size 75th percentile (P75) - sediment	0.2226		
pH - water	0.1815 (ns P > 0.01)	0.065	0.0063
Manganese oxide (MnO) - sediment	0.1362 (ns P > 0.01)	0.054	0.0216

The identification of variables which are the most closely related to the community groups, rather than individual sites, can be conducted using stepwise discriminant function analysis, which attempts to minimise covariance between variables. Using this approach, eleven variables were identified as being the best related to the group structure (Table 4.5). Two of these variables, pH and MnO, were not significantly correlated with the species' ordination matrix created using principal axis correlation. The variables selected first by stepwise discriminant analysis (Table 4.5) are those that tend to have a higher correlation with the species ordination matrix from principal

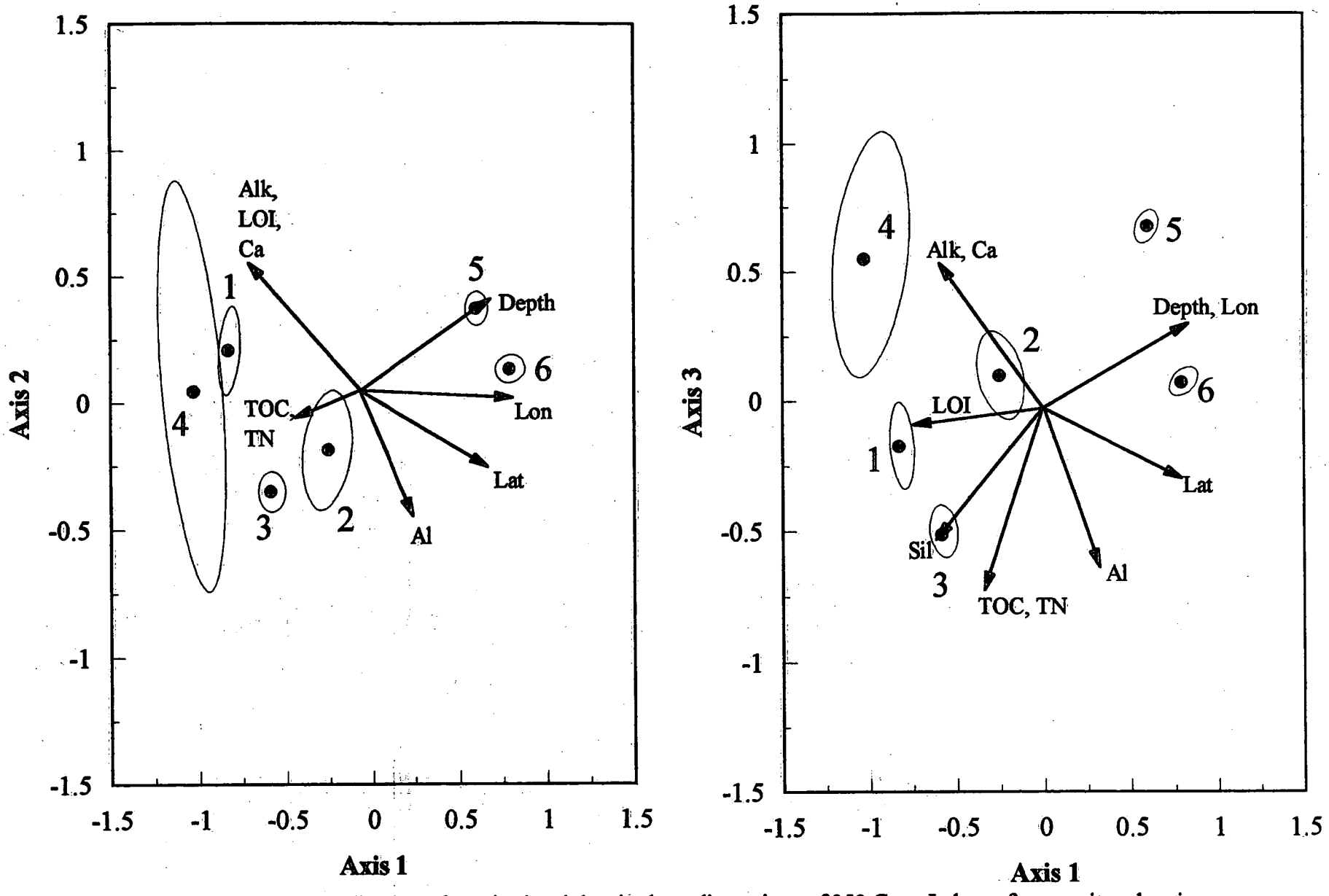


Figure 4.6. Multi dimensional scaling ordination of species level data in three dimensions of 252 Great Lakes reference sites showing group centroids from cluster analysis and 90% probability ellipses for each group of sites (stress = 0.19048). The contribution of environmental variables and the axis are illustrated.

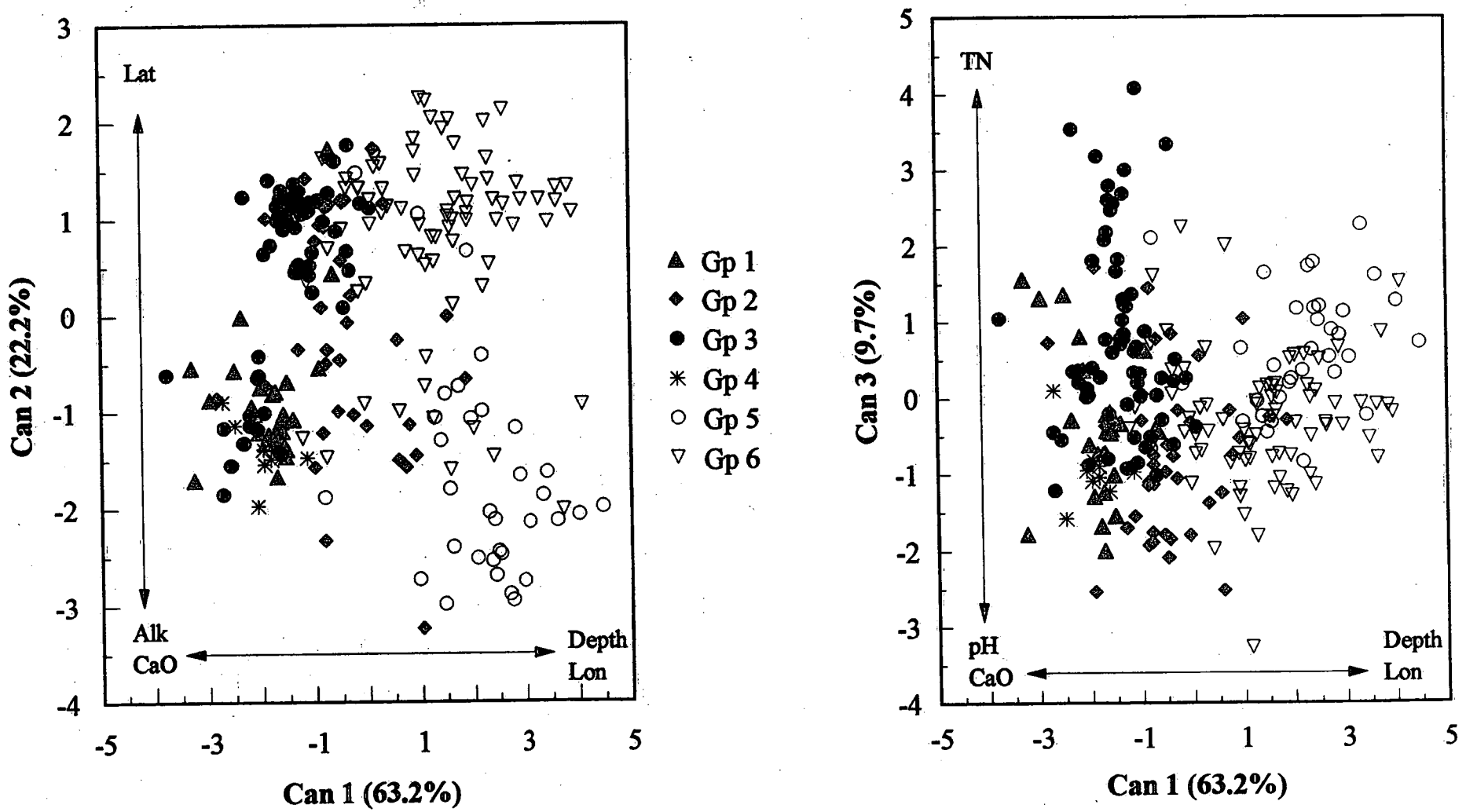


Figure4.7. Discriminant analyses of 252 Great Lakes reference sites using 11 environmental variables. The sites are identified as belonging to one of six groups formed from cluster analysis of species level data.

axis correlation. We have plotted the sites in discriminant space (Figure 4.7) with the eleven variables selected by the stepwise analysis (Table 4.5). The discriminant plot shows separation between the sites. For example, the deeper sites representing community groups 5 and 6, are oriented to the left of the figure on the first discriminant function. There is considerable overlap in some groups. For example, group 4 cannot be discriminated from group 1, suggesting that the difference between these two site groups is mostly attributed to biological differences, perhaps the recent colonisation of sites by *Dreissena* (zebra mussels).

4.2.4 Building predictive models of community structure

The objective of establishing the relationship between the habitat attributes of the reference sites and species composition, is to develop a model that allows a prediction to be made of the type of invertebrate assemblage that should occur at any unimpaired site in the Great Lakes basin. The comparison of the sampled assemblage with the assemblage predicted to be present allows an assessment of degree of impairment of the benthic invertebrate assemblage to be made.

Table 4.6. Error rate estimates for species level discriminant models constructed using three sets of variables.

	Stepwise discriminant model	Principal axis correlation model	Optimal Model
Variables used	11 - (depth, lat,lon, alkw, pH, TN, K_2O CaO, MgO, MnO, SiO_2)	18 - (depth, lat,lon, alkw, TN, TP, TOC, LOI, CaO, Al2O3, SiO2, K2O, MgO, Na2O, Sil, San, Cly, Psz75)	12 - (depth, lat,lon, alkw, pH, TN, TOC, K_2O CaO, MgO, MnO, SiO_2)
Cross validation Error rate for 252 sites	32.4 %	35.8%	30.1%
Error rates (%) for 20 site subset			
Gp 1 (n=2)	0	0	0
Gp 2 (n=3)	0	33	0
Gp 3 (n=5)	40	60	40
Gp 4 (n=1)	0	0	0
Gp 5 (n=3)	0	0	0
Gp 6 (n=6)	33	33	33
Average error rate (cross validation)	12%	21%	12%

The predictive model is based on discriminant function analysis (DFA). This is a statistical method which enables one to distinguish between two or more "groups" using a set of discriminating variables. In this case, the "groups" are those established based on species composition and are defined as *community types*. The *community types* are the numeric guidelines for the invertebrate community component of the biological sediment guidelines. The *discriminating variables* are a set of habitat attributes which are minimally affected by human activity. Discriminant function analysis distinguishes between these groups by forming one or more linear combinations (discriminant functions) of the discriminating variables used in the analysis to maximise the separation of the groups. This allows sites to be classified with a set of classification variables. Once a set of variables is found which provides a satisfactory discrimination for sites with known group membership (reference data set), a set of classification functions can be derived that will permit the classification of new sites with unknown memberships. One of the earliest applications of this approach to relating biological groups to environmental data was by Green and Vascotto (1978).

In the case of the Great Lakes reference data base, the discriminant model was built using three variable selection processes: (1) variables identified by stepwise discriminant analysis; (2) variables identified through principal axis correlation with the species ordination matrix; and, (3) an iterative process using variables from both stepwise discriminant analysis and PCA. The performance of each model was assessed based on the error rates for the classification in the SAS procedure DISCRIM. The estimate of error count is calculated by applying the classification criterion derived from a training sample to a test set and counting the number of misclassified observations. The group-specific error rate is the proportion of misclassified observations in the group. The total error rate is estimated through a weighted average of the individual group-specific error rate estimates whereby the prior probabilities are used as the weights. The results (Table 4.6) for three models show little difference in total error rates between the stepwise (error rate 32.4%) and optimal model (error rate 30.1%) which used the 11 stepwise variables plus total organic carbon (TOC). The model based on variables selected from principal axis correlation was less accurate with a total error rate of 35.8%.

A second independent approach was used to test each of the models. A total of twenty sites were removed from the reference data set. Sites were removed randomly from within each of the six groups to ensure that the test data set would represent each of the six community groups. The performance of the different models shows that the stepwise model was acceptable. Only four of the 20 sites were incorrectly predicted using the model and the overall error rate was 12%. The model which used 18 variables significantly correlated with ordination structure (from principal axis correlation) was less accurate; the total error rate using this model was 21%, six sites were incorrectly predicted. A number of alternative models were constructed using various combinations of variables identified by stepwise and principal axis correlation. The variables used in these models were selected as they were biologically meaningful e.g., particle size and variables associated with sources of carbon. None of these models showed any improvement over the stepwise model. The optimal iteration had the same error rate as the stepwise model (Table 4.6). The addition of further variables, such as particle size distribution and surrogates for food availability, resulted in higher error rates and were not considered further.

The optimal model uses 12 variables that are easily measured such as geographic location (latitude and longitude), simple sediment attributes (total organic carbon, total nitrogen and oxides of potassium, calcium, magnesium, manganese and silica), and general limnological conditions (water depth, alkalinity and pH of the water 0.5 m above the sediment water interface). We have selected this as the standard model, it predicts 88% of the sites to the correct group; this is a more than acceptable error rate and equal to or better than could be achieved using other models.

4.2.5 Alternate models

The models described above treat the entire Great Lakes basin as a single geographic unit. However, the site classification identified a strong spatial signal to the faunal group structure (Table 4.3, Figure 4.3). The dominance of geographic location could be masking other smaller spatial scale factors. Therefore, we investigated the structure of communities on an individual lake basis.

We also investigated the validity of using a coarser taxonomic resolution than species. Considerable effort is required to identify organisms to the level of species or genus. Based on

our experience, identification of individual organisms in a sample from the family level to the lowest taxonomic level (usually species or genus) at least doubles the processing time. Identification to the lowest taxonomic level also requires a level of taxonomic expertise that is often difficult to obtain.

4.2.5.1 Individual lake models

The classification of sites and development of predictive models was undertaken for each lake using the following number of sites: Lake Ontario - 28 sites; Lake Erie (including L. St Clair) - 42 sites; Lake Huron (including Georgian Bay and the North Channel) - 114 sites; Lake Michigan - 37 sites; and, Lake Superior - 31 sites.

The resulting number of groups formed from the invertebrate data matrices and the predictive models are presented in Table 4.7. The models for lakes Ontario, Erie and Huron have lower error rates than the all lake model (30.1% - Table 4.6), whereas the upper lake models (Michigan and Superior) do not perform as well. The major problem with the individual lake models is that in several cases the number of sites per group is small, of the 15 groups formed from all the lakes, seven have less than 10 sites. Small group size reduces the ability to distinguish impairment (Reynoldson 1996). Therefore, at present, we would recommend the use of the all lake model. Accumulation of more reference sites may allow the development of individual lake models.

Table 4.7. Predictive models formed for individual lakes.

Lake	No. sites	No. community types (groups)	Predictive model error rate (cross validation)	Number and type of predictor variables	
Ontario	28	3	14.5%	6	(Lat, Lon, Depth, Alk, TiO_2)
Erie	42	2	16.7%	4	(Lat, Depth, Sand, TiO_2)
Huron	114	3	12.4%	4	(Lat, Depth, Alk. K_2O)
Michigan	37	3	34.8%	4	(Lat, Depth, Silt, Clay)
Superior	31	4	38.2%	4	(Lat, pH, Fe_2O_3, SiO_2)

4.2.5.2 Taxonomic level

There have been ongoing discussions regarding the level of taxonomic detail required for bioassessment. Freshwater biologists have usually suggested that identification to the lowest taxonomic level, either the genus or the species level, is desirable. The supporting argument for this view is, that, as there is considerable variation in the responses of different species to environmental stress, it is important to differentiate responses at this level (Resh and Unzinger 1975). However, in the Rapid Bioassessment Protocols developed in the United States (Plafkin *et al.* 1989), identifications to the family level are usually recommended. In addition, comparisons of the effects of taxonomic level on identification of environmental stress have been documented in several papers in the marine benthic literature. In two papers, Warwick (1988, 1993) has shown that identification at the family, and even phyla, level were as effective as species level, in identifying pollution gradients. Warwick presents the argument that anthropogenic effects tend to modify communities at higher taxonomic levels than natural environmental variables; the latter tend to influence fauna by species replacement rather than species elimination. In an examination of 10 freshwater data sets Bowman and Bailey (1997) found that genus level identification did not provide a strikingly different description of community patterns than higher levels (e.g., family, order) of taxonomic identification. Multivariate methods of analyses can use all the organisms present in the invertebrate community, and thus, are more sensitive than other univariate or graphical methods of analyses. If taxonomic identification to the level of family is acceptable for the determination of stress, this observation is of considerable importance to agencies or organisations required to conduct bioassessments or biomonitoring as the cost-saving will be considerable. For the Great Lakes data base, we examined the performance of family level classification for the identification of site groups and in model development.

Distribution and abundance

A total of 39 families of invertebrates have been recorded in this study (Table 4.8). Of these families, three are very common (> 80%) in occurrence, i.e., the Chironomidae (midge larvae), the Tubificidae (worms) and the Sphaeriidae (fingernail clams). A further three families are also common (>50%) occurrence; i.e., the Naididae (worms), the Pontoporeiidae (amphipod)

and the Spongillidae (sponges). Almost half the families (18), are considered rare and occur at less than 10% of the sites.

Table 4.8 Distribution and abundance of invertebrate families found at 252 Great Lakes reference sites.

	No of sites found	% occurrence		Total No collected	% of total	% of total - Spongillidae
Chironomidae	237	94.05	Spongillidae	67267.3	82.50	
Tubificidae	228	90.48	Tubificidae	3465.2	4.25	24.28
Sphaeridae	223	88.49	Pontoporeiidae	3149.8	3.86	22.07
Naididae	170	67.46	Chironomidae	2113.9	2.59	14.81
Pontoporeiidae	141	55.95	Dreissenidae	1847.8	2.27	12.95
Spongillidae	136	53.97	Sphaeridae	1324.1	1.62	9.28
Valvatidae	87	34.52	Lumbriculidae	545.4	0.67	3.82
Lumbriculidae	80	31.75	Naididae	418.4	0.51	2.93
Enchytraeidae	76	30.16	Enchytraeidae	323	0.40	2.26
Sabellidae	61	24.21	Asellidae	272	0.33	1.91
Asellidae	58	23.02	Sabellidae	153.4	0.19	1.07
Hydridae	46	18.25	Valvatidae	142.4	0.17	1.00
Hydrobiidae	41	16.27	Gammaridae	100	0.12	0.70
Glossphoniidae	40	15.87	Planorbidae	67.4	0.08	0.47
Dreissenidae	39	15.48	Hydridae	62.2	0.08	0.44
Planorbidae	38	15.08	Ephmeridae	59	0.07	0.41
Leptoceridae	37	14.68	Hydrobiidae	44.4	0.05	0.31
Gammaridae	37	14.68	Leptoceridae	33.2	0.04	0.23
Ceratopogonidae	29	11.51	Physidae	26.2	0.03	0.18
Ephmeridae	28	11.11	Chaoboridae	24.8	0.03	0.17
Chaoboridae	26	10.32	Ceratopogonidae	24.4	0.03	0.17
Physidae	23	9.13	Glossphoniidae	21.2	0.03	0.15
Bithyniidae	13	5.16	Unionidae	13.4	0.02	0.09
Lymnaeidae	8	3.17	Erpobdellidae	9.2	0.01	0.06
Caenidae	8	3.17	Bithyniidae	8	0.01	0.06
Unionidae	7	2.78	Lymnaeidae	6.6	0.01	0.05
Sialidae	6	2.38	Hydroptilidae	3.4	0.00	0.02
Hydroptilidae	5	1.98	Taliridae	3	0.00	0.02
Erpobdellidae	4	1.59	Caenidae	2.8	0.00	0.02
Macrobiotidae	2	0.79	Sialidae	2	0.00	0.01
Viviparidae	1	0.40	Phryganeidae	1	0.00	0.01
Baetiscidae	1	0.40	Macrobiotidae	0.8	0.00	0.01
Helicopsychidae	1	0.40	Helicopsychidae	0.4	0.00	0.00
Limnophilidae	1	0.40	Limnophilidae	0.4	0.00	0.00
Molannidae	1	0.40	Empididae	0.4	0.00	0.00
Phryganeidae	1	0.40	Viviparidae	0.2	0.00	0.00
Empididae	1	0.40	Baetiscidae	0.2	0.00	0.00
Pyralidae	1	0.40	Molannidae	0.2	0.00	0.00
Taliridae	1	0.40	Pyralidae	0.2	0.00	0.00

The most abundant family was the family containing the freshwater sponges (Spongillidae) representing over 80% of the organisms found. This group is frequently excluded from invertebrate enumerations because they are colonial animals and are difficult to compare with other organisms. The other most abundant families were the Tubificidae and Pontoporeiidae, representing >20% of total animals found (excluding sponges). Two other families were abundant (>10% of the total), i.e., 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).

Classification and Ordination

Sites were classified using cluster analysis and a final number of groups was established by examination of both the dendrogram tree structure and the distribution of sites in ordination space (Figure 4.8). Five groups of sites were selected from the data; further subdivision resulted in groups with a small number of sites. For example, a sixth group was formed by the splitting of group 5 (Figure 4.8) and, at the seventh split (8 groups), one group of 13 sites, one group of three and one group of nine sites were and species levels were utilized, there is a strong spatial component to the site groups (Table 4.9).

Table 4.9 Geographic distribution of sites in five reference groups formed with family level data.

Lake	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5
Erie	7	22	0	0	12
Georgian Bay	36	3	17	0	5
Huron	5	3	2	6	1
Michigan	3	7	7	20	0
N. Channel	24	1	11	0	1
St Clair	0	1	0	0	0
Ontario	7	1	4	1	15
Superior	1	0	28	1	0

Group 1 is characterized by lower numbers of organisms (Figure 4.9) with the dominant organisms being the chironomids. However, the chironomids are a widespread family and are

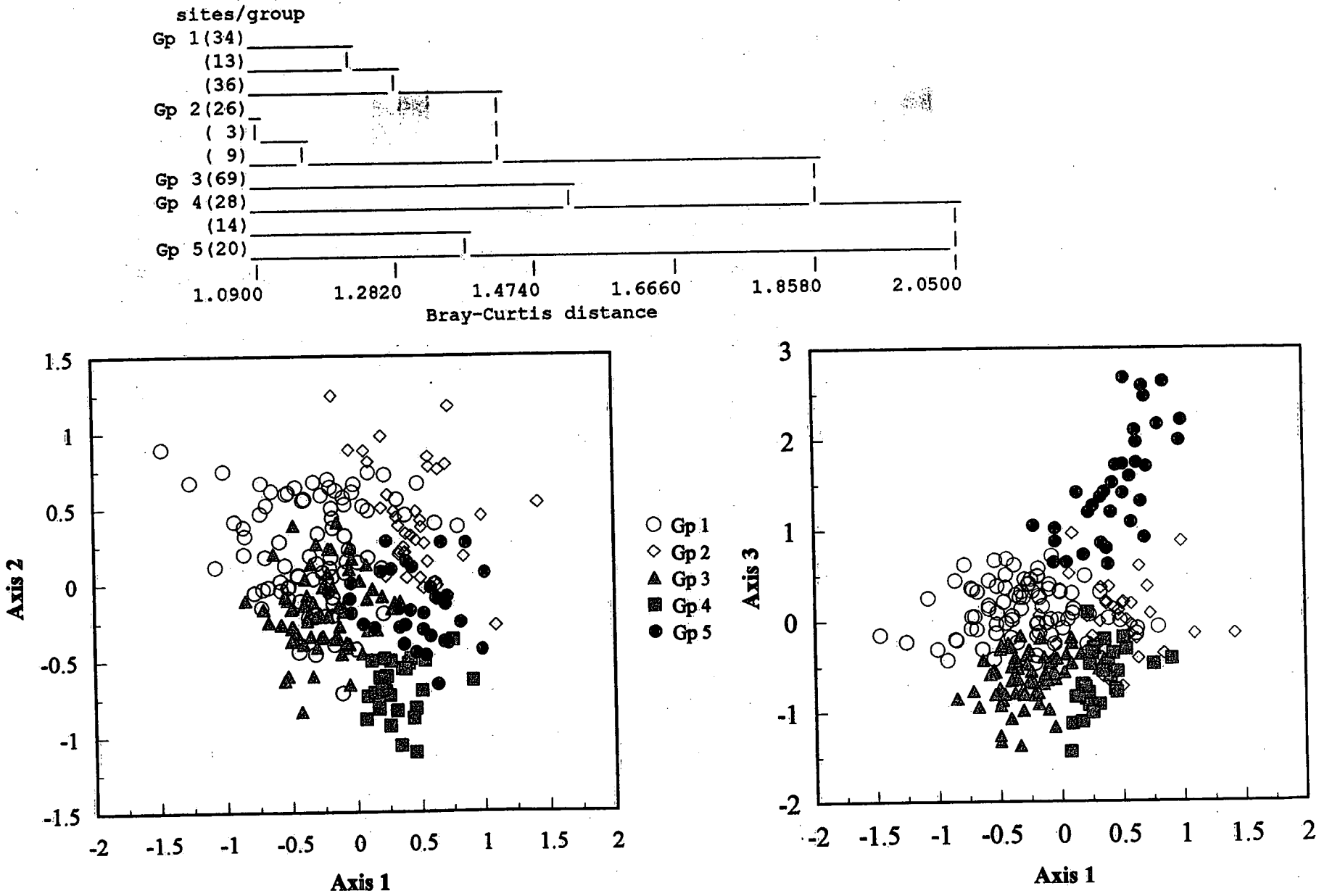


Figure 4.8. Dendrogram of 252 Great Lakes reference sites using family level taxonomic data and showing 10 groups and the number of sites in each group.

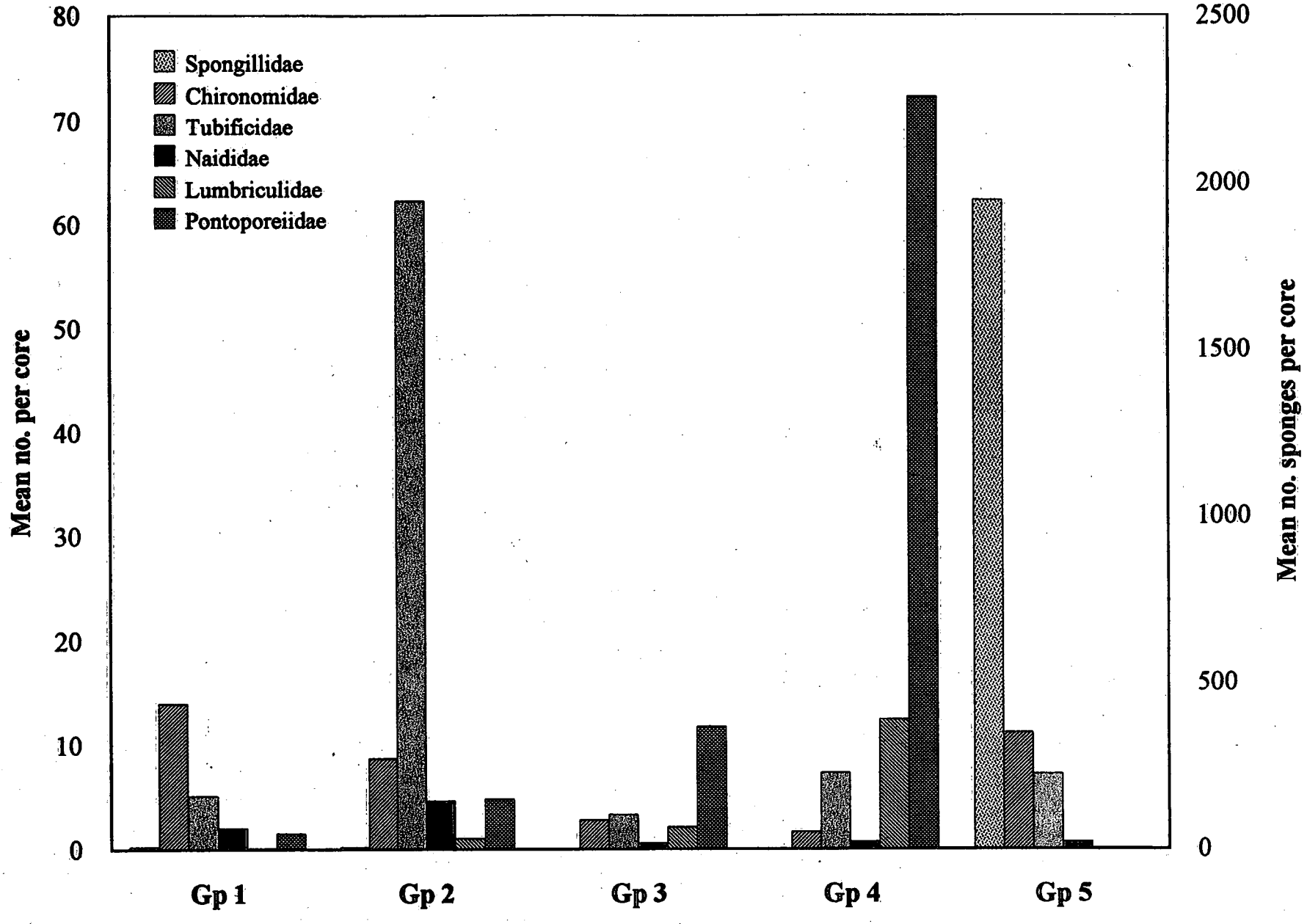
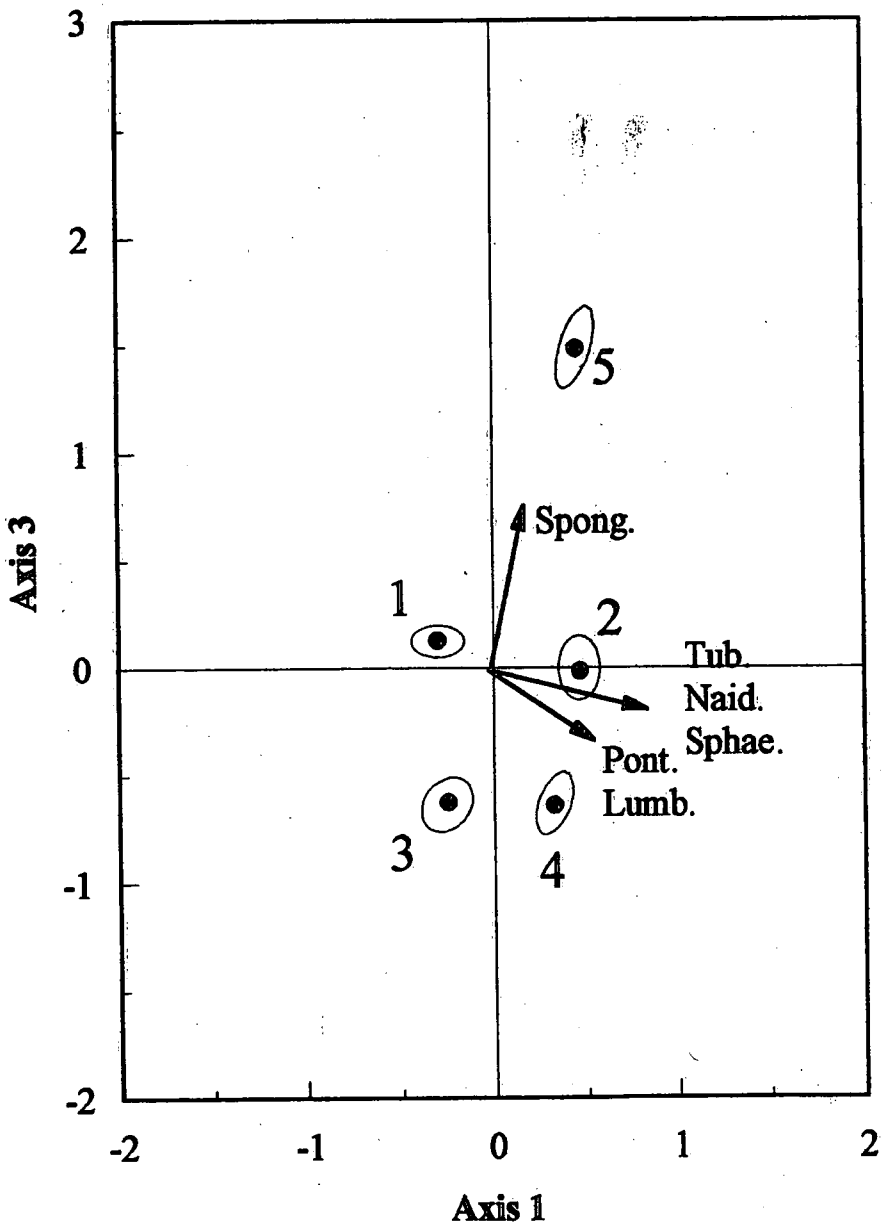


Figure 4.9. Abundance of selected families in five groups formed from 252 Great Lakes reference sites.



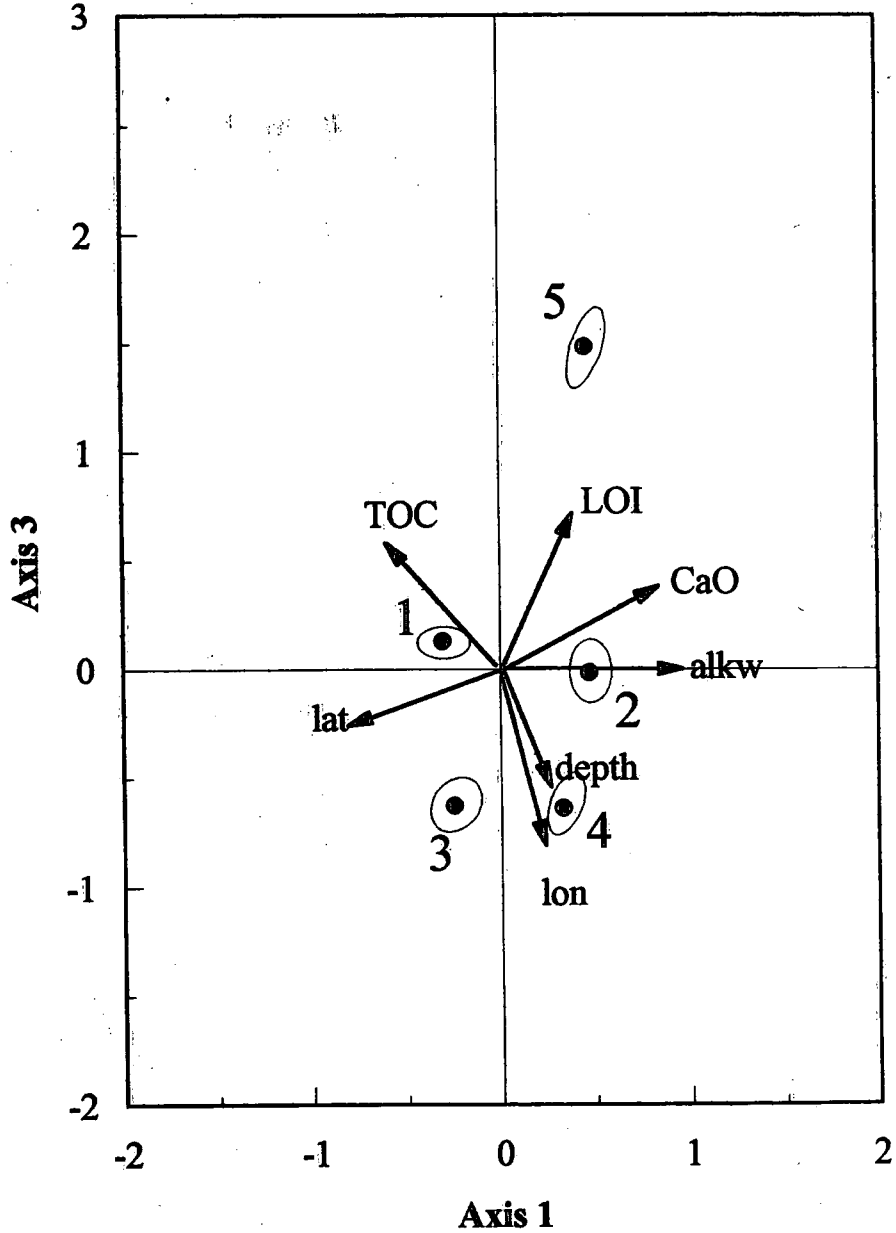


Figure 4.10. Multi-dimensional scaling ordination of family level data in three dimensions of 252 Great Lakes reference sites showing group centroids from cluster analysis and 90% probability ellipses for each group of sites (stress = 0.13879). The contribution of family and environmental variables and the axis are illustrated.

found at most sites (94.0% - Table 4.8). This assemblage of families is characteristic of southwestern Georgian Bay and much of the North Channel (Table 4.9).

Group 2 is dominated by tubificid and naid oligochaetes (Figure 4.9). 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 throughout the other Great Lakes (Table 4.8). 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 higher alkalinity which may be a surrogate for dissolved minerals, including nutrients (Figure 4.10).

Group 3 is characterized by sites where the Pontoporeiidae are dominant (Figure 4.9) and occur in significantly ($P < 0.05$) greater numbers than in Groups 1 and 5 (Figure 4.9). The Chironomidae are the second most abundant family in this group of sites. This assemblage of organisms is characteristic of sites in Lake Superior (93%) and many of the more exposed sites in Georgian Bay (28%) and the North Channel. These sites are associated with deeper water and less organic material in the sediment (Figure 4.10).

Twenty of the 28 Group 4 sites (71%) are in Lake Michigan (Table 4.9). This group is dominated by two oligotrophic families, the Pontoporeiidae and Lumbriculidae (Figures 4.9, 4.10) and these families occur in significantly greater ($P < 0.05$) numbers than in other groups. These sites also represent a deeper water assemblage of organisms (Figure 4.10).

Finally, Group 5 is unique in that it is dominated by sponges. The sites that compose Group 5 are characteristically in sheltered areas such as Long Point Bay, Lake Erie, the Bay of Quinte and Presque Isle Bay in Lake Ontario and Severn Sound in Georgian Bay (Table 4.9). Chironomids are also abundant in this group but these sites tend to be associated with sediment containing a higher organic content (Figure 4.10).

Models

The strategy employed for developing predictive models for assigning a test site (new) to a group using family level was the same as that used at the species level. Three approaches were used for selecting environmental variables in the discriminant function analysis. First, a stepwise discriminant analysis was performed; second, a model was built using all the variables identified by

principal axis correlation as being significantly ($P < 0.01$) related to the family level ordination vectors; third, an iterative removal and replacement process was used in which ten combinations of variables were used.

Table 4.10 Error rate estimates for family level discriminant models of 252 reference sites constructed using three sets of variables.

	Stepwise discriminant model	Principal axis correlation model	Optimal model
Variables used	11 - (depth, lat, lon, alkw, pH, TN, CaO, MgO, Na2O, Al2O3, silt)	20 - (depth, lat, lon, alkw, pH, TN, TOC, LOI, CaO, Al2O3, Fe2O3, TiO2, P2O5, SiO2, K2O, MgO, Na2O, Silt, Sand, Cly,)	6 - (depth, lat, LOI, Al2O3, Alkw, silt)
Total Error rate (cross validation)	31.1%	33.9%	30.0%
Sites predicted correctly	172	167	169
Error rates (%) for 20 site subset:			
Gp 1 (n=8)	38	38	38
Gp 2 (n=4)	50	50	50
Gp 3 (n=3)	0	0	0
Gp 4 (n=3)	0	0	0
Gp 5 (n=2)	50	50	50
Average error rate (cross validation)	28%	28%	28%

The results indicate that the ability of different models to predict site groups are very consistent (Table 4.10). Using all the sites (252), the total error rate varied between 30.0 (6 variables) and 33.9% (20 variables). Using the same calibration data set of 20 sites as in the species level models, the total error rates were similar (28%).

There is little apparent difference between species and family level classifications and predictive models. The best species level models had total error rates of 30.1 - 35.8%, the family level models 30.0 - 33.9%, and, with calibration sub-sets, the species-level model error rates were 12 - 20% and the family-level model error rates 28%. Based on the performance of the models in detecting site differences within the reference site data set, family level identification would appear

to be sufficient. However, it is possible that family level models may be less sensitive to detecting change from reference. This will be examined at a later date.

4.2.6. Sources of Error

There are three major sources of error associated with the construction of community-based models. First, methodological error related to the collection and sorting of samples and the identification of organisms can occur. The second source of error is the inherent variability of invertebrate communities in the natural environment and whether estimates of the community taken from one point in time can be considered representative. The third relates to the potential variability in the estimates of the habitat descriptors taken from single field measurements. We have examined these sources of error separately.

4.2.6.1. Sorting and identification

The errors associated with sorting are related to efficiency and consistency. In this project, the numbers of samples collected necessitated the use of more than one sorter. Over the time frame of the project, four individuals sorted and picked invertebrate samples. In order to ensure consistency in sorting and picking, a number of QA/QC measures were taken. When a new individual began sorting and picking, the residue material was re-picked by another more experienced sorter. This was maintained for the first five samples processed or until the new individual had an acceptable collection efficiency arbitrarily set at 90% recovery. Subsequent random monthly checks of performance were conducted on each sorter. The results show a very high recovery rate from 94.8 - 98.7%, well within the acceptable range (Table 4.11).

Table 4.11 Efficiency of sample picking.

Individual	Average Sorting Efficiency	Number Random Checks
Picker 1	98.7%	3
Picker 2	94.8%	6
Picker 3	95.3%	12
Picker 4	97.5%	7

The error in identification was determined by comparing the accuracy rate for the major groups. All species-level identifications were completed by one person, Mr. Craig Logan. Error rates, as determined by the number of misidentified specimens determined by experts, was less than 10%, with the exception of the Chironomidae. However, as familiarity with the group was acquired, this error rate also dropped well below 10% (Table 4.12).

Table 4.12. Accuracy of species identifications at NWRI taxonomy laboratory.

Taxonomic Group	Error rate	Group expert
Mollusca	2.3%	G. Mackie
Oligochaeta	8.0%	R.O. Brinkhurst, D. Spencer
Chironomidae batch 1	19.6%	B. Bilyj
batch 2	14.0%	
batch 3	4.2%	
Other taxa	7%	B. Bilyj

4.2.6.2 Measurement error

As the prediction is dependent on measured estimates of habitat variables, measurement variability in those estimates may introduce error into the outcome of the predictive models. To estimate the degree of error associated with field and laboratory estimates of habitat variables triplicate samples and/or measurements were taken at 47 sites. The coefficients of variation (CV) were calculated for each variable at each site and then an average CV for all the sites. We have presented (Table 4.13) the average, maximum and minimum CV's for the 12 variables used in the optimal model (Table 4.6). Of the 43 variables measured, cadmium was the most variable (CV 50.3%), five variables showed no variation (latitude, longitude,pH, alkalinity and depth). Of the variables used in the predictive models (Tables 4.6 and 4.10), the average CV does not exceed 20% although occasionally they are higher (Table 4.13). Whether, this is due to patchiness in the field sampling error or measurement error cannot be determined from these data.

Table 4.13. Sampling and measurement error in predictor variables as indicated by coefficient of variation determined for 47 sites.

Variable	Average	Minimum	Maximum
latitude	0.0	0.0	0.0
longitude	0.0	0.0	0.0
depth	0.0	0.0	1.0
alkalinity (n=45)	0.0	0.0	0.2
pH	0.0	0.0	0.0
TN	19.7	0.1	122.9
TOC	17.2	0.4	155.9
K2O	3.9	0.0	22.2
CaO	10.5	0.1	100.8
MgO	7.3	0.0	96.1
MnO	11.3	0.0	135.3
SiO2	1.9	0.1	8.9

4.2.6.3 Seasonal and annual variability

The majority of the reference sites were visited only once due to the geographical scale of the study design. Therefore, the predictive models developed from this reference data base are restricted to the sampling period and geographical area encompassed by those 252 sites. The median sampling date for the data base was September 11th with the earliest sampling date occurring on July 15th and the latest, on October 26th. Because community assemblages of benthic invertebrates vary as a result of life cycle patterns, the validity of a predictive model built from samples taken in the fall season requires assessment. We examined two aspects of temporal variation and its impact on the accuracy of the predictive model as follows: (1) seasonal patterns which largely affect life cycles and reproductive strategies; and, (2) annual variation which is associated more with climate and weather patterns.

Our primary concern about the effects of temporal variability are in relation to the accuracy of the predictive model and how changes in the abundance of taxa will affect the classification of a site. A subset of sites was sampled both seasonally and annually to determine what effect sampling at different times of the year or in different years has on the assemblage of organisms at a site. Temporal changes in the species assemblage of selected sites were examined to determine whether the site varied from the group to which it was assigned based on the 252

site-model. If no such change occurred then temporal change is no greater than the normal spatial variability observed in that reference group. We determined the seasonal and annual stability of the communities found at sites by examining the temporal behaviour of the site in species ordination space. Sites sampled both annually and seasonally were tracked in the same ordination space as the reference sites used to form the group to which the seasonal site belonged. A site was deemed to be part of the same group (i.e., the same species assemblage) if it remained within a 90% probability ellipse constructed around those sites in the reference group. All estimates of the effects of temporal variation were assessed at the species level.

Annual variation

Annual changes in the benthic invertebrate community structure more likely reflect differences in the community due to the effects of climate and weather, especially the timing of the spring warming period and its effect on warming patterns in the lakes. The life cycles of most invertebrates are related to temperature patterns and, therefore, annual differences in the warming pattern in water can have a major effect on populations and community structure.

Annual variation was examined by determining the similarity of sites over the three-year period of the study. Eleven of the 16 sites sampled in each of the three years (Table 3.1) were included as part of the 252 site reference data base in each of the three years, the other five sites had been screened out. Similarly all nineteen sites (Table 3.1) sampled in two years were part of the reference data base. The effect of annual variation in the benthic community on the assessment of the status of a site was determined by locating the sites in ordination space. If annual variation is likely to confound the interpretation of changes in a community, then these variations will be greater than those due to normal variation. We consider a site that remains within a 90 % probability ellipse to have the same invertebrate assemblage; therefore, annual variation is only of concern if a site sampled from year to year moves outside the 90% probability ellipse. Each of the sites sampled annually for three years was plotted in ordination space and examined. This is illustrated for two sites (104 and 113) in Figure 4.11. Both of these sites were

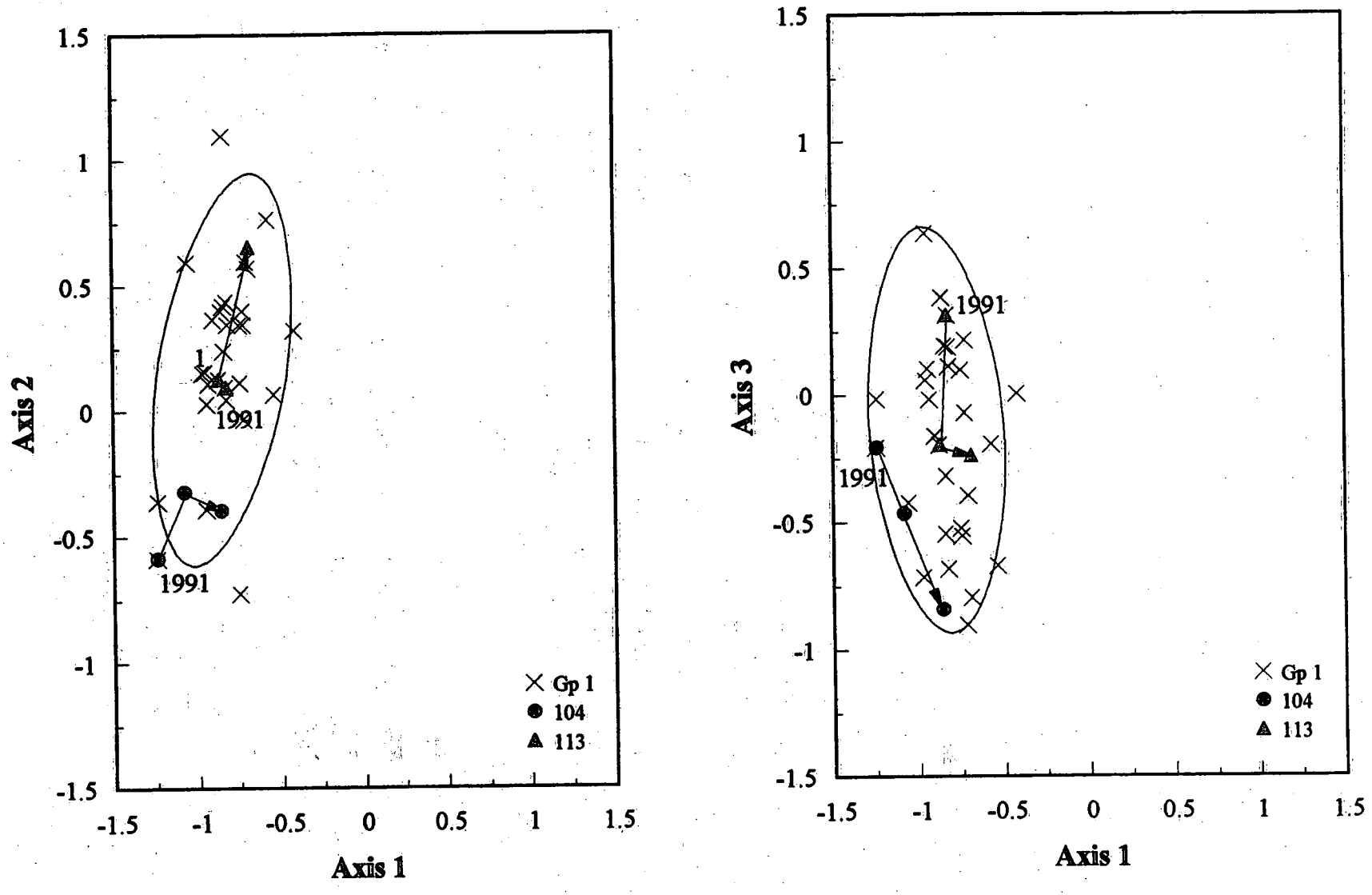


Figure 4.11. Variation of two sites in species level HMDS ordination space over a three year period. (90% ellipse shown for Gp 1 reference sites).

members of Group 1 in 1991. The figure shows the reference sites comprising Gp 1 (X) and the 90% probability ellipse for those sites. The annual samples for the two sites are also shown, with their trajectory from 1991 to 1993. Both sites show similar trajectories. Site 113 remains within the Group 1 reference ellipse. Site 104 falls just outside the ellipse in 1991 (on axis 2) but remains within the ellipse in the next two years.

Table 4.14. Summary of consistency of group membership from year to year at selected reference sites.

	Samples with same assemblage (No. samples collected / No. with same assemblage)					
Period	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Total
3 years	6 / 5		27 / 24			29/33 (87.9%)
2 years	14 / 12	2 / 1	16 /12	4 / 4	2 / 1	30/38 (78.9%)

This was done for the 11 sites for which three annual samples were taken and the 19 sites sampled in two of the three years. These data are summarized in Table 4.14. Twenty-nine of 33 (87.9%) samples were consistently within the reference group to which they were originally assigned. Of the 19 sites sampled in two years, 30 of 38 (78.9%) samples were within the reference group to which they were originally assigned. Given that one would normally expect 10% of site to fall outside the 90% probability ellipse, we are confident that annual variation is little or no greater than the spatial variation and is not a confounding factor in using the predictive models developed for community structure in the nearshore environment of the Great Lakes.

Seasonal variation

Annual variation in communities is largely related to external factors such as the timing and degree of warming in the lakes. Seasonal changes in the benthic invertebrate community are attributes of the organism's themselves and are based on life history strategies and resource exploitation. The effects of season on site group membership was examined using data from four sites sampled approximately monthly from April to October in the second and third year of the study, 1992 and 1993 (Figure 3.3). The sites were selected as a compromise among geographic distribution, habitat and sampling logistics and included one site in each of Lakes Erie and Ontario, and, two sites in different ecodistricts in Georgian Bay. As seasonal changes are more

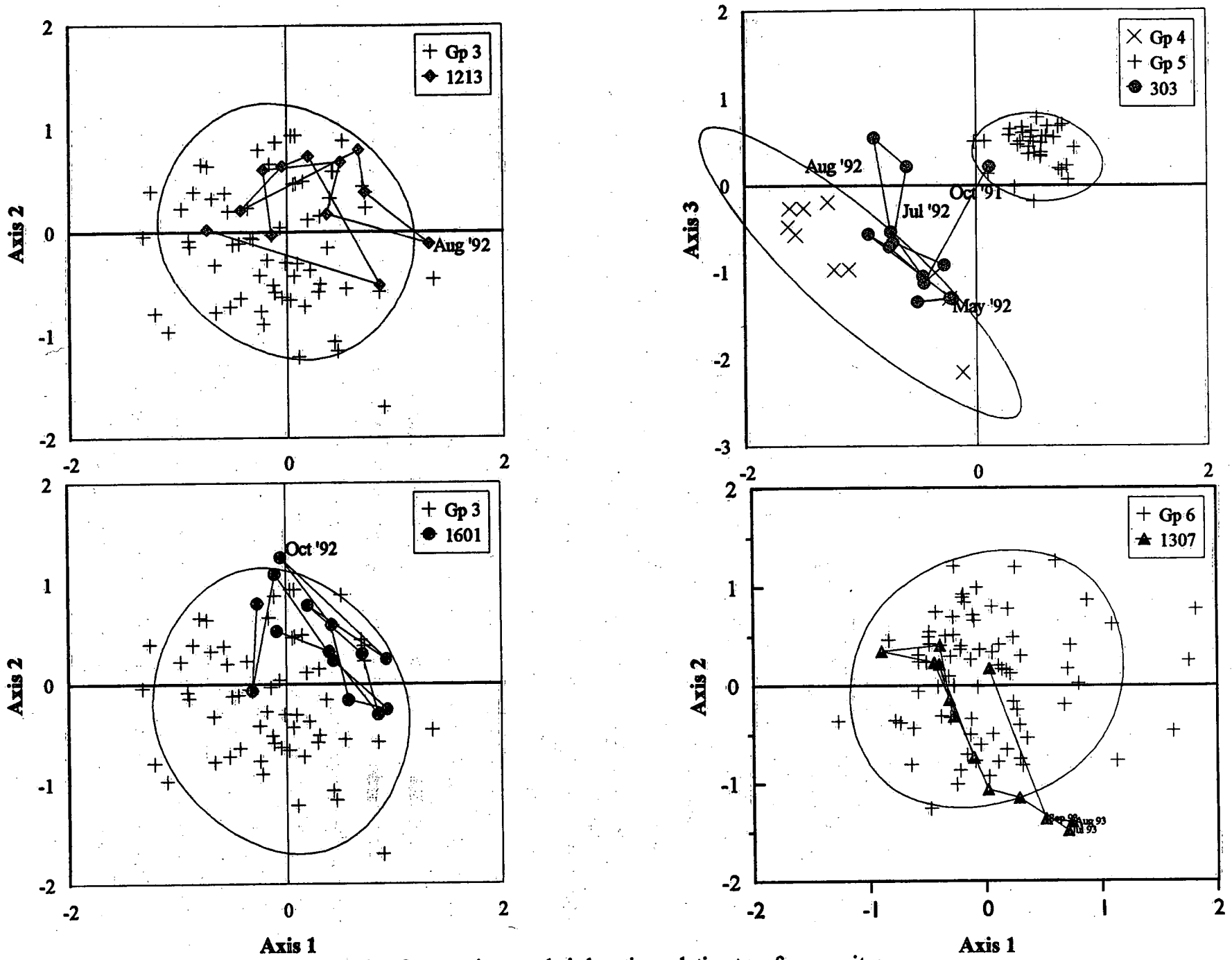


Figure 4.12. Effects of seasonal variation for two sites on their location relative to reference sites, as indicated by the 90% probability ellipse constructed from the reference sites.

likely to affect community structure at the species level, we have examined this level of taxonomic resolution.

To assess the effects of seasonal variation on community structure, we plotted each of the sites in ordination space together with the reference sites from the community group to which they were assigned from the fall sample. We evaluated the importance of seasonal variation by determining the period over which the community would be assessed as equivalent to the reference community. If all samples from the site remained within the 90% probability ellipse of the reference community, we would conclude that seasonal variability is no greater than the observed spatial variability accounted for by the predictive model.

To illustrate seasonal changes, we have presented results from the sites (Figure 4.12). The change in species composition and abundance is described by their movement in ordination space. Arrows have been drawn to reflect the changing monthly position of each of the sites for the two study years. The seasonal changes at Sites 1601 and 1213, located in southern Georgian Bay, result in each site moving out of the reference ellipse (90% probability ellipse) on a single occasion, October 1992 for 1601 and August 1992 for 1213. There was a seasonal pattern, shown by the axes along which these sites are moving, to the changes occurring at these sites which was related to changes in the abundance of four chironomid genera *(Procladius, Tanytarsus, Cladopelma* and *Polypedilum*) and one tubificid worm (*Aulodrilus*). Site 1307, was located in the Kingston Basin in eastern L. Ontario. In 1992 all the sites were within the refernce ellipse, the April 93 sample was marginal, however, in May 1993 there was an increase in numbers of *Diporeia* (6.6 -18.6 / core), this increase continued through June - September (31.2 - 43.2 / core), with the result that those samples collected between June and September were outside the reference ellipse (Figure 4.12, Table 4.15), but by the October sample the numbers were within the normal range (7 / core). At the fourth site, located off Long Point in L. Erie, (303) a quite different pattern was observed. Site 303 was sampled in the fall of 1991, 1992 and 1993. The 1991 community was classified as belong to Group 5 and can be seen to be part of that community (Figure 4.12). Colonisation of the site by *Dreissena* during 1992 resulted in the gradual movement of this site from Group 5 to a new group (Group 4) as the numbers of *Dreissena* increased. With the exception of three samples, May, July and August 1992 all the

seasonal samples were within the variability observed in Group 4. The September 1993 reference sample classified the site as part of Group 4 which represents a small group of nine reference sites dominated by the zebra mussel (*Dreissena*) (see section 4.2.2).

Table 4.15. Effect of seasonal variation on the assessment of four Great Lakes reference sites as being determined equivalent to reference (+ sites located inside 90% probability ellipse; - sites located outside the 90% ellipse; n.s. - not sampled).

Site	303		1213		1307		1601		monthly summary (equivalent to reference)
	1992	1993	1992	1993	1992	1993	1992	1993	
April	+	+	+	+	+	-	+	+	88%
May	-	+	+	+	+	+	+	+	88%
June	+	+	+	+	+	-	+	+	88%
July	-	+	+	+	+	-	+	+	75%
Aug	-	+	-	+	+	-	+	+	62%
Sept	+	+	+	+	n.s.	-	+	+	86%
Oct	n.s.	n.s.	-	+	+	+	-	+	67%
	9/12 (75%)		12/14 (86%)		8/13 (62%)		13/14 (93%)		

The effect of seasonal variation on the location of sites within each reference group is summarised in Table 4.15 for the four sites. We have assumed that Site 303 should be compared with reference sites belonging to Group 4 because of the appearance of *Dreissena*. The percentage of sites equivalent to reference have been summarised monthly, again an error of 10% is expected given that the probability ellipse is 90%. Samples from most months are similar to the matching reference group and suggest that the communities are generally stable through the year, with the exception of site 1307 in 1993 where for 5 of 7 months the community was different to reference because of the increase numbers of *Diporeia*. In fact there is more variation in August and September, which is closest to the period during which the reference samples were obtained. While seasonal variability may have been expected to be greater this result is likely because many of the dominant taxa found within the lakes (*e.g.*, the oligochaetes and molluscs) are resident in the lakes year round. The ordination methods used to assess community assemblages are more

sensitive to the disappearance of taxa rather than changes in abundance. Therefore we are confident that the reference database has year round application, although we would suggest that, where possible, samples be taken from mid July - mid October.

4.3 Responses of Benthic Invertebrates to Reference Sediments in Whole-Sediment Laboratory Bioassays

The three-year data set for the laboratory bioassays with benthic invertebrates was examined to determine if any site should be eliminated as a reference site due to a poor response in one or more end points. The criteria for elimination of any site as a reference site was less than 50% survival for any one species for a particular site in any given year. This critical evaluation resulted in 18 sites being removed from the reference site database. In all 18 cases *H. azteca* survival was less than 50% and in 5 of those *C. riparius* also had less than 50% survival. 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. Sediment bioassays were only conducted at 238 of the 355 sites visited, results from the first year of study suggested that testing was only required on every other sample taken.. After screening these data the number of reference sites used in the data set for each species was as follows: *C. riparius* (220); *H. azteca* (220); *Hexagenia* spp. (170); and *T. tubifex* (170).

A preliminary analysis was conducted to determine what relationships existed between each individual endpoint and attributes of the test sediment. Simple Pearson correlation coefficients were calculated and Bonferroni probabilities determined (Table 4.16)

Survival, which showed little variability, was not related to the measured sediment characteristics. Of the sub-lethal endpoints reproduction in *T. tubifex* (fed and unfed) and growth in *H. azteca* showed little relationship with the measured variables. Two endpoints seemed to be related to sediment attributes, growth in *C. riparius* and *Hexagenia* spp. Growth in *C. riparius* was negatively correlated with growth, the other variables with which growth was correlated were highly correlated ($P < 0.01$) with the clay content, causality with any one (or more) sediment characteristics cannot be inferred from these data and requires experimental investigation. Using

multiple regression (11 variables) the strongest relationship only produced an r^2 value of 0.294 for *C. riparius* growth. Growth in *Hexagenia* (fed test) was correlated with 10 variables, positively with silt (r = 0.47) and negatively with sand (r = -0.42). The importance of the silt content to the burrowing mayfly is well known. The other variables that showed a correlation with growth are also strongly correlated ($P < 0.01$) with the silt and sand content. Again, a multiple regression model was a poor predictor of growth in *Hexagenia* ($r^2 = 0.210$, 10 variables).

Table 4.16. Pearson correlation coefficients between sediment attributes and lethal and sub-lethal bioassay endpoints. (Those variables with $P < 0.05$ shown).

Bioassay Endpoint	Variables (Pearson 'r')
Lethal	
C. riparius	none
H. azteca	none
Hexagenia spp	none
Hexagenia spp	V (0.26)
T. tubifex (unfed)	none
T. tubifex (fed)	none
Sub-lethal	
C. riparius growth	Clay (-0.38), Fe_2O_3 (-0.30), P_2O_5 (-0.30), Zn (-0.30), Cu (-0.28), Pb (-0.27)
H. azteca growth	none
Hexagenia growth (unfed)	CaO (0.47)
Hexagenia growth (fed)	Silt (0.47), SiO_2 (-0.46), Sand (-0.42), LOI (0.42), TN (0.35), TOC (0.32), CaO (0.29), Cr (0.29)
T. tubifex hatch (unfed)	none
T. tubifex cocoons (unfed)	none
T. tubifex young (unfed)	none
T. tubifex hatch (fed)	Na_2O (0.27)
T. tubifex cocoons (fed)	none
T. tubifex young (fed)	Na_2O (0.30), LOI (-0.28)

These analyses conducted to determine the correlation of each end point for each species with sediment characteristics such as particle size distribution, TOC, loss on ignition (LOI), MgO, SiO_2, TP, TN, etc., for the reference sites show only weak relationships between two endpoints and sediment characteristics. In addition, when the range in response for each endpoint in a variety of sediments was compared to the range in response for the same endpoint in only one reference sediment (i.e., Long Point), few differences were noted. It was therefore concluded that the range in each endpoint observed for the reference sediment data base represents the natural range in the responses of each organism in laboratory bioassays.

Descriptive statistics for the mean, median, standard error (S.E.), standard deviation (S.D.), maximum and minimum values, range and coefficients of variation (CVs) for each measured end point with four species of benthic invertebrates exposed to 170 to 220 reference sediments collected throughout the Great Lakes over a three-year period are given in Table 4.17. Frequency diagrams for each end point are also presented in Figure 4.13 for *C. riparius*, *H. azteca* and *Hexagenia* and Figure 4.14 for the oligochaete worm, *T. tubifex*. A similar set of descriptive statistics and frequency diagrams for the range in responses for each species exposed to one reference sediment (LP) in quality assurance studies during the 1991-93 period of the study are presented in Table 4.18 and Figures 4.15 and 4.16.

Table 4.17. Variability in endpoints in bioassays with four species of benthic invertebrates exposed to reference sediments from the Great Lakes.

	Chironomus riparius[1]		*Hyalella azteca*[1]		*Hexagenia* spp.[2]		*Tubifex tubifex*[2]			
	% Survival	Growth mg d.w./ larvae	% Survival	Growth mg d.w./ juvenile	% Survival	Growth mg d.w./ nymph	% Survival	% Hatch	No. Coc./ Adult	No. Young/ Adult
Mean	85.5	0.35	86.8	0.49	95.9	2.97	98.3	58.1	9.8	28.1
Median	86.7	0.33	90.7	0.50	95.9	2.98	98.3	58.1	9.8	28.2
S.D.	8.9	0.07	9.9	0.13	5.2	1.02	4.7	10	1.3	9.1
Max.	100	0.60	100	0.80	100	6.40	100	91.0	14.5	48.9
Min.	53.3	0.16	50.0	0.10	66.0	0.50	60	19.5	4.8	1.1
CV	10.4	21.3	11.4	27.0	5.5	34.3	4.8	17.3	13.1	32.2

[1] n = 220 reference sites [2] n = 170 reference sites

Table 4.18. Variability in endpoints of four species of benthic invertebrates exposed to one reference sediment (LP) in laboratory bioassay conducted over three years (n = 46).

	Chironomus riparius		*Hyalella azteca*		*Hexagenia* spp.		*Tubifex tubifex*			
	% Survival	Growth mg d.w./ larvae	% Survival	Growth mg d.w./ juvenile	% Survival	Growth mg d.w./ nymph	% Survival	% Hatch	No. Coc./ Adult	No. Young/ Adult
Mean	87.4	0.37	91.7	0.59	97.1	5.00	98.9	56.7	11.1	36
Median	89.4	0.36	93.3	0.58	98.0	4.75	100	57.7	11.0	37
S.D.	8.3	0.07	7.2	0.14	4.1	0.99	0.7	5.8	0.8	8.7
Max.	98.7	0.55	100	0.85	100	7.5	100	63	12	52
Min.	62.2	0.26	61.3	0.17	80	3.4	95	33	9.0	22
CV	9.5	17.7	7.7	23.5	4.2	20.8	7.1	10.2	7.3	24.2

As the objective 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 differs from the response(s) of a similar cohort of organisms to a negative control or reference sediment, the data from the reference sites were used to establish three categories of responses to test

Table 4.19. Comparison of toxicity using Minimum Detectable Difference (MDD) versus twice the standard deviation of mean (S.D.)

	Mean for response	MDD	Warning Level for Potential Toxicity based on Minimum Detectable Difference (MDD)	Warning Level for Potential Toxicity based on 2X S. D. of Mean*
Chironomus riparius				
% Survival	85.5	19.3%	< 69.3%	67.7%
Growth	0.35	15.9%	< 0.29	0.20
Hyalella azteca				
% Survival	86.8	20.5%	< 69.6%	66.9%
Growth	0.49	29.2%	< 0.35	0.22
Hexagenia spp.				
% Survival	95.9	11.5%	< 85.9%	85.5%
Growth	2.97	-	-	0.8
Tubifex tubifex				
% Survival	98.3	10.6%	< 87.7%	88.9%
% Hatch	58.1	22.1%	< 45.2%	38.0%
No. Coc./Worm	9.8	3.1%	< 7	7.1
No. Young/Worm	28.1	19.9%	23	9.8

* more conservative estimate of toxicity

sediments. The three categories were: non-toxic, potential toxicity ('grey' area of low to moderate toxicity) and toxicity. The delineations for the three categories were developed from

endpoint measured in all reference sediments. For each endpoint, the nontoxic category was set at two standard deviations (2 x SD) below the mean for the reference data base; 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 standard deviations (3 x 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 SD represent the warning of potential toxicity and may indicate sediment(s) which have low or moderate toxicity and, therefore, some detrimental effects. Additional weigh-of-evidence such as impaired benthic invertebrate communities at sites which fall in the category of potential toxicity would emphasize the need for further study or remedial action.

Table 4.20. Criteria for determination of toxicity for nearshore sediments of the Great Lakes.

	1 Non-toxic*	2 Warning of Potential Toxicity minus twice SD	3 Toxicity minus three times SD
Chironomus riparius			
% Survival	≥ 67.7	67.7 - 58.8	< 58.8
Growth	0.49 - 0.21	0.20 - 0.14	< 0.14
Hyalella azteca			
% Survival	≥ 67.0	66.9 - 57.1	< 57.1
Growth	0.75 - 0.23	0.22 - 0.10	<0.10
Hexagenia spp.			
% Survival	≥ 85.5	85.5 - 80.3	< 80.3
Growth	5.0 - 0.9	0.8 - 0.0	-
Tubifex tubifex			
% Survival	≥ 88.9	88.9 - 84.2	< 84.2
% Hatch	78.1 - 38.1	38.0 - 28.1	<28.1
No. Coc/Worm	12.4 - 7.2	7.1 - 5.9	< 5.9
No. Young/Worm	46.3 - 9.9	9.8 - 0.8	< 0.8

*upper limit for non-toxic category is set using 2 x SD of the mean and indicates excessive growth or reproduction

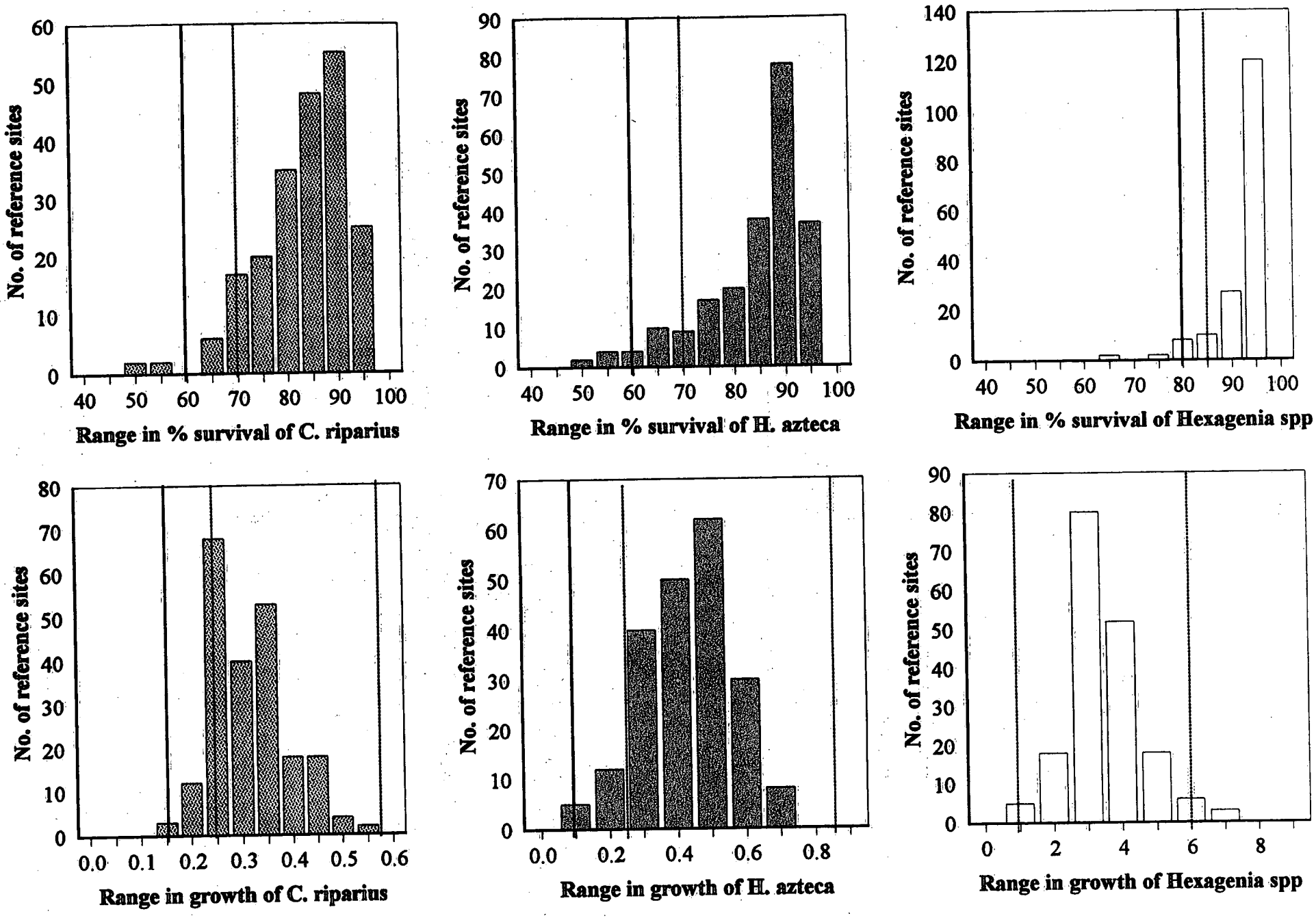


Figure 4.13. Frequency histograms of the responses of three species of invertebrate from sediments from 170 - 220 reference sites in the Great Lakes (dotted line 2 SD, solid line 3 SD).

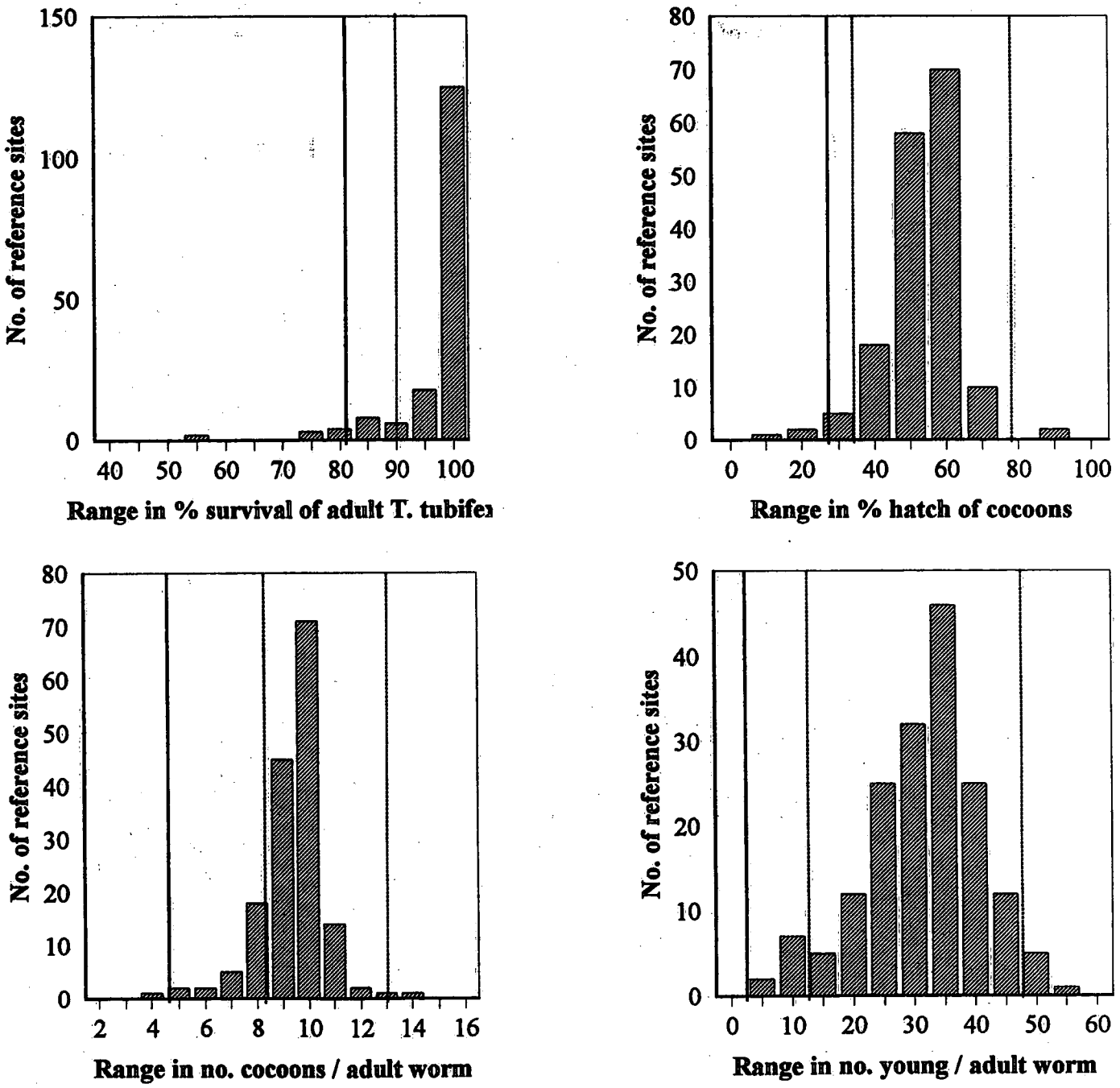


Figure 4.14. Frequency histograms of the responses of *Tubifex tubifex* sediments from 170 reference sites in the Great Lakes (dotted line 2 SD, solid line 3 SD).

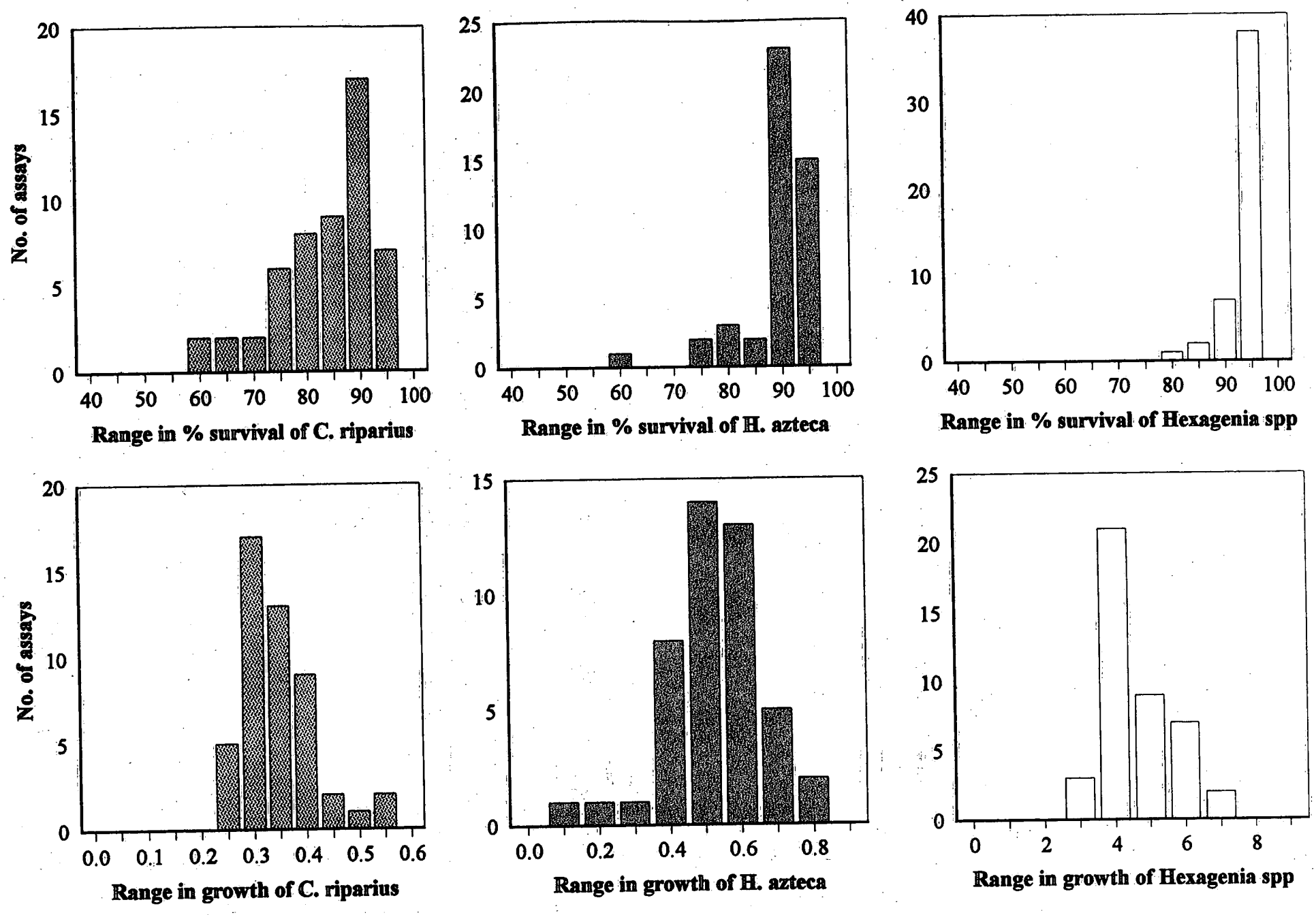


Figure 4.15. Frequency histograms of the responses of three species of invertebrate in repeated bioassays with sediment from Long Point, Lake Erie. (dotted line 2 SD, solid line 3 SD).

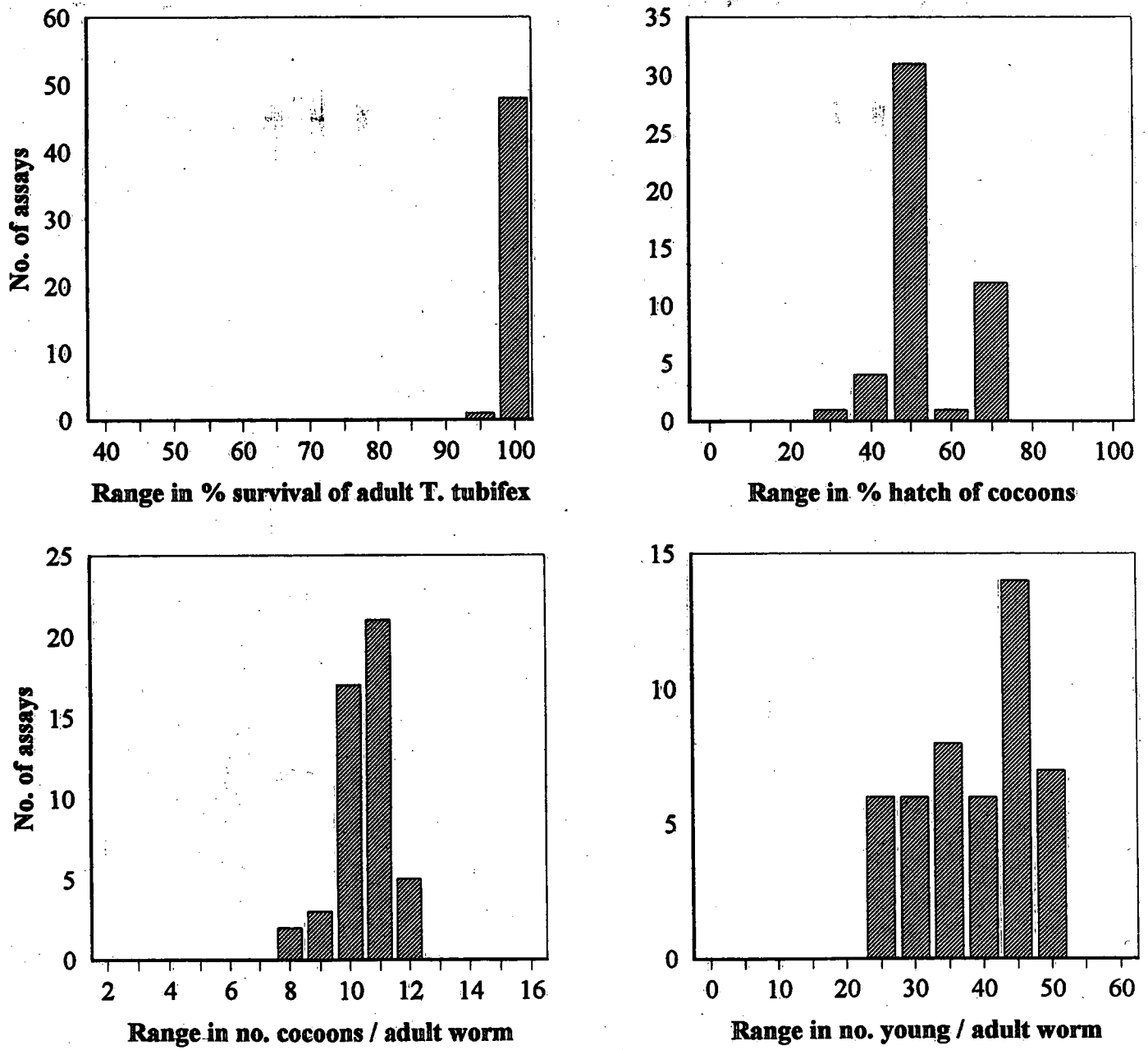


Figure 4.16. Frequency histograms of the responses of *Tubifex tubifex* in repeated bioassays with sediment from Long Point, Lake Erie. (dotted line 2 SD, solid line 3 SD).

For comparative purposes, a formula which incorporates the probability of Type I and Type II errors was also used [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 (α), statistical power ($1-\beta$) and population variance 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 α and β 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 (α) and Type II (β) errors of 0.10 are suitable because the costs of either remediating a non-contaminated sediment or not remediating a contaminated sediments would be equal from either an environmental or a financial viewpoint. Comparison of the MDDs with the criteria based on two and three times the standard deviation of the mean (Table 4.20) showed little difference so the more conservative estimate of toxicity was used in setting the biological criterion for each toxicity end point.

Bioassay results for each species are discussed separately below.

4.3.1 *Chironomus riparius*

Mean percent survival of *C. riparius* in 220 reference sediments was 85.5 with a range of 53.3 to 100% and a CV of 10.4%. 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 $\geq$70% for survival of *Chironomus* spp. in uncontaminated sediments (negative controls or reference sediments) used in toxicity tests. Our results show that this criterion is achieveable in the majority of sediments collected from reference areas in the Great Lakes. The number of reference sediments for which % survival was <70% was well within the 1 in 20 expected to fall outside the 95% confidence limits for any given test. Percent survival in repeated bioassays with one particular reference sediment (QC sediment collected from Long Point, Lake Erie) demonstrated

the same range in sensitivity, i.e., values ranged from 62.2 to 98.7% with a mean survival of 87.4% and a CV of 9.5%.

A MDD of 19.3% was calculated for this species using a Type I error of 0.1 (α) and a Type II error of 0.1 (β). This MDD resulted in a value of < 69.3% survival as a statistical indication of potential toxicity (Table 4.19). The value set at two times the standard deviation of the mean was 67.7% survival (Table 4.20). These values compare quite favourably with the acceptability criterion of >70% survival set by USEPA (1994) for control sediments. A conservative estimate of toxicity to *C. riparius* set at 3X the S.D. would therefore result in a level of <60% survival indicating toxicity with a 1 in 333 chance of incorrectly identifying a toxic sediment.

Growth of larval chironomids in a variety of reference sediments with a range of physico-chemical characteristics was variable: dry weight of individual 4th instar larvae at test termination (10-d) ranged from 0.16 to 0.60 mg with a mean of 0.35 mg and a CV of 21.3%. We were unable to correlate this variability in growth with sediment characteristics, 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. A similar range in growth of midge larvae (i.e., 0.26 to 0.55 mg dry wt. per individual) was observed in reference sediment from Long Point (LP), providing evidence that the physiology of the animals in any given test or a particular cohort of cultured animals may also play a role in their range of growth responses.

The MDD determined for growth of larval *C. riparius* in this study was 15.9% which results in a warning level of potential toxicity for dry weight of individual midge larvae to be set at 0.29 mg or less (Table 4.19). Twice the S.D. of the mean yielded a warning level of 0.20 mg d.w. per larvae (Table 4.20). When the range of responses for growth in the LP sediment is considered, this lower value of <0.20 mg d.w. per larvae appears to be more realistic as a trigger for toxicity. When growth in the 10-d exposure is reduced to a level of <0.14 mg d.w. per individual larva (3X S.D. Table 4.20), sublethal toxicity is suggested with a 1 in 333 chance of error (99.3%). This value is thus set as the level for toxicity (Category 3) in a 10-d test.

4.2.2 *Hyalella azteca*

As with midge larvae, survival of juvenile *H. azteca* in 220 reference sediments was in the range of 50.0 to 100%, with a mean of 86.8% and a CV of 11.4%. However, in 18.4% of the reference sediments used, 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). Survival of *H. azteca* in the repeated bioassays with LP sediment was also below 70% in several tests. The duration of the amphipod test in this study was 28-d which may account for the slight increase in control mortality compared to the 10-d USEPA (1994) acute lethality protocol. Our results suggest that the minimum acceptable criterion of ≥70% survival in uncontaminated sediment is appropriate for 28-d tests with this species.

The MDD of 20.5% calculated for this species using a Type I error of 0.1 (α) and a Type II error of 0.1 (β) resulted in a value of < 69.6% survival for a test sediment to be declared statistically toxic (Table 4.19). A limit set at two S.D. below the mean results in a warning of toxicity to be set at 66.9% survival or less. Based on these calculations, it is recommended that the level of warning for potential toxicity to survival of *H. azteca* be set at 66.9% (Table 4.20). Percent survival less than 58% (3X the S.D. of the mean) indicates a toxic sediment with a 1 in 333 chance of being incorrect.

The growth of 3 to 9 day-old *H. azteca* in reference sediments over a 28-d exposure was more variable than growth in the midge bioassay and ranged from 0.10 to 0.80 mg dry wt. per juvenile, with a CV of 27.0% (Table 4.17). A negative correlation with % clay in the sediments was noted. However, similar variability in growth (Table 4.18; 0.17 to 0.85 mg d.w.) occurred in tests with the LP sediment. Based on these results, as well as the value of 0.35 mg d.w. per juvenile calculated using the MDD (30.0%) or 0.10 mg d.w. per juvenile calculated using three times the S.D. of the mean, the limit for growth below which a sediment is considered to cause sublethal toxicity to juvenile *H. azteca*, was set at the more conservative value of <0.10 mg d.w. per individual juvenile (Table 4.20).

4.2.3 *Hexagenia* spp.

Percent survival of the mayfly nymph *Hexagenia* spp. was high in all types of sediment, ranging from 66.0 to 100% with a mean of 95.9% and a CV of 5.5% (Table 4.17). Excellent survival was also noted in the LP reference sediment (Table 4.18). The acceptablility criterion for survival of this species in clean sediment can, therefore, be set quite high, i.e., ≥ 85% survival in control sediments. A warning of potential toxicity can also be set quite high at 80.3-85.5% survival. Percent survival of mayfly nymphs less than 80.3% is a conservative estimate of toxicity based on 3X the S.D. of the mean (Table 4.20).

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 34.3% (Table 4.17). Positive correlations with LOI, TOC, TN, TP and SiO2 as well as negative correlations with % sand and % silt were noted in single regressions (Table 4.16). Growth in LP sediment was slightly less variable and ranged from 3.4 to 7.5 mg dry weight per individual with a CV of 20.8%. Because variability in growth was large and a MDD could not be calculated (225%), a warning limit of 0 to 0.8 mg d.w. per nymph using 2X S.D. has been set for this species (Table 4.20). As negative growth (weight loss) has been observed in some whole-sediment toxicity tests conducted in our laboratory, a negative value for growth of *Hexagenia* nymphs places the test sediment into Category 3, toxic.

4.2.4 *T. tubifex*

Percent survival of adult *T. tubifex* was usually 100% (Table 4.17) in all bioassays with reference sediments; only 3.6% of sediments tested produced any mortality (between 10 and 20%). Based on these results, the acceptability criterion for % survival of adult worms in non-toxic sediments can be set quite high, i.e., >88.9% (Table 4.20). Percent hatch of cocoons was also fairly high and constant with a mean of 58.1 ± 10 and a CV of 17.3% (Table 4.17). The acceptability criterion for % hatch of cocoons is set at >38.0% (Table 4.20). The number of cocoons produced per adult worm ranged from 4.8 to 14.5, with a mean of 9.8 and a CV of 13.1%. The range in this response in LP sediment was somewhat narrower (9.0 to 12 cocoons

per adult worm) and the mean was higher, i.e., 11.1 cocoons per adult worm. LP sediment is an organically enriched sediment which may account for the slightly higher reproductive output. Nevertheless, an acceptability criterion of 7 cocoons per adult worm can be set using either a calculated MDD or twice the S.D. of the mean. The number of young produced per adult worm was more variable than cocoon production, with a mean of 28.1 ± 9.1 and a CV of 32.2%. Similar ranges in production of young were noted for the LP sediment used routinely in all bioassays i.e., 36 ± 8.7. Based on these results, a conservative estimate for a warning of toxicity is set at <9.9 young per adult worm with toxicity indicated when production of young falls below 0.8 young per adult worm (Table 4.20).

5.0 DEVELOPMENT OF GUIDELINES

5.1 Guidelines for Determination of Nearshore Sediment Quality

This database developed on invertebrate community structure from 252 reference sites and on ten toxicity test endpoints from 220 or 170 reference sites is a unique resource that has allowed us to derive numeric expressions of the normal variability observed in these biological measures. This understanding of normal variation allows us to identify changes that are outside the normal response range, indicating that the system is responding to stress, rather than to normal environmental variability.

The guidelines developed below for both invertebrate community structure and toxicity are based on measured variation outside the expected range.

5.1.1. Invertebrate community structure

The process of determining whether an invertebrate community is impaired at a potentially contaminated site involves the following:

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

(2) running the discriminant model developed from the reference data base with data from the test site(s) to assign the test site(s) to one of the reference community groups;

(3) comparing a test site(s) invertebrate community to the community from the reference group to which the test site(s) were predicted.

There are a number of approaches to comparing reference and test sites, for the purposes of making decisions on impairment. Traditionally, these have involved comparing control and test sites using univariate statistical methods, on a taxon by taxon basis, or using variables such as number of taxa, or other community attributes thought to incorporate higher level community function (*e.g.* ratio of shredders/burrowers). However, our selection of multivariate statistical methods to examine patterns in invertebrate assemblages and define community assemblages, because they are unbiased and incorporate information on all taxa makes such methods equally appropriate for determining whether a test community is equivalent to reference and for setting

guidelines that describe the degree of impairment in the community. The use of a multivariate approach allows the incorporation of information on all the taxa, it makes no *a priori* assumptions about important taxa, it removes the element of subjectivity inherent in many indices, and it allows a probability based approach to be used.

A large water quality survey on rivers 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 (Clarke *et al* 1992). The study (Clarke *et al* 1992) produced a simplification of the continuum of responses in sites ranging from good to poor biological quality. It was seen as an appropriate mechanism for obtaining a simple statement of biological quality which allows broad comparisons in either space or time that are useful for management purposes. From a management perspective it is desirable to assign a degree of impairment. This can be done by setting response categories from mild to severe impairment. In the study by Clarke *et al.* (1992), a number of schemes for categorising the response were considered and tested. The threshold between unstressed and stressed sites (band A) was set at the 90% probability level (SD = 1.64) for number of taxa and the BMWP score and 95% for the average score per taxon (ASPT). In Australia the threshold is set at 2 SD's from the reference site mean for the number of taxa. Finally, 95% is frequently set as the limit for determining a biological effect for univariate data and single community descriptors (Lowell, 1997). The strategy employed to in the UK (Wright 1995) to discriminate between degrees of impairment was to quantify the difference between the threshold for stressed and non-stressed sites and the most impaired site and to divide that into three equal size bands. As wriught (1995) argued that there was no logical basis for an aletrnative scheme for dividing up the continuum of sites.

Setting guidelines for invertebrate community structure at a test site

We have adopted a similar approach for defining degrees of impact using a multivariate approach. The reference invertebrate assemblage is described by its distribution in ordination space, and the assemblage at any given site is characterised by it position in the XY space (Figure 5.1). The greater the similarity between sites the closer together they are in XY space. Using this

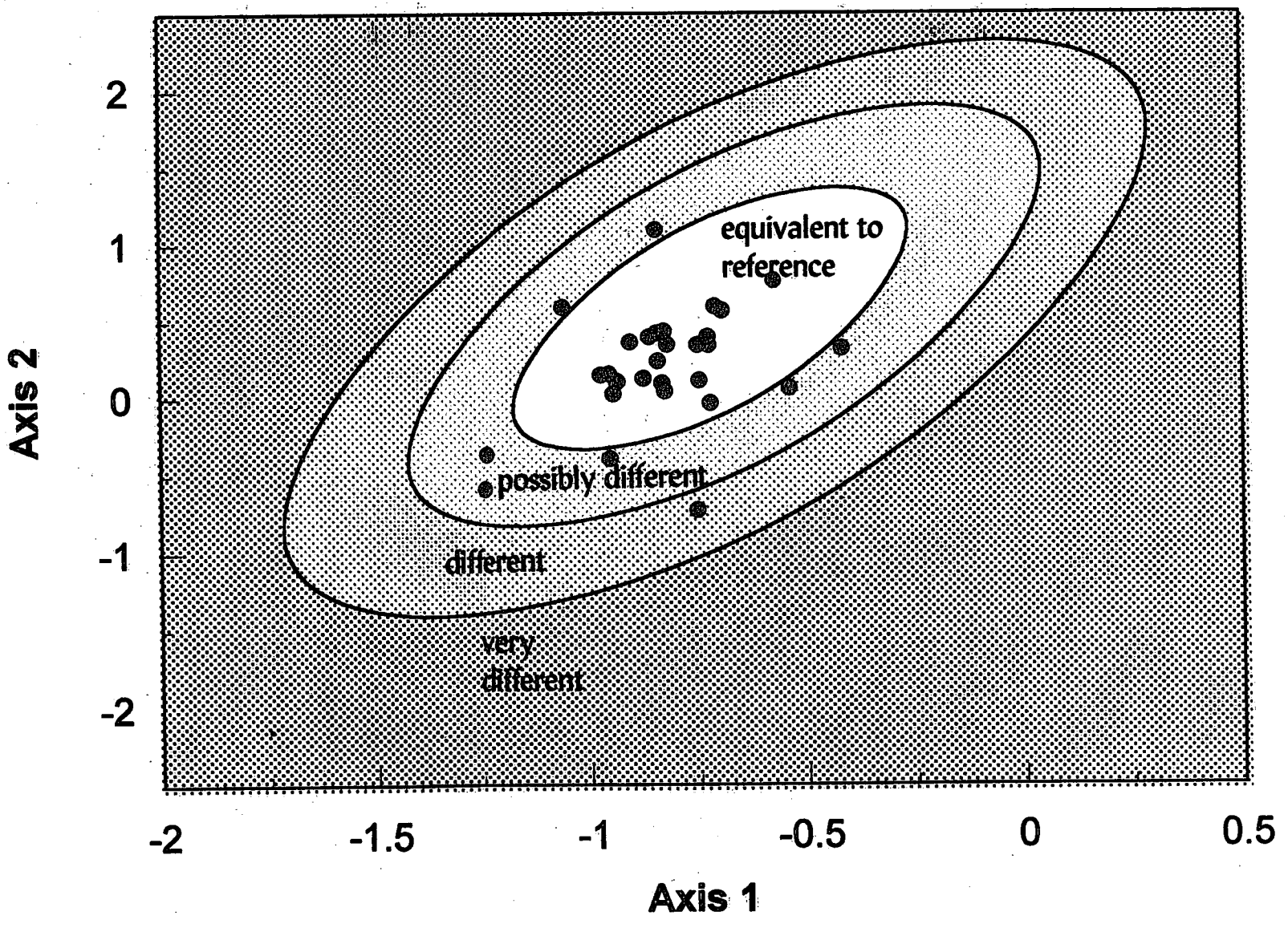


Figure 5.1. Impairment stress levels derived for reference sites in HMDS ordination space. Bands, based on 90, 99 and 99.9% probability ellipses, are identified as A (equivalent to reference), B (possibly different), C (different) and D (very different).

approach to setting numeric guidelines for an invertebrate assemblage all the reference sites are plotted in XY space together with a test site(s). The likelihood of the test site being the same as the reference sites is quantified by constructing probability ellipses for the reference sites ONLY. We have selected a 90% probability ellipse as representing the first band, the threshold between a site being considered equivalent to reference, the rationale for using the 90% ellipse rather than the more typical 95% was based on the fact that a multivariate approach will tend to be noisier than univariate measures individual measures and therefore a more conservative threshold was deemed appropriate. Sites located in ordination space inside this smallest ellipse (90% probability) would be considered as equivalent to reference and therefore unstressed. Two other probability ellipses are used (Figure 5.1), that are equal in width, to describe further divergence from the reference state, following the argument used by Wright and co-workers (Clarke *et al* 1992, Wright 1995). Sites between the smallest (90%) and next ellipse (99% probability) would be considered possibly different, there is a 1 in 10 chance that sites will fall in this band through normal variability; sites between the 99% and the largest ellipse (99.9% probability) are considered different, there is a 1 in 100 chance that these sites would incorrectly be described as different; and finally, sites located outside the 99.9% ellipse are designated as very different. The observed differences may represent either a response to anthropogenic stress or possibly a natural stress.

5.1.2 Whole Sediment Toxicity Tests

Three categories of toxicity were developed for nearshore sediments in the Great Lakes based on the results from the 166-208 reference sediments. The categories are - non-toxic, potential toxicity and toxic. The delineations for each species and endpoint derived from whole-sediment exposure tests in reference sediments are presented in Table 22. An upper limit is provided in the non-toxic category for growth based on twice the standard deviation of the mean response for the data base. Although sublethal responses derived from whole-sediment toxicity tests are usually considered to be lower values (i.e., growth or reproduction is reduced in comparison to a control), in areas of eutrophication or high nutrient impact, an increase in

reproduction could have a negative impact on the structure and function of benthic invertebrate communities in aquatic ecosystems by allowing some species to dominate the habitat or utilize resources at the expense of other species. 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 of growth and reproduction may not be considered indications of toxicity, such observations should be taken into consideration in any managerial decisions made regarding the remediation of sediments.

The use of twice and three times the standard deviation below the mean for each endpoint was chosen because it is considered to be a more conservative delineation of toxicity than the *Minimum Detectable Differences* (MDD's) also determined in this study. The MDD's calculated for all endpoints in the data set ranged from 11.5 to 20.5% for the lethality endpoint (% survival) and 3.1 to 29.2% for sublethal responses such as growth and reproduction (with the exception of the data for *Hexagenia* growth in which a MDD could not calculated).

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 would translate to a 36 to 57% reduction in the fecundity of the species (Cooper 1965; France 1992).

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 the absence 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.. Based on these considerations, the limits set for the determination of the toxicity of fine-grained sediments in the Great Lakes (Table 4.20) are both conservative yet realistic estimates.

Setting guidelines for toxicity at a test Site

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). 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 two SD 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 SD below the reference data base mean, a score of 2 or more will be given. A score of 4 is indicative of a non-toxic sediment and a score of 12 severe toxicity. If one test indicates potential toxicity the score will be 6:

Acute Toxicity Score = Cr_{su} score + Ha_{su} score + Hl_{su} score + Tt_{su} score

Using the rationale described for the invertebrate assemblage structure we have examined the range of scores at reference sites for which all tests are available and calculated the average score, and the range. Using 2 SD as the normal range one can expect at reference sites, then sites

equivalent to reference will score either 4 or 5. The remaining range from 6-12 we have divided equally into potentially toxic, toxic and severely toxic bands (Table 5.1). The same approach has been used to rank the 6 chronic endpoints, growth of *H. azteca*, *C. riparius* and *Hexagenia* and % hatch, number of cocoons per adult worm and number of young per adult worm, based on the chronic toxicity score calculated:

Chronic toxicity score = $Cr_{gw} + Ha_{gw} + Hl_{gw} + Tt_{hatch} + Tt_{cc/ad} + Tt_{yg/ad}$.

Table 5.1. Toxicity bands based on scores for individual test endpoints established from 166 reference sites.

	Acute Scores	Chronic scores
Mean	4.2	6.3
SD	0.5	0.6
Bands		
Non toxic	4 - 5	6 - 8
Potentially toxic	6 - 7	9 - 11
Toxic	8 - 9	12 - 14
Severely toxic	10 -12	15 - 18

5.2 The guidelines in operation

To demonstrate the application of these biological guidelines we present the results from studies undertaken in co-operation with the Ontario Ministry of Environment. Results from two Areas of Concern (AOCs) were examined for benthic invertebrate assemblage structure and toxicity to four species in benthic invertebrates in laboratory toxicity tests. The two AOC were Collingwood Harbour, Georgian Bay, Lake Huron, and Severn Sound, Georgian Bay, Lake Huron.

5.2.1 Collingwood Harbour

In the fall of 1992 and spring 1993, in collaboration with Environmental Protection Branch, Ontario Region, Environment Canada and with the support of the Collingwood Harbour

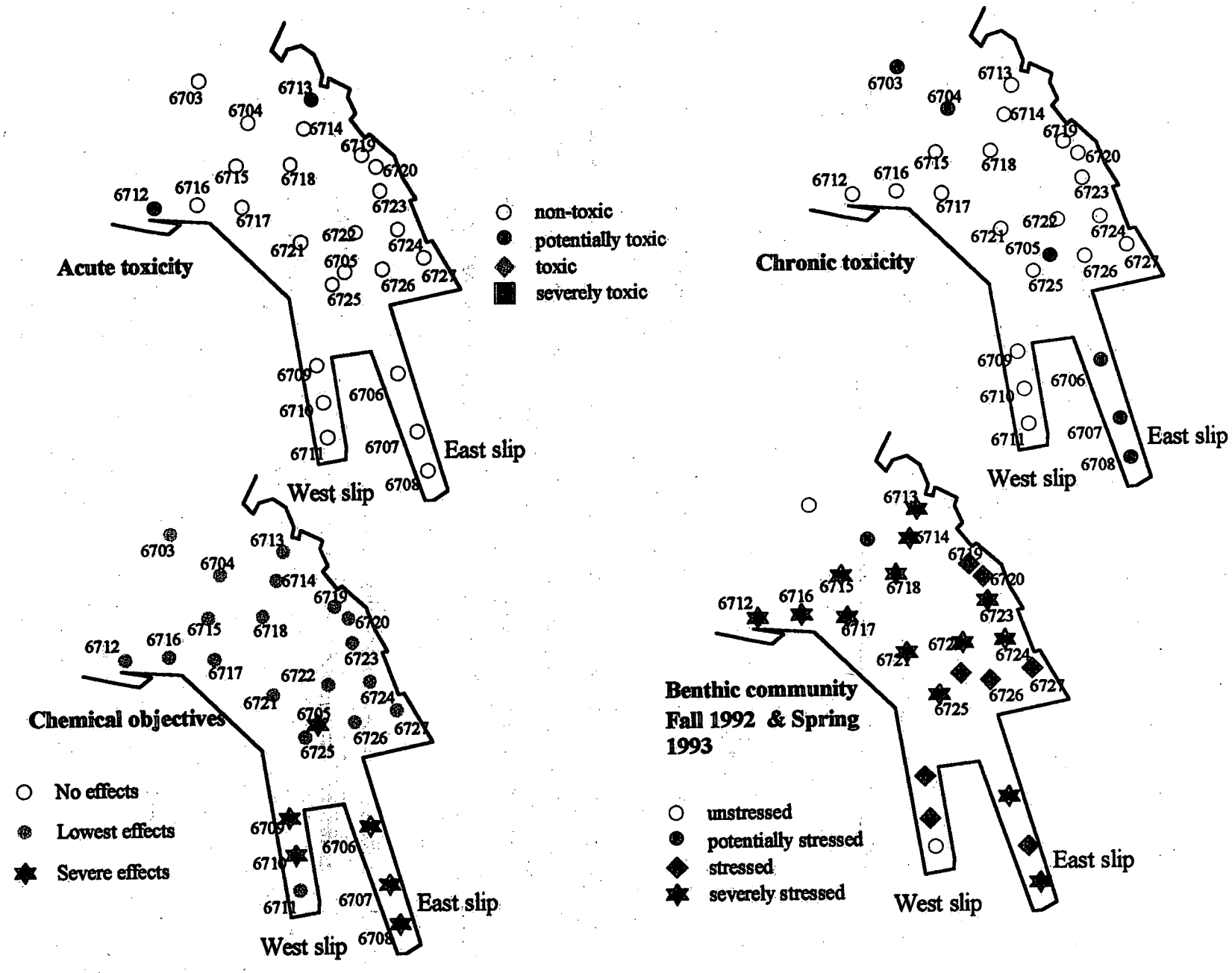


Figure 5.2. Assessment of sediment toxicity and impaired benthic communities in Collingwood H. in Fall 1992 and Spring 1993.

RAP team, extensive sampling of the sediments in Collingwood Harbour was conducted by personnel from NWRI. The data collected included samples for invertebrate assemblage structure, sediment toxicity tests and sediment and water chemistry. Six sites (6706, 6707, 6708, 6709, 6710 and 6711) were located within the east and west boat slips where high metal concentrations had been previously noted. The remaining 19 sites (6703, 6704, 6705, 6712-6727) were located in the inner Harbour (Figure 5.2). The results were assessed using the data rom the 252 reference sites in Lakes Ontario, Erie, Michigan, Superior and Huron, using the methods described previously (Sections 4 and 5). Sediment toxicity scores were calculated and the invertebrate assemblage structure compared to the reference communities. The assessment of the sediment based on these biological guidelines is described below.

5.2.1.1 Sediment chemistry

Selected physical and chemical parameters were measured in sediment collected both before and after dredging and are presented in Table 5.2. The values are averages for sites located in each of the slips and for areas within the inner Harbour. Both the east and west boat slips of Collingwood Harbour had been known previously to be heavily contaminated with metals and these data confirmed that concentrations of copper, zinc and lead were very high in sediments collected from these areas in 1992. Some areas in the inner Harbour also had elevated levels of these contaminants.

Table 5.2. Concentration ($\mu g.g^{-1}$) of selected variables in Collingwood Harbour.

Variable (OMEE severe effect conc.)	Reference Sites	East Slip 1992	West Slip 1992	Inner Harbour 1992/3
Cu (110)	21.5	3042	401	41
Zn (820)	99.3	10750	1401	161
Pb (250)	39.1	802	260	78
Fe_2O_3	4.0	17.4	5.6	3.2
Sand %	19.7	37.5	15.3	11.7
Silt %	51.8	44.1	66.0	68.0
Clay %	27.4	17.2	18.6	20.3
TP	650	1085	1425	937
P_2O_5	0.2	0.02	0.34	0.28

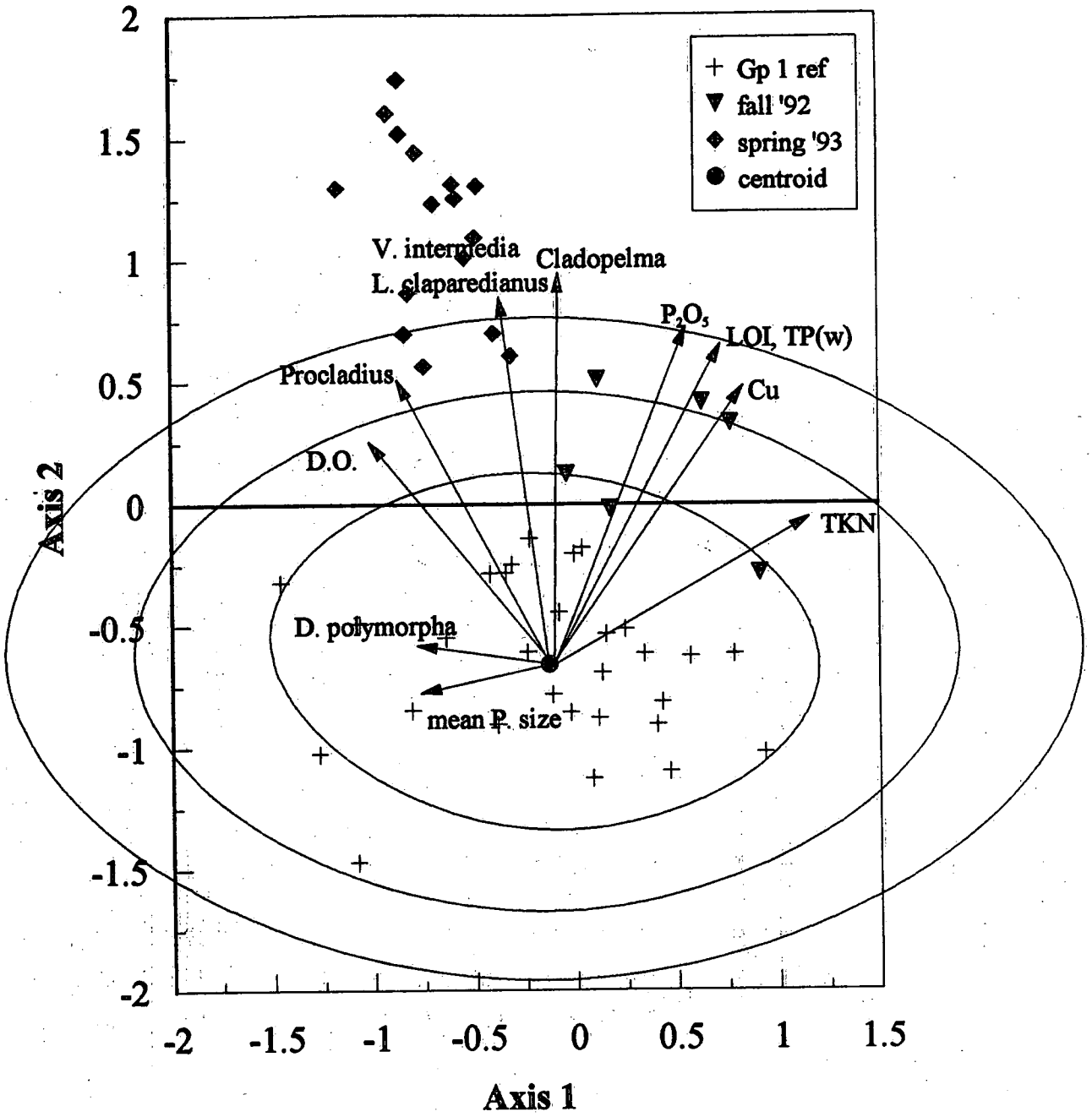


Figure 5.3. Ordination of Collingwood Harbour sites from Fall 1992 and Spring 1993 with reference sites, probability ellipses (90%, 99%, 99.9%) constructed around reference sites only. Taxa and habitat vectors are also illustrated.

Table 5.3. Collingwood H. sites, mean values for toxicity tests endpoints (values below criteria are shown in bold).

Site	CrSu	CrGw	HxSu	HxGw	HaSu	HaGw	TtCc	TtHt	TtSu	TtYg	Lethal	Sub-lethal
6703	88.0	0.38	98	6.87	89.3	0.72	5.6	21.6	95	5.5	4 (non-toxic)	11(potentially toxic)
6704	81.3	0.38	98	8.11	94.7	0.75	6.1	32.8	100	5.7	4 (non-toxic)	9 (potentially toxic)
6705	80.0	0.43	100	5.78	90.0	0.66	5.5	25.0	100	3.6	4 (non-toxic)	11(potentially toxic)
6706	72.0	0.39	98	5.62	93.3	0.50	6.9	27.9	100	5.6	4 (non-toxic)	9 (potentially toxic)
6707	86.6	0.33	100	6.17	90.7	0.42	6.5	30.2	100	7.2	4 (non-toxic)	9 (potentially toxic)
6708	82.6	0.36	94	5.35	94.7	0.53	6.3	25.7	100	5.6	4 (non-toxic)	10 (potentially toxic)
6709	68.0	0.46	100	3.86	94.7	0.53	9.1	35.8	100	10.6	5 (non-toxic)	8 (non-toxic)
6710	78.6	0.40	100	5.04	88.0	0.60	8.0	38.0	100	10.6	4 (non-toxic)	7 (non-toxic)
6711	85.3	0.40	100	4.56	84.0	0.50	7.2	46.2	100	3.6	4 (non-toxic)	7 (non-toxic)
6712	89.3	0.52	80	10.12	94.7	0.70	6.7	30.0	60	25.8	7 (potentially toxic)	8 (non-toxic)
6713	76.0	0.51	100	10.93	88.0	0.66	8.7	40.5	80	25.1	6 (potentially toxic)	6 (non-toxic)
6714	86.6	0.61	100	10.59	84.0	0.74	11.4	56.6	100	33.8	4 (non-toxic)	6 (non-toxic)
6715	85.3	0.49	100	10.94	82.7	0.76	10.8	53.2	100	31.5	4 (non-toxic)	6 (non-toxic)
6716	90.6	0.65	100	10.58	89.3	0.79						
6717	89.3	0.64	94	10.40	86.7	0.87	10.8	57.5	100	38.4	4 (non-toxic)	6 (non-toxic)
6718	86.6	0.66	98	11.16	93.3	0.82	11.6	54.9	100	36.7	4 (non-toxic)	6 (non-toxic)
6719	88.0	0.45	100	8.56	92.0	0.62	10	46.2	100	18.6	4 (non-toxic)	6 (non-toxic)
6720	90.6	0.56	100	6.70	89.3	0.74	10.9	35.0	100	9.2	4 (non-toxic)	8 (non-toxic)
6721	84.0	0.58	98	9.97	89.3	0.74	11.2	81.3	100	29.3	4 (non-toxic)	6 (non-toxic)
6722	88.0	0.55	98	9.11	77.3	0.71	10.7	42.7	100	18.6	4 (non-toxic)	6 (non-toxic)
6723	96.0	0.35	100	7.98	90.7	0.71	9.8	37.6	100	10.7	4 (non-toxic)	8 (non-toxic)
6724	76.0	0.51	98	8.53	90.7	0.66	10.6	33.2	100	15.6	4 (non-toxic)	7 (non-toxic)
6725	90.6	0.37	84	8.34	94.7	0.61	9.6	51.0	100	19.4	5 (non-toxic)	6 (non-toxic)
6726	92.0	0.34	96	7.98	72.0	0.75	9.8	47.4	100	12.2	4 (non-toxic)	6 (non-toxic)
6727	92.0	0.38	98	6.28	85.3	0.56	9.4	35.7	95	11.4	4 (non-toxic)	7 (non-toxic)
No. above non-toxic	1	10	2	25	0	2	7	13	0	12		

5.2.1.2 Sediment toxicity

Sediment bioassays were conducted according to the protocols described in section 3.4. The actual results for each endpoint are presented in Table 5.2 and we have also determined the score for both acute and chronic toxicity tests (see section 5.1.2). Of the four test species, *H. azteca* showed the least response, only two sites were outside the normal range, and in both cases growth was slightly enhanced. Both *C. riparius* and *H. limbata* showed enhanced growth. Only the worm *T. tubifex* indicated consistent negative effects. Cocoon production was reduced at seven sites, hatching was reduced at 13 sites and the number of young produced was reduced at 12 sites. This suggest that the sediment was primarily affecting egg maturation and inhibiting embryogenesis. Based on scoring the toxic responses (Table 5.3) two sites indicated potential toxicity based on acute endpoints, and six sites based on chronic endpoints. These sites are shown in Figure 5.2, and are located in both the inner harbour and the east slip.

5.2.1.3 Invertebrate assemblage structure

Forty-three species of benthic invertebrates have been identified from the 25 stations sampled in Collingwood Harbour. Two classes of oligochaetes (worms), the Naididae and the Tubificidae, are the dominant groups of organisms found in the area followed by the Porifera (sponges) and the Chironomidae (midge larvae). Each of the other groups of organisms comprise less than 5% of the total number of organisms found.

The condition of the benthic invertebrate assemblage in the Harbour was determined by the steps outlined in Figure 1. The predictive models described in Section 4 were used with the habitat data from Collingwood and the 25 sites predicted to one of the six groups established for the Great Lakes (Table 5.4). The similarity of the Collingwood sites to the reference sites was determined by plotting the reference sites and the Collingwood Harbour sites in ordination space, as described in Section 5.1.1 (Figure 5.1). The location of a test site is a measure of its similarity to a group of reference sites. Sites were assigned to one of four stress bands by their proximity to the reference group.

Those sites predicted to Gp 1 (Table 5.4) are shown to illustrate the process used to asses community structure (Figure 5.3).

Table 5.4. Collingwood H. sites, assessment of invertebrate assemblage structure and environmental attributes.

Site	Predicted	Probability	Status	Potential stressors: - within 1SD of referene mean, + more than 1 S.D away from reference mean, ++ more than 2 SD away from reference mean			
				Nutrient	Metal	Physical	Season
6703	1	0.517	unstressed	-	-	-	spring
6704	1	0.627	potentially stressed	-	-	-	spring
6705	1	0.676	stressed	+	++	-	spring
6706	4	0.839	severely stressed	+	++	-	spring
6707	4	0.970	stressed	+	++	-	spring
6708	4	0.812	severely stressed	+	++	-	spring
6709	1	0.343	stressed	+	++	-	spring
6710	1	0.558	stressed	+	++	-	spring
6711	1	0.532	unstressed	+	++	-	spring
6712	1	0.416	severely stressed	-	-	-	spring
6713	1	0.686	severely stressed	-	-	-	fall
6714	1	0.582	severely stressed	-	-	-	fall
6715	1	0.570	severely stressed	-	-	-	fall
6716	1	0.590	severely stressed	-	+	-	fall
6717	1	0.549	severely stressed	+	+	-	fall
6718	1	0.548	severely stressed	-	-	-	fall
6719	1	0.512	stressed	-	+	-	fall
6720	1	0.475	stressed	-	+	-	fall
6721	1	0.555	severely stressed	-	-	-	fall
6722	1	0.477	severely stressed	-	+	-	fall
6723	1	0.485	severely stressed	-	+	-	fall
6724	1	0.573	severely stressed	-	+	-	fall
6725	1	0.683	severely stressed	-	+	-	fall
6726	1	0.545	stressed	-	+	-	fall
6727	1	0.447	stressed	-	+	-	fall

Only two sites were identified as unstressed (Figure 5.3), Site 6703 the site furthest from the contaminated boat slips and Site 6711, the least contaminated site in the slips. The other sites sampled in the Fall of 1992, show a trend of increasing stress moving toward the boat slips, particularly the more highly contaminated East Slip (Figure 5.3). All 16 sites sampled in the Spring of 1993 were either stressed or highly stressed.

However, from their response (Figure 5.3) the change in the benthic assemblage is different to that observed in the fall samples. The position of these sites in ordination space shows a gradient along one ordination vector, associated with two species, *Vejdovskyella intermedia* and *Limnodrilus claparedianus*. These two oligochaete worms appear in higher numbers than at the refrence sites, in the case of *Vejdoskyella*, the mean abundance at reference sites is 0.17 per core, at the Collingwood sites in the Spring 1993 samples numbers ranged from 5.6 - 219.9 per core.

Examination of the relationship of the environmental variables to the community structure (Figure 5.3), based on the orientation of environmental vectors in ordination space, supports the interpretation that the invertebrate assemblage is responding to nutrient enrichment as well as metal contamination. The data show that both metal vectors (Cu in Figure 5.3) and nutrient vectors are oriented similarly (TPw and TKN). We have also categorised habitat stressors as either nutrient related (e.g. Total Phosporus, nitrate-nitrite in the water), metals (metal levels in the sediment) or physical (particle size). If these are outside the range observed at the reference sites this may indicate that type of stress. Again it is difficult to discriminate between nutrient effects and metal stress as many sites show both to be possible (Table 5.3).

5.2.1.4 Interpretation of Results

None of the sites meet all the criteria using the Ontario chemical sediment guidelines (Table 2.2) for defining sediment impairment (Persaud *et al.* 1992), and a number of sites, in the east slip (6706, 6707, 6708) and one in the west slip (6709), exceed the low effects criteria for all variables and the severe effects criteria (Figure 5.2) for some of the variables (e.g., copper, zinc, arsenic, etc.). Using the methods developed in this study, there was good concordance between the chemical and biological data for the most severely contaminated sites i.e., both the in situ data on community structure and the data from the laboratory toxicity tests indicated toxicity at the sites with the highest concentrations of contaminants. While the community structure indicates stressed or

Table 5.5. Summary of biological sediment assessment for Collingwood Harbour.

Site	Assessmentcommunity	Assessment lethality	Assessment chronic toxicity	Assessment Nutrients	Metals
6703	unstressed	non-toxic	potentially toxic	-	-
6704	potentially stressed	non-toxic	potentially toxic	-	-
6705	stressed	non-toxic	potentially toxic	+	++
6706	severely stressed	non-toxic	potentially toxic	+	++
6707	stressed	non-toxic	potentially toxic	+	++
6708	severely stressed	non-toxic	potentially toxic	+	++
6709	stressed	non-toxic	non-toxic	+	++
6710	stressed	non-toxic	non-toxic	+	++
6711	unstressed	non-toxic	non-toxic	+	++
6712	severely stressed	potentially toxic	non-toxic	-	-
6713	severely stressed	potentially toxic	non-toxic	-	-
6714	severely stressed	non-toxic	non-toxic	-	-
6715	severely stressed	non-toxic	non-toxic	-	-
6716	severely stressed			-	+
6717	severely stressed	non-toxic	non-toxic	+	+
6718	severely stressed	non-toxic	non-toxic	-	-
6719	stressed	non-toxic	non-toxic	-	+
6720	stressed	non-toxic	non-toxic	-	+
6721	severely stressed	non-toxic	non-toxic	-	-
6722	severely stressed	non-toxic	non-toxic	-	+
6723	severely stressed	non-toxic	non-toxic	-	+
6724	severely stressed	non-toxic	non-toxic	-	+
6725	severely stressed	non-toxic	non-toxic	-	+
6726	stressed	non-toxic	non-toxic	-	+
6727	stressed	non-toxic	non-toxic	-	+

severely stressed communities at many sites, more careful examination of these data together with the laboratory toxicity tests and chemistry suggest that:

•the divergence from reference state in the Spring 1993 is largely a seasonal effect due to large numbers of one species of oligochaete;

•that the only sites which community structure and toxicity tests corroborate a sediment related stress are 6704, 6705, the East slip and sites 6712 and 6713;

•that despite very high contaminant levels (e.g., Cu concentration in the east Slip ranged from 2121 - 4170 ug/g and Zn from 7527 - 13943 ug/g), much of this material was

not bioavailable, either because of nutrient enrichment which leads to binding to organic material, or the form of the metal .

The fact that these biological guidelines are available allowed a specific assessment of the risk posed by contamination that cannot be made using chemical guidelines, and in the case of Collingwood Harbour reduced the area where remediation or restoration would be required from the entire inner harbour to one boat slip and two small nearshore areas.

5.2.2 Severn Sound

Sampling was conducted in August 1994 at 21 sites in the vicinity of Penetanguishene, one of the Great Lakes Areas of Concern (Figure 5.4). Again samples were taken for community structure assessment and sediment toxicity. Habitat attributes were also measured.

Table 5.6. Summary of biological sediment assessment for Severn Sound.

Site	Gp / Probability	Assessment - community	Assessment - acute toxicity	Assessment - chronic toxicity
6728	2 / 0.433	unstressed	not done	not done
6730	3 / 0.893	stressed	non-toxic	potentially toxic
6732	3 / 0.923	potentially stressed	non-toxic	non-toxic
6735	2 / 0.515	unstressed	non-toxic	non-toxic
6736	2 / 0.776	unstressed	non-toxic	non-toxic
6737	2 / 0.601	unstressed	non-toxic	non-toxic
6739	3 / 0.604	potentially stressed	non-toxic	non-toxic
6740	3 / 0.794	unstressed	potentially toxic	non-toxic
6742	3 / 0.929	unstressed	non-toxic	non-toxic
6743	3 / 0.904	unstressed	non-toxic	non-toxic
6745	3 / 0.769	potentially stressed	non-toxic	non-toxic
6746	2 / 0.844	unstressed	non-toxic	non-toxic
6747	2 / 0.783	unstressed	non-toxic	non-toxic
6749	3 / 0.913	stressed	non-toxic	potentially toxic
6752	3 / 0.999	unstressed	toxic	non-toxic
6754	3 / 0.758	stressed	non-toxic	potentially toxic
6755	3 / 0.817	potentially stressed	non-toxic	potentially toxic
6756	3 / 0.851	potentially stressed	non-toxic	potentially toxic
6761	3 / 0.966	potentially stressed	potentially toxic	potentially toxic
6763	3 / 0.895	unstressed	non-toxic	non-toxic
6764	2 / 0.542	unstressed	non-toxic	non-toxic

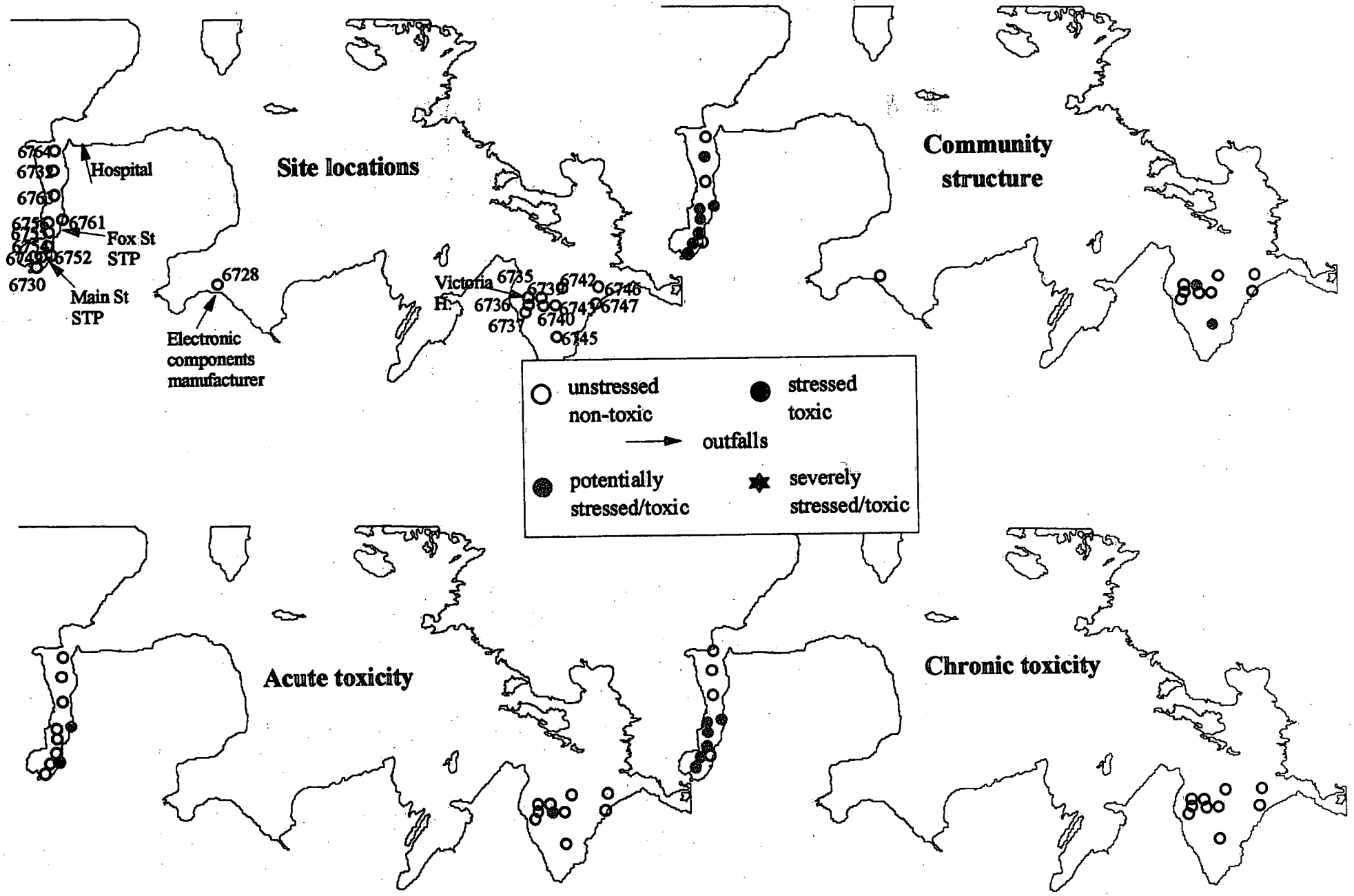


Figure 5.4. Assessment of sediment toxicity and impaired invertebrate communities in Severn Sound in September 1994.

The results for sediment toxicity and community structure are summarised in Table 5.6, using the methods described for Collingwood. Sites are designated as being either: 1 - unstressed/non-toxic; 2 - potentially stressed/toxic; 3 - stressed/toxic, or; 4 - severely stressed/toxic. Three sites are identified as having stressed communities (Table 5.6), all in lower Penetanguishene Bay, where two outfalls are located (Figure 5.4) discharging effluent from the Fox St and Main St sewage treatment plants. At all three sites sediments were identified as potentially toxic, because of reduced hatching and young production of *T. tubifex* (6730, 6749, 6754) and reduced survival of *H. azteca* (6749). Six sites showed potentially stressed communities, four in Penetanguishene Bay, suggesting a response gradient from the outfalls, and two in Sturgeon Bay, in the vicinity of the Victoria Harbour WPCP outfall. One site adjacent to the Victoria H. WPCP outfall showed potential acute toxicity (Figure 5.4) because of reduced survival of *H. azteca* (57.4% survival). All six sites showing chronic potential toxicity were located in Penetanguishene Bay (Figure 5.4), in the vicinity of the Main St and Fox St outfalls, the toxicity was a result of reduced hatching and young production of *T. tubifex*. The other responses were reduced survival of *H. azteca* and *C. riparius* (6752).

Table 5.7. Abundance (no. per core - 34.2cm^2) of selected species at reference sites and Severn Sound test sites.

Taxa	Gp 3 mean (reference)	6730	6749	6754	6755	6756	6761	6763
D. hoyi	0.27	0	0	0	0	0	0	0
Heterotrissocladius sp	0.02	0	0	0	0	0	0	0
P. casertanum	0.99	2.4	0	0	3.8	2.6	0.2	2.2
Chironomus spp	0.85	0.6	0.2	0	0.4	0.2	0	0.2
Procladius spp	3.18	1.2	12.2	11.2	8.4	5.8	1	6
M. speciosa	1.05	1.2	0	132	62	33.6	0	7

The impacts observed in Severn Sound can be clearly related to the sewage outfalls, particularly the two in lower Penetanguishene Bay. The three sites closest to the Main St outfall have stressed communities that correspond to contaminated sediments as

shown by the potentially toxic response at the six sites in the same area and acute toxicity observed at the site closest to the Main St outfall (6752). The main response of the assemblage is loss of those species more associated with oligotrophic conditions (e.g. *D. hoyi, Heterotrissocladius* spp) and increased numbers of those species associated with more eutrophic conditions, e.g., the clam *P. casertanum*, the midges *Procladius* spp, *Chironomus* spp and the polychaete worm *M.speciosa* (Table 5.7).

Examination of some of the environmental data from these sites (Table 5.8) shows that at the sites with stressed communities (6730, 6749, 6754) there was little indication of any particular sediment-associated variable being elevated above background value. From the examination of the response of sites relative to the reference sites in ordination space, the stressed communities are associated with a Kjeldahl Nitrogen vector (Figure 5.5). Zinc, which was elevated (478 ug/g) at the one site (6752) where acute toxicity was observed, seems to show no relationship with the community response.

In summary, there is evidence of a slight toxic response in the vicinity of the outfalls in Penetanguishene Bay, however the major response in the resident invertebrate communities appears to be associated with enrichment and eutrophication.

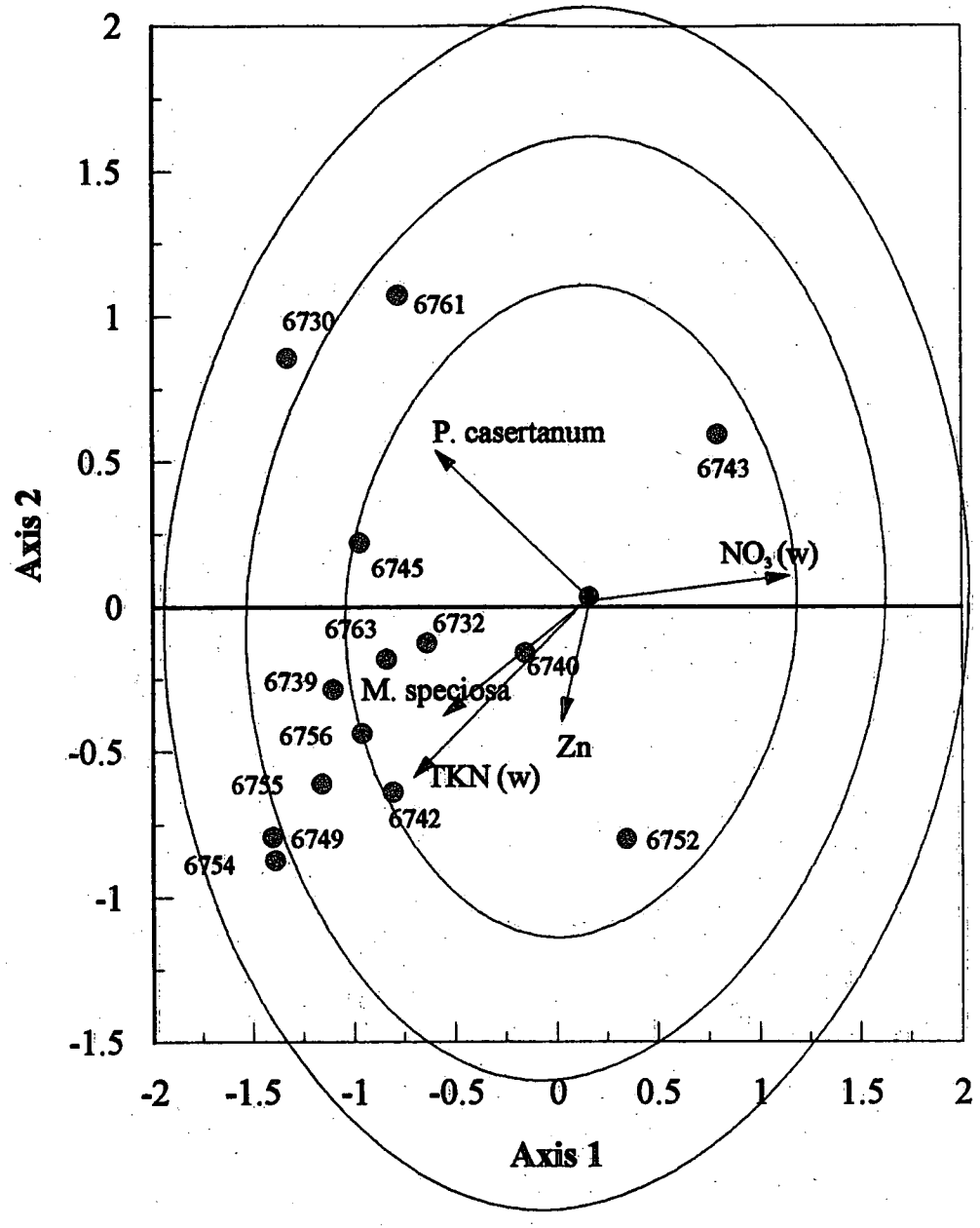


Figure 5.5. Ordination of Severn Sound sites predicted to Gp 3, probability ellipses (90%, 99%, 99.9%) constructed around reference sites only (reference sites not shown). Taxa and habitat vectors are also illustrated.

Table 5.8. Values for selected environmental variables for Severn Sound and matched reference sites.

Variables	Gp 3 Reference sites	6730	6732	6739	6740	6742	6743	6745	6749	6752	6754	6755	6756	6761	6763
Nutrient	Mean (SD) n=64														
T P (mg/L)	0.02 (0.01)	0.04	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.04	0.03	0.04	0.03	0.03	0.02
NO3 (mg/L)	0.13 (0.11)	0.02	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.14	0.03	0.09	0.07	0.01	0.02
LOI (%)	12.05 (6.84)	19.49	16.73	10.21	14.44	16.92	14.98	15.41	18.4	36.08	12.5	14.1	15.22	29.22	17.92
TOC (%)	3.00 (2.08)	7.01	5.79	3.69	3.77	5.88	5.27	5.54	5.48	12.27	4.97	5.61	5.85	12.84	6.16
TP (ug/g)	799 (910)	964	964	925	846	885	772	822	792	2820	941	828	927	1018	540
TN (ug/g)	3020 (2164)	6267	5467	3704	4694	5615	5794	5997	5409	16240	4695	5062	5239	6145	4505
DO (mg/L)	7.78 (1.60)	12	5.4	10.2	10.7	9.3	9.1	10.3	10.1	10.7	7.9	8.5	7.8	11	9.6
Metals															
As (ug/g)	9.4 (8.4)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Cu (ug/g)	26 (15)	34	40	17	21	28	23	21	37	283	40	42	42	35	46
Ni (ug/g)	50 (50)	21	36	20	25	34	26	23	23	23	23	29	29	24	38
Zn (ug/g)	147 (84)	159	196	106	132	177	144	124	143	478	152	172	169	197	216
Pb (ug/g)	50 (29)	50	81	23	30	40	33	26	58	121	69	80	71	60	96
Cr (ug/g)	55 (29)	58	127	47	50	61	53	50	62	82	59	77	94	57	167
Cd (ug/g)	1.0 (0.7)	0.8	0.9	0.6	0.4	0.4	0.6	1	0.4	3.1	1	1.2	1	1	1.5
Physical															
% sand	30.12 (35.82)	7.62	0	24.33	2.31	0.53	3.53	24.82	8.4	22.01	9.04	8.11	6.23	34.64	10.45
% silt	36.22 (24.19)	48.38	34.58	42.39	52.95	45.08	43.94	59.04	68	54.03	70.11	73.06	63.13	39.14	59.38
% clay	32.83 (20.85)	44	65.42	33.28	44.74	54.39	52.53	16.14	23.6	23.95	20.84	18.83	30.64	26.22	30.17
temp.	15.6 (2.9)	21	19.7	19.7	19.7	19.6	19.6	19.5	22.7	21	20.3	19.8	20	20.5	20.6
Response Variables															
tknw (mg/L)	0.26 (0.14)	0.638	0.377	0.391	0.443	0.408	0.413	0.45	0.415	0.494	0.436	0.402	0.4	0.427	0.407

5.3 BEAST Software

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 BEnthic Assessment of SedimenT, the software automates the methodology outlined in this paper. 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.

5.3.1 Conceptual Software Design

The conceptual design for the BEAST calls for seven modules surrounding a central core of data. A method of automating the entry of data to be compared to the reference data base is the first module. Data in the BEAST is stored in an easily accessible, standard format to limit the problems normally associated with complex data sets. Once data to be analyzed by the BEAST have been entered, data handling and statistical modules are required. One module would predict the reference group membership of each test site using established predictor variables. The next is responsible for combining each test site with the appropriate group of reference data. The analysis of a site's assemblage structure is the fourth module, with the final three modules responsible for graphic analysis and comparison of the BEAST results.

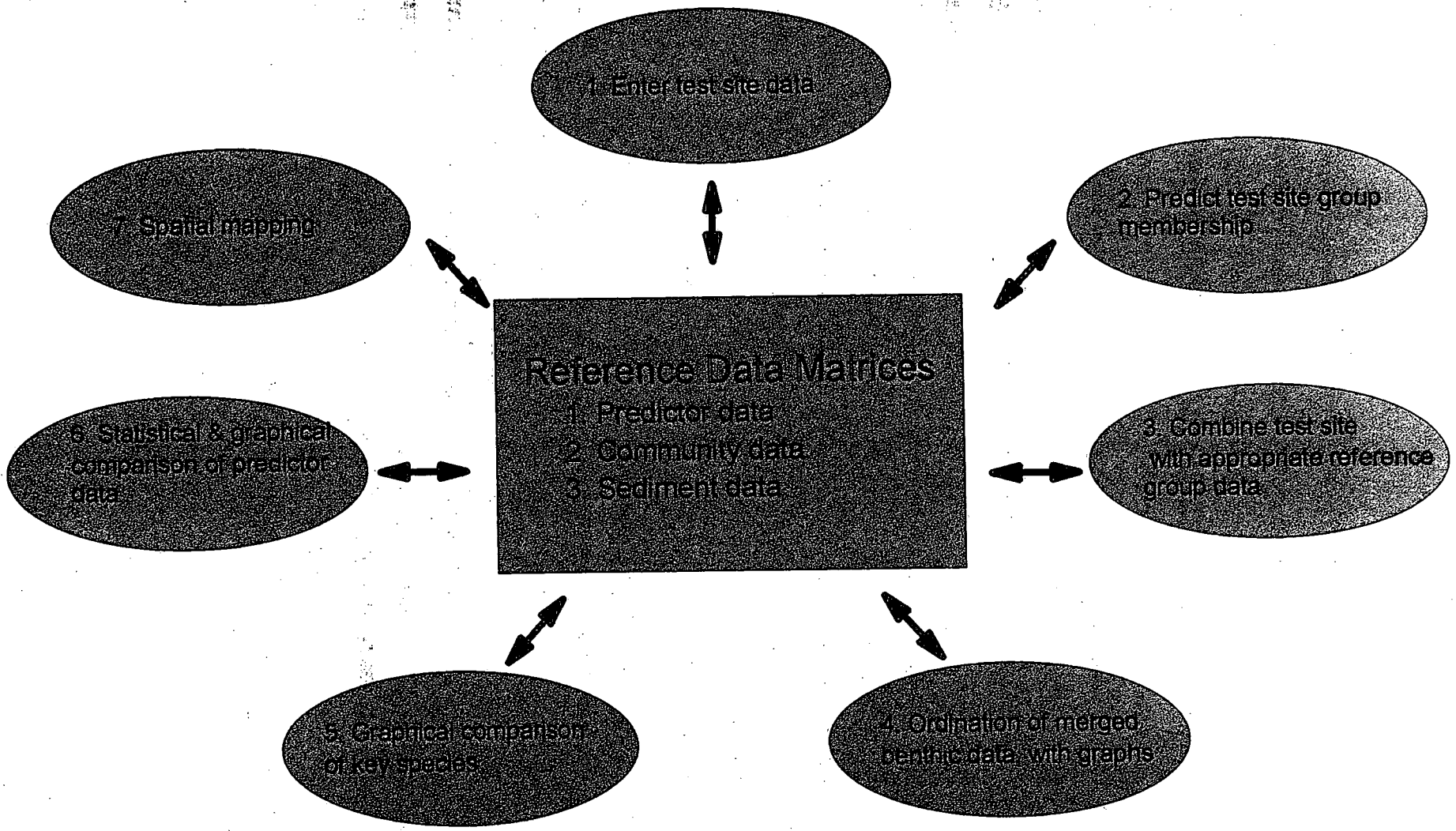


Figure 5.6. Conceptual structure of the BEAST software.

5.3.2 How to use the BEAST

MicrosoftTM Access file formats have been adopted for information storage and retrieval. A widely available Relational Data Base, Access is designed to accommodate the kind of large, complex data sets common to benthic analysis. All of the data related to BEAST operation are stored in this format.

Entry of Test data to the BEAST is achieved through the Benthic Data Information System (BDIS). Developed using MicrosoftTM Access, BDIS is an automated data entry/management tool which provides a simple graphic user interface (GUI). Data entry errors are reduced significantly by providing validation routines to ensure that data fall within acceptable ranges, and conforms to previously established formats and standards.

Unlike may other software packages, the manual generation of complex input files for analysis is also eliminated by BDIS. Test data can be entered and then selected by a user and a data base file containing all of the information, in the proper format for successful BEAST analysis, automatically generated and placed in the appropriate location.

The BEAST is also designed to maintain any number of reference data sets, without the need for continual updating of the software itself. When a new reference data base is developed, the resulting Access file can simply be placed in the same directory as other reference data base files within the BEAST file structure. Once there, it is automatically available for analysis in the BEAST

The BEAST maintains information for various analysis projects in a hierarchical format. The first step in analysis is the creation of a Project with a unique name. Projects in the BEAST act as a container, establishing which reference and test data bases are to used each time the project is opened, and storing the results of any analysis undertaken. Any number of projects can be maintained within the BEAST at any one time, and can be deleted when they are no longer needed.

A project in the BEAST also has a sub-set of containers within it called scenarios. Scenarios represent variations on the analysis of test data contained within a single project.

Although the BEAST supplies an optimal set of predictor variables for Multiple Discriminant Analysis, some cases may occur where these variables are not available to the user. In these cases, the user must employ alternate variables, and the results for each of these discriminant models is stored as a scenario. This permits the user to compare the error rates of various discriminant analyses, and select the most accurate for use in the BEAST.

Results from BEAST analysis can be viewed several different ways. Error Rates and Probabilities of Prediction are generated in a table format. Using the RAISON mapping engine, thematic maps of group membership and toxicology for each site can be produced. Bar graphs comparing key species and environmental variables of a test site to the average of the related reference group can be generated. Finally, bivariate probability ellipse plots showing a test site's location in ordination space with relation to associated reference sites can be displayed.

6. CONCLUSIONS

This report is the first description of an approach for developing site specific guidelines tailored to a specific geographic region. It provides numeric, statistically based, guidelines that incorporate normal variability into the decision making process. The biological measures used for the guidelines were selected to incorporate those that are most likely to be effected by contaminants associated with particulate material. These are the invertebrate fauna residing in the fine grained sediment, and that live in the sediment and ingest sediment particles, and laboratory test species that reside in sediment. The community-based criteria utilise ecological information that relates the species to their environment by means of predictive models that link habitat to community structure. The bioassay criteria are based on the normal response of the test endpoints (survival, growth and reproduction) to normal sediment variability and the guideline values for determining a toxic response account for this heretofore unaccounted variation.

These guidelines address the fundamental question of sediment contamination; is it effecting biological processes? In our opinion this is a major step forward in the management of sediment contamination and will assist in making decisions regarding sediment disposal and the need for remediation. Software developed for the application of these guidelines removes the data and labour intensive statistical steps required to use these guidelines. This software, the BEAST, will be available in the spring of 1998 and will be a major component of a system for setting site specific guidelines for assessing soft sediment contamination in the Laurentian Great Lakes.

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8. APPENDICES

8.1 Workshop participants

8.1.1 Design workshop, January 1991, Cambridge, Ontario

Gary Ankley- US EPA Duluth, Minnesota

Peter Chapman- EVS consultants, North Vancouver, BC

Kristin Day- National Water Research Institute, Environment Canada, Burlington, Ontario

John Giesy- Michigan State University, East Lansing, Michigan

Kees van de Guchte- RIZA, Lelystadt, Netherlnds

Richard Johnson- Swedish EPA, Uppsala, Sweden

Gail Krantzberg- Ontario Ministry of Environment, Toronto, Ontario

Paul Mudroch- Environment Canada, Ottawa, Ontraio

Richard Norris- University of Canberra, Canberra, Australia

David Rosenberg- Fisheries and Oceans Canada, Freshwater Institute, Winnipeg, Manitoba

Alena Mudroch- National Water Research Institute, Environment Canada, Burlington, Ontario

Trefor Reynoldson- National Water Research Institute, Environment Canada, Burlington, Ontario

Michael Zarull- National Water Research Institute, Environment Canada, Burlington, Ontario

8.1.2 Review workshop participants, May 18-22nd 1992, Cambridge, Ontario

Gary Ankley- US EPA Duluth, Minnesota

Robert Bailey- University of Western Ontario, London, Ontario

Amanda Brady- Environment Canada, Ottawa, Ontario

Peter Chapman- EVS consultants, North Vancouver, BC

Kristin Day- National Water Research Institute, Environment Canada, Burlington, Ontario

David Dolan- International Joint Commission, Windsor, Ontario

Richard Johnson- Swedish EPA, Uppsala, Sweden

Gail Krantzberg- Ontario Ministry of Environment, Toronto, Ontario

Paul Mudroch- Environment Canada, Ottawa, Ontario

Richard Norris- University of Canberra, Canberra, Australia

David Rosenberg- Fisheries and Oceans Canada, Freshwater Institute, Winnipeg, Manitoba

Alena Mudroch- National Water Research Institute, Environment Canada, Burlington, Ontario

Trefor Reynoldson- National Water Research Institute, Environment Canada, Burlington, Ontario

Richard Scroggins- Environment Canada, Ottawa, Ontario

Michael Zarull- National Water Research Institute, Environment Canada, Burlington, Ontario

8.2 Species list for Great Lakes reference sites.

ORDER	Family	Formal Name
COELENTRATA	HYDRIDAE	*Hydra americana*
AMPHIPODA	GAMMARIDAE	*Gammarus pseudolimnaeus*
	HAUSTORIIDAE	*Diporeia hoyi*
	TALITRIDAE	*Hyalella azteca*
ISOPODA	ASELLIDAE	*Caecidotea intermedius*
		Caecidotea racovitzai
BIVALVIA	DREISSENIDAE	*Dreissena bugensis*
		Dreissena polymorpha
	SPHAERIIDAE	*Musculium partumeium*
		Musculium securis
		Musculium transversum
		Pisidium casertanum
		Pisidium compressum
		Pisidium fallox
		Pisidium ferrugineum
		Pisidium henslowanum
		Pisidium lilljeborgi
		Pisidium nitidum
		Pisidium ventricosum
		Sphaerium nitidum
		Sphaerium simile
		Sphaerium striatum
	UNIONIDAE	*Anodonta grandis*
		Elliptio camplanata
		Elliptio dilatata
		Lampsilis radiata
GASTROPODA	BITHYNIIDAE	*Bithynia tentaculata*
	HYDROBIIDAE	*Amnicola limosa*
		Amnicola walkeri
		Gillia altilis
		Probythinella lacustris
	LYMNAEIDAE	*Fossaria obrussia*
		Physella heterostropha
		Pseudosuccinea columella
	PHYSIDAE	*Physella integra*
	PLANORBIDAE	*Armiger crista*

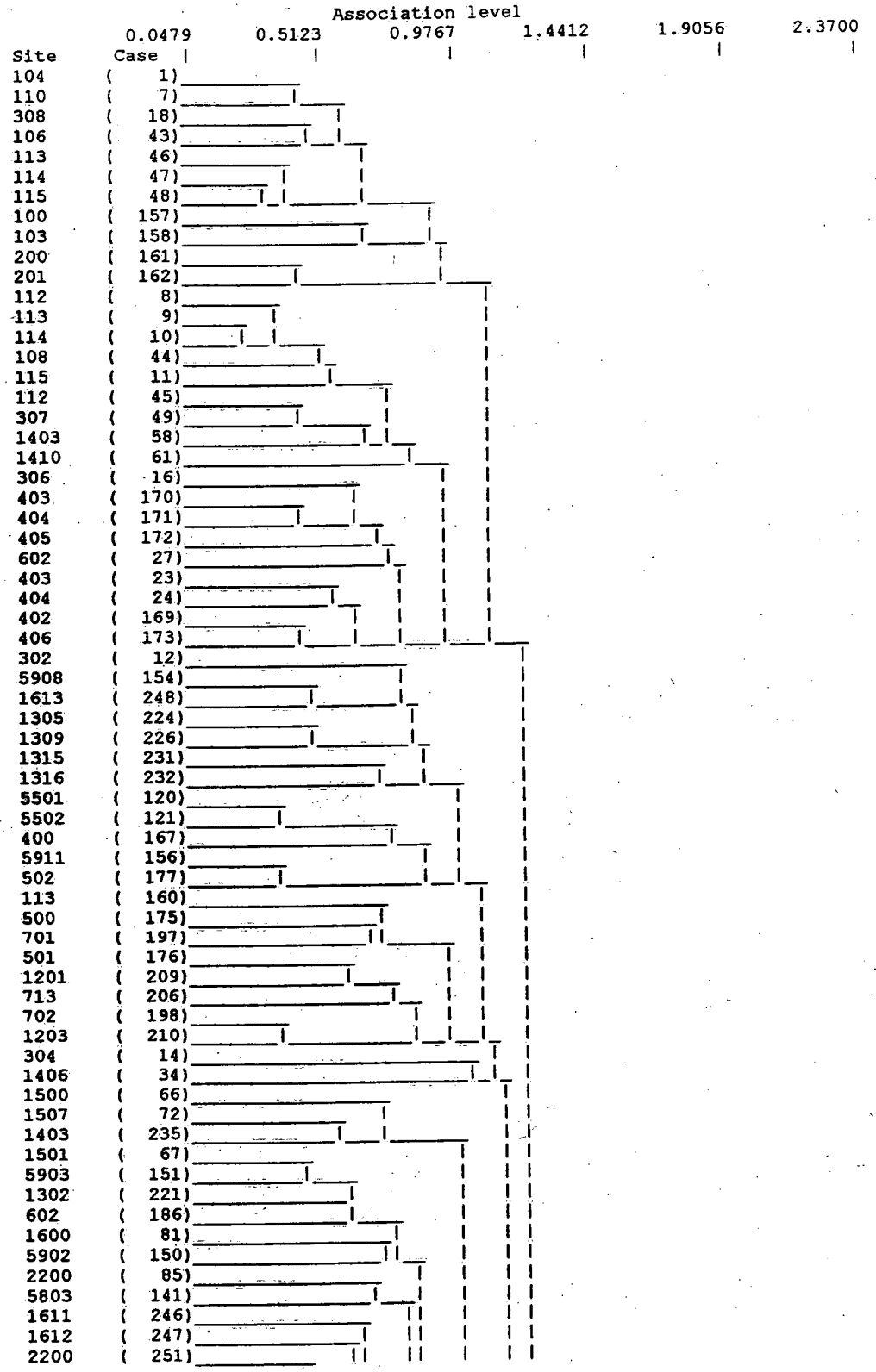
GASTROPODA	PLANORBIDAE	*Gyraulus circumstriatus*
		Gyraulus deflectus
		Helisoma anceps
		Promenetus exacuous
	VALVATIDAE	*Valvata lewisi*
		Valvata piscinalis
		Valvata sincera
		Valvata tricarinata
	VIVIPARIDAE	*Campeloma decisum*
HIRUDINEA	ERPOBDELLIDAE	*Nephelopsis obscura*
	GLOSSIPHONIIDAE	*Alboglossiphonia heteroclita*
		Gloiobdella elongata
		Glossiphonia complanata
		Helobdella stagnalis
	PISCICOLIDAE	*Piscicola milneri*
OLIGOCHAETA	ENCHYTRAEIDAE	*Pristina aequiseta*
	LUMBRICULIDAE	*Eclipidrilus lacustris*
		Lumbriculus variegatus
		Stylodrilus heringianus
	NAIDIDAE	*Arcteonais lomondi*
		Chaetogaster diaphanus
		Dero digitata
		Nais elinguis
		Nais pseudobtusa
		Nais simplex
		Nais variabilis
		Ophidonais serpentina
		Piguetiella michiganensis
		Pristina leidyi
		Pristinella acuminata
		Pristinella osborni
		Ripistes parasita
		Slavina appendiculata
		Specaria josinae
		Stylaria lacustris
		Uncinais uncinata
		Vejdovskyella intermedia
	TUBIFICIDAE	*Aulodrilus americanus*
		Aulodrilus limnobius

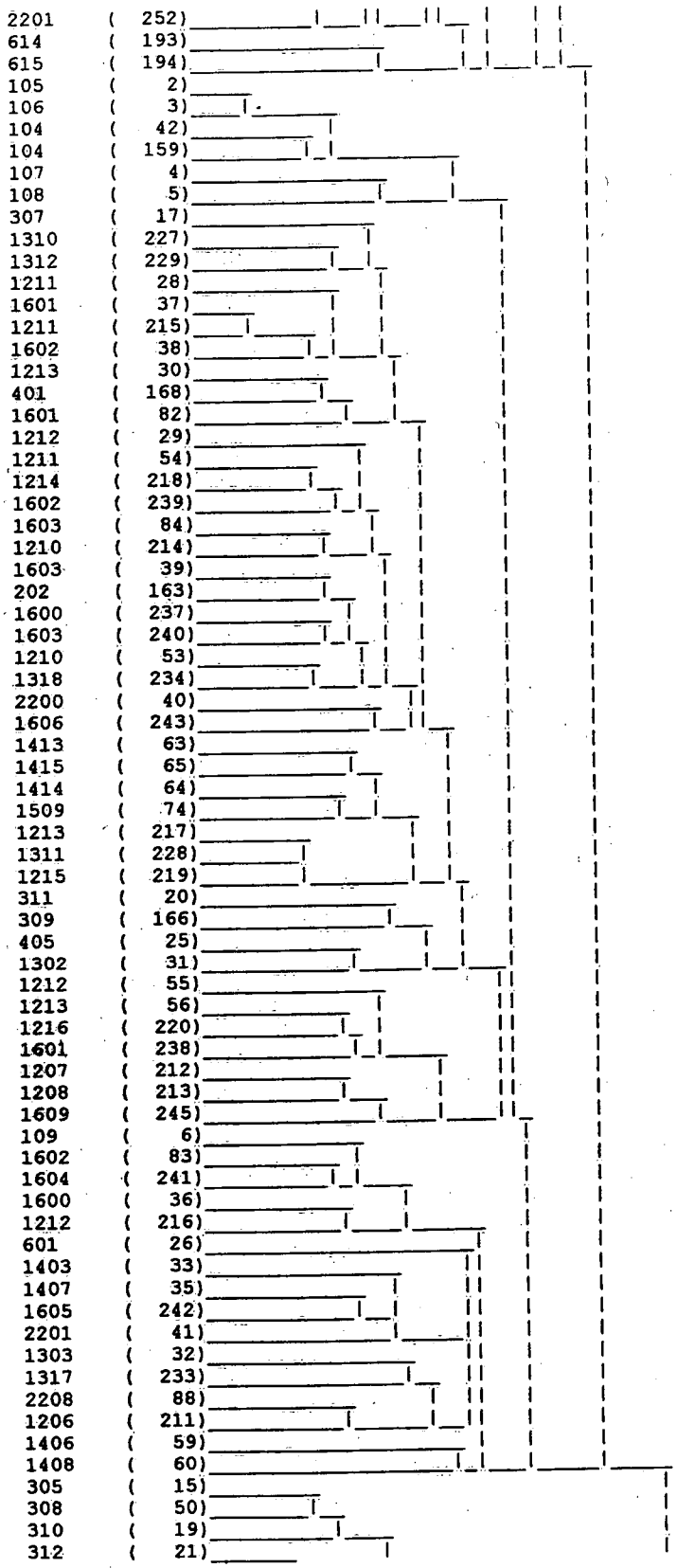
OLIGOCHAETA	TUBIFICIDAE	*Aulodrilus pigueti*
		Aulodrilus pluriseta
		Branchiura sowerbyi
		Ilyodrilus templetoni
		Isochaetides freyi
		Limnodrilus cervix
		Limnodrilus claparedianus
		Limnodrilus hoffmeisteri
		Limnodrilus profundicola
		Potamothrix bedoti
		Potamothrix moldaviensis
		Potamothrix vejdovskyi
		Quistadrilus multisetosus
		Rhyacodrilus montana
		Spirosperma ferox
		Spirosperma nikolskyi
		Tasserkidrilus kessleri
		Tasserkidrilus superiorensis
		Tubifex tubifex
POLYCHAETA	SABELLIDAE	*Manayunkia speciosa*
DIPTERA	CERATOPOGONIDAE	*Bezzia/Palpomyia spp*
		Mallochohelea spp
		Probezzia spp
		Stilobezzia spp
	CHAOBORIDAE	*Chaoborus spp*
	CHIRONOMIDAE	*Ablabesmyia spp*
		Chironomus spp
		Cladopelma spp
		Cladotanytarsus spp
		Clinotanypus spp
		Coelotanypus spp
		Constempellina spp
		Corynoneura spp
		Cricotopus spp
		Cryptochironomus spp
		Cryptotendipes spp
		Demicryptochironomus spp
		Dicrotendipes spp
		Endochironomus spp
		Epoicocladius spp
		Glyptotendipes spp
		Harnischia spp

DIPTERA	CHIRONOMIDAE	*Heterotrissocladius spp*
		Larsia spp
		Micropsectra spp
		Microtendipes spp
		Monodiamesia spp
		Nanocladius spp
		Nilothauma spp
		Pagastiella spp
		Parachironomus spp
		Paracladopelma spp
		Parakiefferiella spp
		Paralauterborniella spp
		Paratanytarsus spp
		Paratendipes spp
		Polypedilum spp
		Potthastia spp
		Procladius spp
		Prodiamesia spp
		Protanypus spp
		Psectrocladius spp
		Pseudochironomus spp
		Stempellina spp
		Stempellinella spp
		Stictochironomus spp
		Tanypus spp
		Tanytarsus spp
		Tribelos spp
		Zavrelia spp
EPHEMEROPTERA	BAETISCIDAE	*Baetisca lacustris*
	CAENIDAE	*Caenis latipennis*
	EPHEMERIDAE	*Hexagenia limbata*
LEPIDOPTERA	PYRALIDAE	*Acentria niveus*
MEGALOPTERA	SIALIDAE	*Sialis spp*
TRICHOPTERA	DIPSEUDOPSIDAE	*Phylocentropus spp*
	HELICOPSYCHIDAE	*Helicopsyche borealis*
	HYDROPTILIDAE	*Orthotrichia spp*

	HYDROPTILIDAE	*Oxyethira spp*
	LEPTOCERIDAE	*Ceraclea spp* *Leptocerus americanus* *Mystacides spp* *Nectopsyche spp* *Oecetis spp*
	LIMNEPHILIDAE	*Limnephilus spp*
	MOLANNIDAE	*Molanna spp*
	PHRYGANEIDAE	*Phryganea cinerea*
TARDIGRADA	MACROBIOTIDAE	*Dactylobiotus spp*

8.3 Dendrogram of 252 Great Lakes Reference Sites

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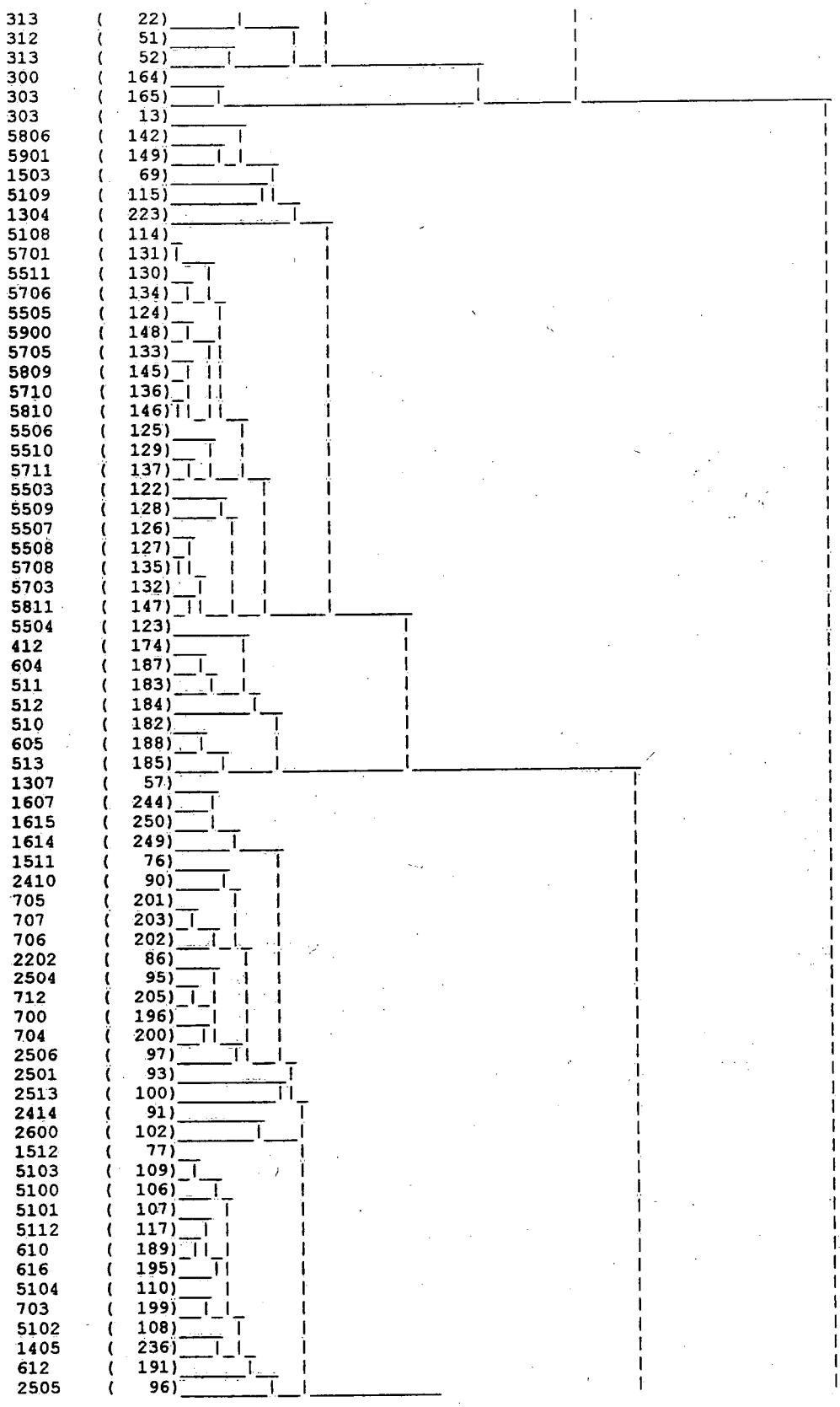
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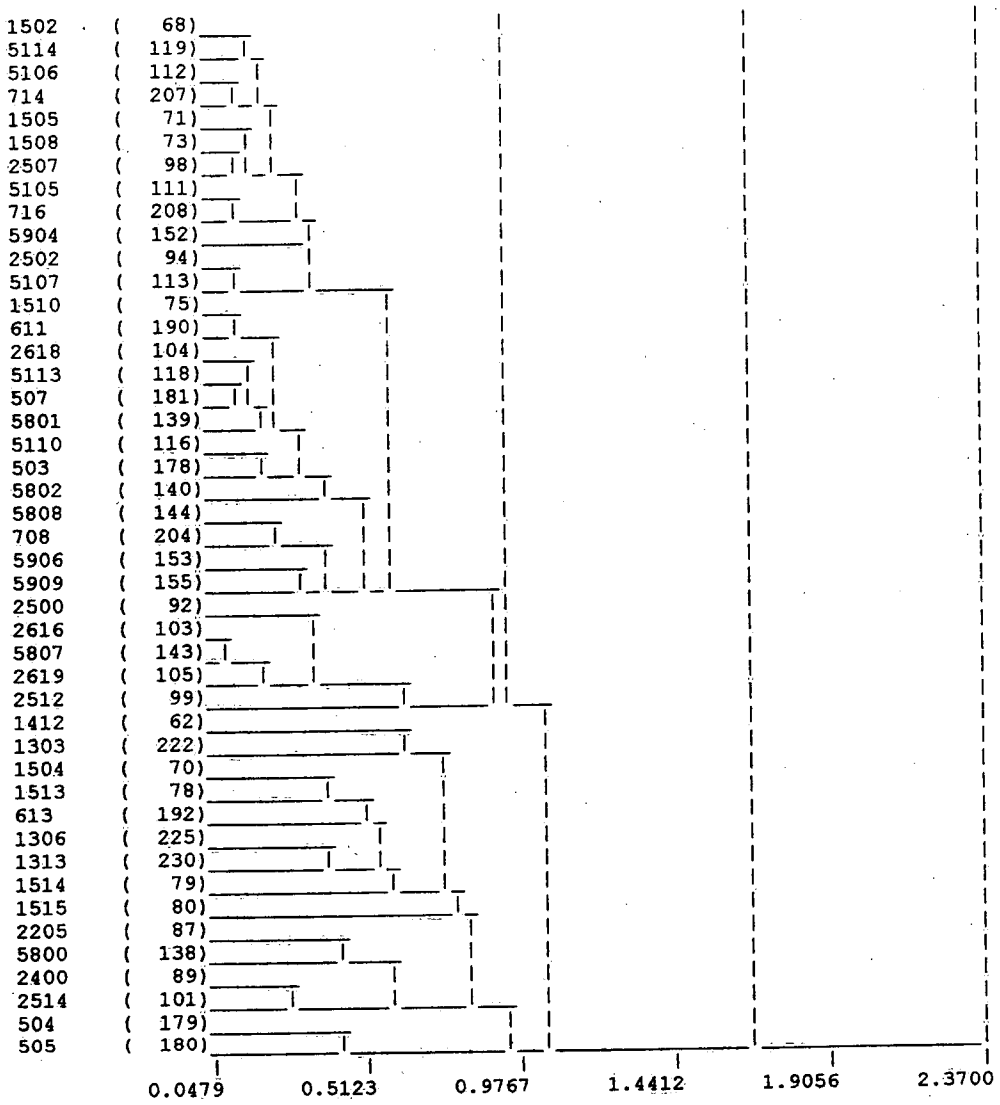
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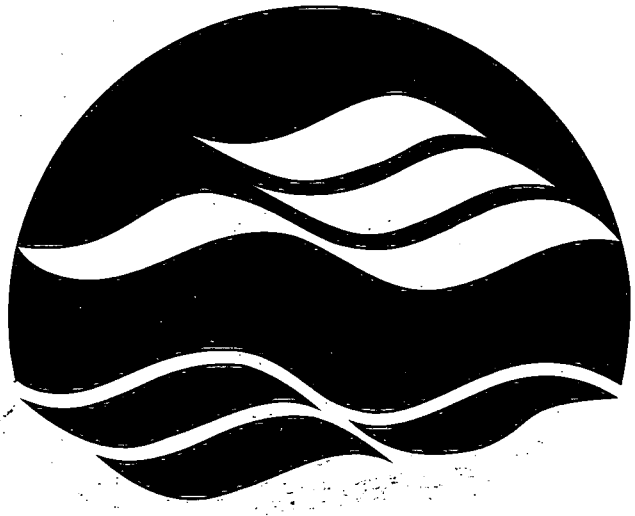

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