DIAGNOSTIC CAPACITY OF LABORATORY MESOCOSMS: RESPONSE OF MACROINVERTEBRATE COMMUNITIES TO SEDIMENT CONTAMINATION

by

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ABSTRACT

There is now widespread recognition that chemical monitoring of the environment is not sufficient given that pollution is essentially a biological phenomenon because of its impact on living organisms. Careful interpretation of the biota, in light of the known ecology of the species involved, is needed to ascertain whether there is impact, and may even indicate the nature of the cause. An approach developed in Great Britain (RIVPACS) provides a method for biological surveillance, conservation, and environmental impact assessment in rivers. It predicts the macroinvertebrate fauna to be expected at a given site based on a small number of environmental features. Using the model, it is possible to predict the benthic community that should occur at a site.

A modified approach to that described in the British Rivers study has been used in the assessment of the Great Lakes. A goal of that study was to predict what the benthic community should look like at a site if it were undisturbed. The approach also provides an appropriate reference for determining the degradation at a site due to anthropogenic contamination. However, when there is divergence from an expected state, as yet it is not possible to define what is/are the causative agent(s). In a site exposed to multiple stressors, no clear statement can be made as to which potential sources should be controlled, other than by inference from chemical analysis.

As divergence from an expected state is due to changes in abundance of species from those predicted, it is reasonable to assume that communities of organisms will have characteristic responses to certain stressors. Therefore, the way in which a site diverges from its expected state may provide useful diagnostic information as to the nature of a stress. This study was intended to complement the predictive model designed for the Great Lakes. The invertebrate communities contained within the sediment of intact box cores, collected from the field and manipulated under laboratory conditions, were used to investigate the directional changes in community composition as a result of contamination.

This study showed that the intact box cores could be maintained in the laboratory with little change in the resident fauna. Despite reductions in the overall abundance of species, which was evident in the separation of the field and control boxes in ordination space, univariate analysis of the most abundant taxa and a number of diversity and richness measures showed that there was little significant change between the field and laboratory boxes on collection, or over time. It was also established that the addition of low levels of nutrients had little effect on the communities.

Clean sieved sediment spiked with cadmium, atrazine or nutrient enriched was added to intact box cores. Upon sampling the boxes, the benthic macroinvertebrate community composition was analysed using the ordination technique Multi-Dimensional Scaling. The consistency and pattern of change in the community composition as a result of the contaminants was identified with respect to the direction in which each treatment moved the community in ordination space. Not all of the contaminants were identified as having an impact when compared to the field data. However, this study did establish that different contaminants did have distinct impacts on community composition.

Intact cores of naturally co-adapted species show potential as a diagnostic tool and are a useful technique for the analysis and identification of sediment contamination. They combine the realism of natural communities from the environment with the power of controlled laboratory experiments. Their use may avoid some of the problems encountered when extrapolating data obtained from *in vitro* studies to natural communities.

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DEDICATION

I dedicate this thesis to my parents for their endless encouragement and support.

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CHAPTER 1

Thesis Outline

1.1 *Current problem*

Efforts to measure and mitigate the impacts of contaminants associated with sediments have arisen from the continuing need to remove and dispose of large volumes of sediment for purposes such as navigation (Reynoldson & Zarull, 1993). The need to quickly and inexpensively assess the potential environmental impacts of dredged material, prior to disposal, resulted in the development of chemical-specific criteria. From these, sediments are usually designated as contaminated on the basis of bulk chemical concentrations. However, the measurement of chemical concentrations in the sediment does not address the question of bioavailability. It is generally believed that it is no longer sufficient to document aquatic pollution in terms of chemical concentrations of the contaminants (Metcalfe, 1989). Although chemical concentrations are important for inventory purposes, from an ecological viewpoint, they are inadequate by themselves as a method of determining and interpreting pollution levels (Chapman & Long, 1983).

Physical, chemical and biological factors controlling the distribution of animals and community composition often act synergistically. When these factors are taken into account, the benthos reflect changing environmental conditions (Barton, 1989). Bulk chemical measurements serve to indicate 'hot spots', where high concentrations of contaminants occur. To provide more direct and conclusive proof of adverse contamination effects, a combination and integration of both chemical and ecological methods are required (Chapman & Long, 1983).

1.2 Recent trends and developments

There is general agreement throughout Europe (Metcalfe, 1989) that there is a need to define reference communities based on chemical, physical and geographical features unrelated to pollution as the first step toward the identification of expected/ best achieved communities for each type of water. An approach developed in Great Britain (Wright *et al.*, 1993), the River Invertebrate Prediction And Classification System (RIVPACS), offers a method for the prediction of macroinvertebrate fauna to be expected at a given site based on a small number of environmental features.

A modified approach to that described by Wright and co-workers (1993) has been used in the sediment assessment of the Great Lakes (Reynoldson and Zarull, 1989; IJC, 1988; 1987). The process relies heavily on the benthic community, and the primary concern is the determination of sediment quality. As a result, a large database has been assembled from reference sites in Lakes Ontario, Erie, Michigan, Superior and Huron. It includes information on the composition of the benthic invertebrate communities, measured environmental variables, and the responses of four species of benthic invertebrates exposed in the laboratory to sediment collected from the same sites.

Reynoldson and co-workers (1995) have shown that the utility of the biological guidelines developed (Reynoldson & Zarull, 1993), and the predictive modelling approaches used, do work. They found that test communities showed expected trends in relation to a range of reference communities.

1.3 Proposed work

Community composition data can be portrayed in a reduced number of dimensions using multivariate ordination techniques, such as Multi-Dimensional Scaling (MDS). In MDS, environmental and biological variables can be related to one another through Principal Axis Correlation (PCC). PCC takes each environmental (or species) attribute and finds the best location of the fitted vector in ordination space. Two pieces of information result:

the direction of the best fit, and the correlation with that direction. Although the correlation coefficient may be used as a rough indicator of the significance of each attribute, the significance of the relationships can be tested through Monte-Carlo permutation tests.

Figure 1.1 gives a diagrammatic representation of an ordination plot. Axis 1 and axis 2 are summaries of the largest and second largest amount of variation in the data set. Hypothetically, the inverted triangles represent samples taken from a reference site and the ellipse its community state. The 3 sites indicated by the crosses, circles and squares were all predicted, based on their environmental conditions, to have a community type similar to that represented by the ellipse. The results represented in figure 1.1a indicate that the sites indicated by the crosses are unimpacted and lie within the defined community type. The circles and squares all lie outside of the ellipse. It can therefore be suggested that there is some stress or impact acting upon these two sites. However, based on this type of analysis no information is provided about the nature of the stresses acting on these two sites, which are obviously quite different.

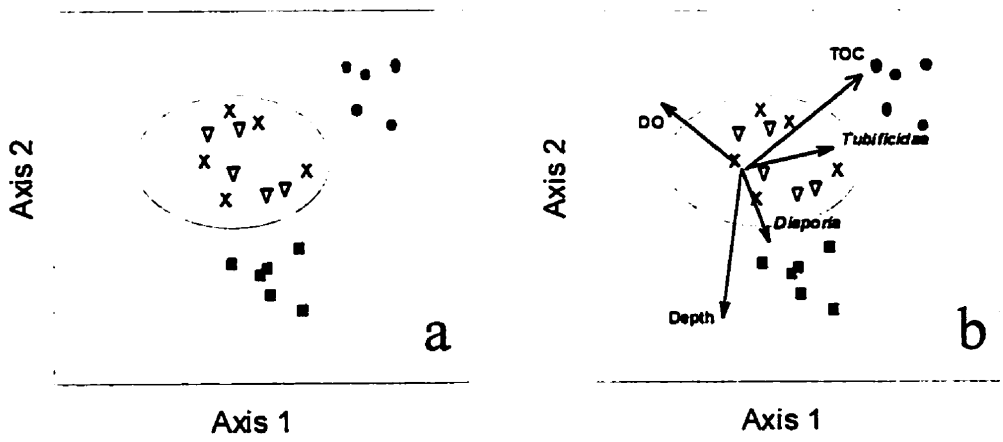

***Fig. 1.1:** Hypothetical ordination plot. Reference group (ellipse), reference sites (inverted triangles), and unknown sites (crosses, squares, and circles).*

In their current form, the guidelines developed in this type of model allow one to determine whether or not the community is impacted (different from the reference community). However, when there is divergence from an expected state, as in figure 1.1a, the causative agents cannot be determined, except by means of correlation with measured environmental variables. Thus, in a site exposed to multiple stressors, no clear recommendations can be made as to the potential sources to be controlled.

Divergence from an expected state is due to differences in the abundance of species and changes in species composition from that predicted. It is, therefore, reasonable to hypothesise that communities of organisms will have characteristic responses to certain stressors. It is believed that the direction in which a site diverges from its expected state may provide useful diagnostic information as to the nature of an impact.

The environmental (and species) variables can be correlated to the position of the sites in ordination space through Principal Axis Correlation (PCC), and their significance identified using Monte-Carlo permutation procedures. These attributes can be displayed on the ordination plot, as illustrated in the hypothetical example (fig.1.1b) in the form of vectors; the arrows indicate the direction in which that environmental variable explains the ordination pattern. Similar vectors can be plotted for the taxonomic data.

The research outlined here is an expansion of the work carried out by Reynoldson and co-workers (1995) to develop a predictive model using benthic macroinvertebrates for the assessment of sediments. It has been shown that community type can be predicted successfully based on the site's environmental variables. The question now arises as to whether changes in community composition can be used to indicate the nature of a stress. An attempt was made in this thesis to address the diagnostic capacity of benthic invertebrate community structure in the assessment of sediment contamination. This was achieved using mesocosms, consisting of intact box cores taken from the field, brought back to the laboratory, and maintained under laboratory conditions. If such methods are to be used successfully, and have any diagnostic capabilities, two questions must be answered:

1. *Can the communities be maintained under laboratory conditions with no significant effect on the resident benthic fauna?*
2. *Do the communities respond predictably and consistently to specific stresses?*

This thesis attempts to answer these questions using boxes collected over two field seasons.

In Chapter 2, an overview of the problem is presented. Current trends in the assessment of sediment contamination, and developments in assessment techniques, are outlined. The utility of indicators in environmental monitoring is introduced, together with the use of multivariate statistical techniques. Both general and specific site descriptions of the area used for this study are given. The community composition of the site and factors influencing the community composition of the site are also considered.

In Chapter 3, an attempt is made to evaluate whether communities within the intact box cores can be successfully maintained under laboratory conditions. To achieve this a number of hypotheses are tested:

- Collection and transportation of the boxes to the laboratory do not significantly alter the community composition, compared to that found in the field.
- The communities within the laboratory boxes do not change significantly over time.
- The communities do not require the addition of nutrients to survive in the laboratory.
- As a result of maintaining the intact boxes at temperatures higher than that observed in field, there are no significant changes in community composition between the laboratory and field boxes.

It has been suggested (Sheehan *et al.*, 1986) that small mesocosms, containing naturally co-adapted communities, exhibit typical ecosystem properties. The ability to maintain such mesocosms would allow sediment contamination to be assessed using the

replicability and statistical power of laboratory techniques while retaining much of the ecological realism of field studies. Multivariate analysis of the complete benthic macroinvertebrate community composition will be compared to the more traditional univariate statistical methods. The most abundant (predominant) species, together with various measures of diversity and richness will be considered in the univariate analyses.

There is a lot of speculation as to the effects of the exotic species *Dreissena* on community composition (Dermott *et al.*, 1993; Griffiths, 1993; Mackie, 1991). *Dreissena* are very patchy in their distribution and tend to migrate to the sides of the intact boxes when maintained in the laboratory. It is therefore important to establish the relationship between *Dreissena* and community composition, if the intact boxes are to be successfully used to evaluate the sensitivity of macroinvertebrate community structure for the assessment of sediment toxicity. The relationship of *Dreissena* has on community composition will also be addressed in this chapter.

It was necessary to make certain assumptions at the beginning of the project. The test boxes and field cores are collected from the same site. It is therefore assumed that upon collection, the boxes are comparable in all physical and chemical aspects and, the community composition of the intact boxes do not differ significantly. Lack of external migration of species, nutrients, and predation is assumed to have negligible impact upon the community structure of the mesocosm, within the time frame of the observations. An attempt is made to address these assumptions, where possible, during the analysis.

Changes in community assemblage as a result of sediment contamination are considered in Chapter 4. The research presented in this chapter is designed to determine whether the communities respond differently to various contaminants. The predictability and consistency of community response to specific stressors is evaluated, and in doing this, the following hypotheses are tested:

- Seasonal changes in community composition of the intact boxes are greater than between- and within-box variation.
- Changes in the community composition caused by the addition of the stressors are greater than between- and within-box variation.
- A consistent pattern, regardless of seasonal effects, is observed in the changes in community composition in ordination space as a result of the different stressors.
- Directional changes in ordination space of the intact boxes can be related to changes in specific species.
- Directional changes in ordination space of the contaminated boxes can be related to changes in the measured environmental parameters.

The utility of the benthic macroinvertebrate community composition for the assessment of sediment toxicity will be addressed using intact box cores. Clean sieved sediment from the sample site will be contaminated with either cadmium, or the pesticide atrazine. Nutrient enrichment will also be applied, as it is perhaps the most common perturbation in Great Lakes sites.

Spatial variation of the community composition is assumed to be similar between and within the boxes. Variation seasonally, and between boxes, will be addressed using univariate statistical techniques. Responses to the different contaminants will be addressed using Multi-Dimensional Scaling (MDS), and displayed graphically in the form of ordination plots. Divergence from the control will be assessed for the treated boxes, and the directional trends, consistent nature and predictability of these divergences considered.

Data from both the 1995 and 1996 sampling seasons will be considered in Chapter 5. The capability of using intact boxes will be assessed using descriptive methods outlined by Reynoldson and Day (1998), to qualify directional changes of community composition as a result of various contaminants. These trends will then be related to the changes in both

species composition and measured environmental variables. In doing this, an attempt will be made to address the diagnostic capacity of the techniques described in this thesis.

The final chapter will tie together the findings of this thesis in a final discussion, highlighting the relationships between sediment quality and community structure and their potential use as a diagnostic tool to predict the type of contamination at a site with an unknown impact. Summary of the methods employed, recommendations and possible future work will also be discussed.

CHAPTER 2

Introduction

2.1 Sediment contamination

Sediments are increasingly being recognised as both repositories and possible sources of contaminants in aquatic systems (Förstner *et al.*, 1986). They tend to become contaminated with both organic and inorganic chemicals, accumulating both nutrients and toxins, which can be the principal cause of environmental degradation in freshwater systems. These contaminants may sorb to particulate matter or remain in solution in the sediment pore-water (Chapman, 1987; Cairns *et al.*, 1984). Sediment contamination is one of the major end results of pollutant discharges into freshwater and marine aquatic environments (Landrum & Robbins, 1990), and has resulted in sediments highly contaminated with metals, persistent toxic organics, and nutrients, representing a significant concern throughout aquatic systems around the world.

Bottom sediments play an important role as sinks in aquatic systems, where a wide variety of chemicals can be stored. Fine-grained sediments, such as silt-clay muds, have a high affinity for agricultural and industrial pollutants, particularly toxic metals (Bolsenga & Herdendorf, 1993). These fine-grained sediments tend to readily sorb with contaminants (Rand, 1995), and thus, together with their high surface area/volume ratio, have the potential for accumulating the highest concentrations of contaminants. However, chemicals are not necessarily fixed permanently in the sediments but may become re-mobilised when physico-chemical conditions change, thus sediments have the potential to be a source of contaminants to the overlying water and to the biota (Calmano *et al.*, 1995;

Baudo & Muntau, 1990). Metals, for example (Förstner *et al.*, 1986), are not necessarily fixed permanently to sediment, but may be recycled via chemical, biological and, physical processes both within the sediment and the water column. Sedimenting particles are subject to degradation, which results in the remobilization of contaminants. These degradation processes may also continue into the sediment layer; causing a difference in the concentrations of some elements between the pore-water and surface waters, and resulting in a flux of metals from the oxic layer in the sediment (Solomons *et al.*, 1987). The mobilisation of accumulated pollutants may also result from anthropogenic influences such as changes in pH due to acid rain (Solomons *et al.*, 1987), reworking of the sediments by benthic organisms and upward mixing of sediments by turbulence (Bennett, 1987).

Fine sediments, known for their ability to absorb both trace metals and relatively insoluble organic compounds (Thomas & Frank, 1987), tend to be deposited in deeper waters where the physical processes necessary to induce resuspension are lacking, or in shallow, low energy areas of restricted circulation. Many such areas are also recipients of urban and agricultural inputs of contaminants. The highest levels of sediment associated contaminants and some of the worst manifestations of their resultant problems are found in these urban-industrial harbours, embayments and river mouths (Zarull & Reynoldson, 1992).

Measurement and mitigation of the impacts of sediment associated contaminants are of particular importance when there is a need to remove and dispose of large volumes of sediment for purposes such as navigation (Reynoldson & Zarull, 1993). From as early as the late 1600's, large vessels have sailed on the Great Lakes (Ashworth, 1991). As vessel size and numbers rapidly increased, new harbours and deeper channels had to be constructed. Maintenance of these harbours and channels, together with the disposal of dredged material, remain an issue. A report produced in 1990 by the International Joint Commission indicated that 24.26 x $10^6 m^3$ of material was removed and disposed of

throughout the Great Lakes between 1980 and 1984. Indeed, an earlier report produced by the International Joint Commission (IJC, 1982) documented volumes of around $1.8 \times 10^6 m^3$ between 1975 and 1979 for the Canadian sectors of the Great Lakes alone. The need to quickly and inexpensively assess the potential environmental impacts of dredged material, prior to disposal, resulted in the development of chemical-specific criteria. Chemical monitoring depends upon knowing what pollutants are likely to be present. With the increasing complexity of industrial effluents, this is becoming more difficult (Hawkes, 1978). As a result, sediments have been designated as contaminated on the basis of bulk chemical concentrations. Although chemical concentrations are important for inventory purposes, as a method of determining and interpreting pollution levels they are inadequate by themselves (Chapman & Long, 1983); the measurement of chemical concentrations in the sediments does not directly address the question of the bioavailability of these contaminants to the resident fauna. These chemical criteria can also tend to be overprotective and thus making, for example, disposal of dredged sediment very costly (Darby *et al.*, 1986).

There has been a developing awareness that chemical objectives alone are insufficient as indicators of the overall "health" of aquatic systems, and that ultimately the biological integrity of the ecosystem is the prime concern (Reynoldson *et al.*, 1989). In order to predict the effects on organisms, there is a need to deal with chemical concentrations in sediments in such a way as to take into account the complex interactions occurring as a result of the increased complexity of industrial effluents (Butcher, 1946). The determination of contaminant concentration *per se* provides little, if any, information on the availability (its bioavailability) of these compounds to the resident biota or their potential adverse effects (Chapman & Long, 1983).

Concern over the degree of environmental protection offered by chemical guidelines prompted the development of alternative approaches to sediment assessment (Reynoldson & Zarull, 1993). There is now widespread recognition (Reynoldson & Zarull, 1993; IJC, 1987; Chapman & Long, 1983) that chemical monitoring alone is not enough, and that

pollution is essentially a biological phenomenon because of its impact on living organisms. Evidence from monitoring indicates that even though water-quality criteria are not exceeded, organisms in or near sediments may be adversely affected at concentrations below those detectable by chemical analysis (Chapman, 1995). Biological guidelines, however, may be less stringent than universal chemical criteria as they incorporate site-specific adaptations and reflect only bioavailable material (Painter, 1992). Invertebrates are useful indicators of water quality, especially in relation to fisheries. However, they may not be sensitive to some pollutants, which affect other water uses. For example, work done by Hawkes (1978) indicated that the presence of low concentrations of the herbicide TBA (trichlorobenzoic acid) had only slight effects on the invertebrate fauna. Although, biological surveillance detects ecological change indicative of water quality changes, it does not identify the specific cause. Bulk chemical measurements serve to indicate "hot spots" where large concentrations of contaminants occur. However, some chemicals known to be toxic are not readily detected with standard analytical techniques. To provide more direct and conclusive proof of adverse contamination effects, a combination and integration of both chemical and biological methods is required (Chapman & Long, 1983). Biological and physico-chemical approaches are essentially complementary in monitoring water and sediment quality, and it is appropriate to detect and assess impact through examination of the biota (Wright *et al.*, 1994). Careful interpretation of changes in the biota, in light of the known ecology of the species involved, may indicate the nature of the cause, which may then be confirmed by physico-chemical tests (Hawkes, 1978). Such an approach provides different types of information, contributing to a full assessment of water quality. In some cases, when biological results do not support the physico-chemical data, they probably indicate contaminants for which tests were not conducted (Hawkes, 1978). Hence, chemical and biological approaches are complementary.

2.1.1 Utility of biological indicators in environmental monitoring and decision making

There are many different ways in which biological studies are used to address water and sediment quality. These include toxicity studies and bioassays for linking concentrations of pollutants to observed effects, and bioconcentration and bioaccumulation studies to assess the exposure of organisms to pollutants and the potential effect higher in the food chain. These can be used to provide base-line information for comparison with impacted areas, to detect changes, and to assess the impact of pollutants (Norris & Norris, 1995).

Unknown factors, which may affect the estimation of sediment toxicity using dose-response relationships, include the efficiency of the extractions and analyses, the availability of the toxicants to the biota, and potential interactions among the toxicants. Therefore, measured contaminant concentrations may not accurately reflect the potential toxicity of the sediments to the organisms (Giesy & Hoke, 1989). While dose-response techniques provide useful information and should be maintained as methods for sediment toxicity evaluation, Chapman and co-workers (1987) suggest that they are limited and do not provide sufficient information to answer all of the pertinent questions regarding the *in situ* toxicity of contaminant mixtures in sediments.

Benthic macroinvertebrates continue to be used for biological monitoring and, indeed, are the group of organisms most widely used for the assessment of both water and sediment health (Resh *et al.*, 1995). They are present in most aquatic habitats and are relatively less mobile than other groups (e.g. phytoplankon, zooplankton, fish, etc.), thereby being more representative of the location being sampled (Reynoldson & Metcalfe-Smith, 1992). Benthic community responses to changes in water quality have long been recognised (Reynoldson, 1984; Sheldon, 1984; Hynes, 1960) and, in general, their taxonomy is well established. They are often considered the most appropriate biological indicators for sediment quality as they are directly associated with contaminants in the sediments through their feeding and behaviour. Animals buried in sediment are exposed to toxicants in both sediment particles and pore waters. Therefore, animals that are adapted to live in fine sediments, such as oligochaetes, bivalve molluscs, chironomids, burrowing mayflies,

etc., are excellent indicators of sediment toxicity (Swift *et al.*, 1996).

Species composition of benthic invertebrate communities has frequently been used in environmental monitoring and assessment (e.g. Canfiefld *et al.*, 1996; Canfiefld *et al.*, 1994; Hawkins *et al.*, 1994; Reynoldson & Metcalfe-Smith, 1992; Furse *et al.*, 1984). Community composition, which provides evidence of impact at one or more trophic levels within the ecosystem, can be easily and relatively inexpensively obtained (Reynoldson, 1984). However, studies involving community composition alone are not sufficient. An absence of macroinvertebrates in sediments does not necessarily indicate sediment toxicity as the causative factor (Painter, 1992; Giesy & Hoke, 1989), and may fail to account for the ecological complexity of the system. Functional tests, such as biological tests of sediment toxicity, can provide quantifiable relationships between contaminants and organisms and can isolate contributing factors. Most of these tests, however, are performed using single species, which may not be indigenous to the area under investigation. Not all species can be tested in the laboratory, and extrapolation between species is highly uncertain. A lack of environmental realism and failure to incorporate ecosystem complexity limits any investigative utility of laboratory tests and possibly their ability to establish actual impairment (Monk, 1983). Nevertheless, laboratory bioassay data for specific contaminants can provide information on toxicity and the potential for bioaccumulation.

Acceptable levels of physical and chemical indicators of water and sediment quality are usually set through toxicity testing. Prediction of effects under field conditions is not easy, because exposure to the contaminant can be variable. Laboratory data allow uncertainties about contaminant effects in the "real" environment to be reduced such that a specific hypothesis can be formulated and tested, either through additional laboratory tests, field studies, or a combination of the two (Chapman, 1995). The relative strengths and weaknesses of field and laboratory approaches make them complementary, and it is clear both field and laboratory methods should be used to examine stress.

A clear link needs to be established between the single (and multiple) species tests conducted in the laboratory under controlled conditions and the potential effects in the ecosystem (Chapman, 1995b). This can be achieved by validating laboratory data in real ecosystems whenever possible (Bacher *et al.*, 1992). Ecotoxicological assessment addresses the effect of chemicals on a range of species and species interactions in the environment. It encompasses both laboratory tests and field assessments, as well as a variety of microcosm and mesocosm experiments (Rand, 1995). The response of a test organism to single compounds or mixtures of compounds is often correlated with that of other species, but seldom is the correlation perfect. Therefore, no single bioassay can be expected to be adequate for the detection of potential adverse effects of complex mixtures of contaminants, due to the different sensitivities and natural histories of bioassay organisms (Giesy & Hoke, 1989).

2.1.2 Trends in biomonitoring

By definition, benthic macroinvertebrates are closely associated with sediments, and so are continuously exposed to sediment contaminants (Canfield *et al.*, 1994). Field surveys of invertebrates have several advantages over laboratory methods for the assessment of sediment toxicity. Indigenous benthic organisms complete most, if not all, of their life cycles in the aquatic environment and serve as continuous monitors of sediment quality. They are relatively sedentary, so are representative of local conditions. They are also relatively easy to collect and are ubiquitous across a broad array of sediment types. Finally, a field assessment of indigenous populations can be used to screen potential sediment contamination.

The idea that certain species can be used as indicators of different types of environmental conditions is well-established (Cairns & Pratt, 1993). The concept of biological indicators of environmental conditions originated with the work of Kolkwitz and Marsson (1909, 1908), who developed the idea of saprobity (the degree of pollution) in rivers as a measure of the amount of contamination by organic matter. Although biological methods

of assessing water quality have been used in Europe since early in the century, and later in America, it was only in the latter half of this century that the possibility of using aquatic organisms as indicators of pollution received serious consideration in the United Kingdom (Hawkes, 1978). Hynes (1960) advocated the use of benthic invertebrates as indicators of pollution. By comparing quantitative samples at various points along the length of a river he was able to assess effects of pollution, even where the levels of pollution were very slight. He reasoned that different types of pollution brought about different ecological conditions.

Toward the end of the 1950's a number of the river authorities in the UK had developed biological methods for classifying pollution conditions of rivers. The Trent Biotic Index, developed from the Saprobien system of Kolkwitz and Marsson (1909, 1908), was a landmark in the development of biological methods for assessing river water quality in Britain, as it was formed on the basis of other systems which were designed by biologists in other areas (Hawkes, 1978). The Trent Index has been largely superseded in the United Kingdom by the Biological Monitoring Working Party (BMWP) score (Chesters, 1980), which was designed to give a broad indication of the biological condition of rivers throughout the UK. Despite the fact that studies have shown that the BMWP score is a reliable indicator of water quality (Armitage *et al.*, 1983), researchers as yet, seem somewhat reluctant to relate these index values directly to levels of pollution (Mason, 1991), which is essential if the indices are to be of direct value to the management of water quality. Similar views are held in both Australia and Canada (Chapman, 1995; Norris, 1995; Norris & Norris, 1995; Resh *et al.*, 1995; Reynoldson *et al.*, 1995; Reynoldson & Zarull, 1993).

Early in its history, the use of stream and lake benthos in biomonitoring focused on the detection of organic pollution, employing structural and taxonomic changes in benthic invertebrate communities as an indicator of anthropogenically and naturally induced stresses (Reynoldson & Metcalfe-Smith, 1992). As with Biotic Indices, Diversity Indices were developed as a measure of stress in the environment (Mason, 1991). This approach

assumes that unpolluted environments are characterised by larger numbers of species, with no single species making up the majority of the community. When the environment becomes stressed, the species sensitive to that particular stress will be eliminated. Warwick & Clarke (1993) suggested that increasing levels of disturbance may either decrease or increase diversity, or it may even remain the same. Most diversity indices take into account the number of species in a sample and their relative abundance, but the sensitivity of the individual species to particular pollutants is not accounted for. Also, no indication of the type of pollutant is provided.

In North America there has been a movement to incorporate cost-effective biological tools into impact assessment (Norris, 1995). Developments have followed along similar lines to those in Europe with respect to the use of indices. These, and other such 'metrics' approaches continue to be widely used in the USA for the analysis of water quality, using several indices (or metrics) presumed to represent ecological features of interest (Resh *et al.*, 1995). As a result, numerous indices have been created and used in benthic monitoring studies. However, many workers believe that such methods result in the loss of information on the composition of the community (Reynoldson & Metcalfe-Smith, 1992), arguing that diversity does not behave consistently or predictably in response to environmental stress.

2.1.3 Identification of impacts

It is difficult to predict the effects of contaminants on an ecosystem from studies based on its individual component parts. The impact of a chemical in the environment may occur at lower concentrations than predicted from standardised single species tests (Dewey, 1986). To be predictive, such tests must be more environmentally realistic (Buikema & Voshell, 1993).

One purpose of biological assessment is to characterise the status of water resources and monitor trends in the condition of biological assemblages that are associated with

anthropogenic perturbation characteristics (Resh *et al.*, 1995). Fundamental to such bioassessment methods is the classification of aquatic systems so that comparisons can be made between reference areas and areas of concern, or test sites with similar characteristics.

Until recently, the development of numerical biological objectives has been considered too difficult due to temporal and seasonal variation inherent in these biological systems (Reynoldson *et al.*, 1995). Reynoldson and co-workers (1997) have defined a reference-condition approach. A reference condition has been defined as the condition which best represents a group of minimally disturbed sites, based on selected physical, chemical and biological characteristics (Reynoldson *et al.*, 1997, Reynoldson *et al.*, 1995). With this approach, an array of reference sites characterises the biological condition of a region; a test site can then be compared to an appropriate subset of reference sites. Using reference communities, defined through the collection of physical, chemical and biological data, methods have been developed (Wiederholm, 1989; Armitage *et al.*, 1987; Corkum & Currie, 1987; Moss *et al.*, 1987; Ormerod & Edwards, 1987; Johnson & Wright *et al.*, 1984) which have demonstrated an ability to predict the community structure of benthic macroinvertebrates using simple habitat and water quality descriptors (Reynoldson *et al.*, 1995).

There has been general agreement within Europe (Metcalfe, 1989) that there is a need to define reference communities based on chemical, physical and geographical features, unrelated to pollution, as the first step toward the identification of expected/best achieved communities for each type of water. These communities could then be used as sediment and water quality objectives, expressed either in terms of whole communities or important discriminating species (Reynoldson & Metcalfe-Smith, 1992). One of the first proposals combining structural and functional methods in aquatic ecosystem management was the Sediment Quality Triad (Chapman & Long, 1983; Chapman, 1986). This approach included measurements of toxicity, community structure and physical and chemical variables. It incorporates measures of sediment chemistry, sediment toxicity, and benthic community structure to describe sediment quality. The approach has been

used to identify and differentiate pollution-degraded areas in marine, estuarine and freshwater sediments, by determining concentrations of contaminants and associated effects (Canfield *et al.*, 1994).

2.1.4 Temporal and spatial variability

Factors controlling the distribution of animals and their community composition often act synergistically, including biotic as well as environmental interactions. When such factors are taken into account, the benthos may reflect changing conditions (Barton, 1989). Spatial and temporal variability at site unimpacted by anthropogenic disturbances must first be understood before disturbance effects can be distinguished from natural variability (Johnson *et al.*, 1993). The interactive effects of environmental change must be understood when predictions about the expected community assemblage are being made (Furse *et al.*, 1984); however, separation of the sources of variability, as opposed to combining their effects, often helps to illustrate the underlying factors causing the observed patterns (Resh & McElravy, 1993).

The value of benthic invertebrates as indicators of both sediment and water quality increases with an increase in the understanding of the ways in which the environmental and methodological factors can affect the results of field studies (Barton, 1989). Benthic animals are not evenly distributed in aquatic systems, and communities can vary a great deal over relatively short distances (Swift *et al.*, 1996). Diversity in feeding, reproduction and morphological and behavioural characteristics makes it difficult to generalise as to the factors responsible for heterogeneity for the entire benthic animal community (Wetzel, 1989).

A great deal of discrepancy exists as to the importance attributed to different environmental influences on community structure. Water quality characteristics such as pH, heavy metal concentration, municipal effluent, and salinity have been suggested as factors effecting the benthic community composition (Jackson, 1993). Other factors

affecting the abundance of benthic invertebrates and their community composition in freshwater lakes include, for example, sediment texture and habitat-specific effects on timing of reproduction (Jackson, 1993; Brinkhurst, 1968). For many species of benthic organisms, clay and silt content of the substrate is an important factor affecting their distribution and abundance (Sauter & Güde, 1996). For example, in substrates with heterogeneous grain size, where tubificids can be selective, many species occur almost exclusively in their particle size preference range (Sauter & Güde, 1996). The occurrence of species is not determined by any one single environmental factor.

Dissolved oxygen is essential to the respiratory metabolism of most aquatic organisms. The dynamics of oxygen distribution in lakes are governed by a balance between exchanges with the atmosphere, photosynthesis, and losses due to chemical and biotic oxidation (Wetzel, 1989). In the profundal zone of many eutrophic lakes, oxygen is a limiting factor for most species (Volpers & Neumann, 1992). Oxygen, as well as being important for the direct needs of organisms, also affects the solubility and availability of many nutrients and toxins, and therefore the productivity of aquatic ecosystems (Wetzel, 1989).

Temperature also has a strong controlling influence on the composition of a community and, indeed, even small changes in temperature can result in a shift in the dominant taxa found at a site. Reynoldson (1987) showed that *Tubifex tubifex* and *Limnodrilus hoffmeisteri* grow only within a narrow temperature range (10-13°C). Thus, thermal stratification during the summer months has a great effect on at least these two species. Not only is it important to address seasonal effects but, where possible, year to year variation should also be considered. Differences from year to year may affect thermal stratification and restrict the annual temperature range of aquatic systems. Valle (1927) recognised causal relationships between temperature and benthos, and significant temporal changes occur in the numbers of all taxa. In freshwater lentic systems, it is known that temperature effects both fecundity and growth of most benthic organisms (Reynoldson, 1990).

The oxygen concentration, water temperature and other physico-chemical variables near the sediment-water interface are also effected by patterns of water movement (Slepukhina, 1996). Mass water movement affects the distribution and development of bottom habitats in lakes through a number of processes. Variation in water movement near the bottom sediments can form heterogeneous sediment distributions. Low mobility bottom water, which can lead to excessive accumulation of organic matter in the sediments, can limit the distribution of oligochaetes. Organic contamination in stagnant conditions can result in the absence of oligochaetes as a result of oxygen depletion (Slepukhina, 1996).

The potentially confounding effects of seasonal changes must be accommodated into the design of biomonitoring programs; knowledge of the life history of the species involved will help in this regard (Johnson *et al.*, 1993). The life history of benthic macroinvertebrates ultimately is defined by factors that govern the survival and subsequent reproduction of a species or population (Johnson *et al.*, 1993). Samples collected just after a recruitment period can seriously over- or under-estimate the relative importance of the particular organism depending upon how the samples are handled (Barton, 1989). Many insects, especially in the nearshore zone, have aquatic stages for only part of the year, so can be easily missed entirely. The lack of temporal consistency and different temporal responses to environmental change introduce variation into the data, which can distort analysis and hinder interpretation (Furse *et al.*, 1984). The existence and importance of strong seasonal variations in life-history patterns, such as emergence and recruitment, must be known in order to avoid erroneous inferences regarding the abundance and distribution of macroinvertebrates (Johnson *et al.*, 1993). Johnson and co-workers (1993) suggest that, in order to get the best representation of benthic organisms in lentic systems, sampling be carried out in the months of September and October. Studies carried out by Reynoldson and co-workers (1995) also indicate that, for their study of the Great Lakes, September and October give the best representation of benthic organisms. However, Furse and co-workers (1984) suggest that the accuracy with which sites could be assigned to a pre-determined taxa group using either environmental

data or differential taxa can be improved by merging seasonal data.

2.1.5 Utility of multivariate analysis for the prediction of biological state.

With increasing frequency, benthic invertebrate communities are used as indicators of environmental degradation or restoration (Clarke & Green, 1988; Cairns *et al.*, 1971) because they broadly reflect environmental conditions. Measuring similarity among samples or groups of samples with respect to the taxa that occur in them is one of the most common problems in ecology (Cushing *et al.*, 1983). Biologists working with benthic invertebrates have long been aware of problems of variability in what they measure, and have emphasised the need to account for the variability of benthic invertebrate counts in their sampling data (Norris & Georges, 1986).

Standard statistical techniques that assume linear relationships among variables have a limited application in ecology due to the generally non-linear response of species to environmental conditions. Ecologists have independently developed a variety of alternative techniques (Ter Braak & Prentice, 1988). Measures such as diversity are much less sensitive than multivariate methods for measuring community change (Gray *et al.*, 1990; Warwick & Clarke, 1991; Warwick *et al.*, 1990), which is no surprise in view of the much greater amount of information multivariate analysis retains. Many environmental problems involve multiple variables and should, therefore, be analysed using multivariate techniques (Green, 1979). With the use of multivariate statistics, subtle changes in the species composition across sites are not hidden by the need to summarise the combined characteristics of the site as a single value, as with indices. Multivariate methods are now accepted by many ecologists, because of their power to both detect and describe subtle patterns of differences on many variables (Cushing et al., 1983). Indeed, multivariate techniques show greater promise than univariate comparisons for detecting and understanding spatial and temporal trends in the benthic fauna (Norris & Georges, 1993). However, univariate methods can provide a robust interpretation of data. If the study were such that there was concern over the response of an individual species, or only

one environmental variable, univariate methods such as analysis of variance or regression should certainly be considered (Hendrickson & Horwitz, 1984). Multivariate approaches allow patterns in the species abundance and co-occurrence to be summarised (Jackson, 1993). However, there is no reason why univariate methods cannot be used to further analyse the data set once patterns in the data have been identified using multivariate techniques (Norris, 1995).

The use of multivariate approaches in ecology is often motivated by the desire to assess and describe similarity (Goodall, 1973; Orloci, 1973). Such approaches are often viewed as "objective techniques", allowing greater understanding of the community assemblage and relationships with corresponding environmental conditions (Jackson, 1993). Indeed, in the past few years considerable advances have been made by applying multivariate statistics to large data matrices and relating benthic community structure to key environmental variables.

The complexity of benthic communities has lead many researchers to adopt multivariate approaches to summarise patterns of species abundance and co-occurrence. Techniques for data reduction (classification and ordination) have aided the progress toward deriving predictive relationships between macroinvertebrate community and environmental factors of lotic systems (Furse *et al.*, 1984; Moss *et al.*, 1987; Wright *et al.*, 1984).

British, Canadian, Australian and American efforts to classify aquatic systems take into account the biological consequences of different habitat characteristics, such as sediment type, depth, temperature, pH, oxygen, and organic content, etc. (Resh *et al.*, 1995). In the UK approach, for example, habitat characteristics are used to predict the fauna expected at a test site. Multivariate analysis of biotic and environmental features may have considerable practical application, and it was with this in mind that the British Rivers Study was developed (Furse *et al.*, 1984). An approach developed in Great Britain (Wright *et al.*, 1993; Armitage *et al.*, 1987), the River Invertebrate Prediction And Classification System (RIVPACS), has many applications for biological surveillance,

conservation and environmental impact assessment in rivers, and offers a method for the prediction of macroinvertebrate fauna to be expected at a given site based upon a small number of environmental features. Moss and co-workers (1987) described the techniques used in RIVPACS for predicting the probability of capture of taxa at a site with known physical and chemical characteristics. Using these techniques, it is possible to estimate the reference communities for a set of environmental conditions in order to predict the benthic community that should occur at a site.

To date, European efforts to classify aquatic systems have primarily been on running-water systems. However, similar strategies have been applied to lakes. A study of numerous lakes in Sweden was carried out by Johnson & Wiederholm (1989), using similar techniques to those described by Armitage and co-workers (1987). Assemblages of profundal zoobenthos were classified using two-way indicator species analysis (TWINSPAN), ordinated by Canonical Correspondence Analysis (CCA), and related to the physico-chemical factors using CCA, discriminant analysis and regression. The analysis showed the species assemblage amongst the profundal zoobenthos to be a good indicator of lake type. The work carried out by Johnson & Wiederholm (1989) was the first of its kind using such techniques on lentic communities. More recently, a modified approach to the RIVPACS system, described by Wright *et al.* (1993), and the Sediment Quality Triad approach used by Chapman & Long (1983) and Chapman (1986) have been presented for use in sediment assessment in the Great Lakes (Reynoldson & Zarull, 1989; IJC, 1988, 1987). The relative sensitivity of such methods of assessment of environmental integrity are yet unknown (Calow, 1989). Efforts carried out in the Laurentian Great Lakes were part of a project to develop sediment criteria based on benthic communities and toxicity response of selected benthic invertebrates (Reynoldson *et al.*, 1995). The fundamental approach here of reference site classification, development of predictive models, prediction of test sites, and comparison with guidelines derived from the reference site can be used in any type of environment for the estimation of ecological integrity. The approach simply requires the selection of appropriate biological and environmental data matrices (Reynoldson & Zarull, 1993).

2.1.6 Development of a predictive model for sediment assessment in the Great Lakes.
A modification of the approach used in the British Rivers study (Wright *et al.*, 1984) has been used in sediment assessment of the Great Lakes (Reynoldson & Zarull, 1989; IJC, 1988; 1987). The process relies heavily on the benthic community. Reynoldson and co-workers (1995) conducted studies for biological guidelines for sediment. The BEnthic Assessment of SedimenT (The BEAST) used a multivariate approach, which predicts community composition from environmental data. The ultimate goals of the study were to develop a method to determine the need for, and the success of, remedial action predicting what a benthic community should be like at a site if it were undisturbed. The approach allows appropriate site-specific biological objectives to be set from measured habitat characteristics. It also provides an appropriate reference for determining the degradation at a site due to anthropogenic contamination.

In the BEAST model, cluster analysis and an ordination technique, Non-metric Multi-Dimensional Scaling (NMDS), were employed in the analysis of community composition and membership groupings of the reference sites. Multiple Discriminant Analysis (MDA) was then employed to assess and predict site groupings using environmental variables. The accuracy of the predictions from the discriminant model was then confirmed by performing several validation runs on subsets of data. The technique was more sensitive than the currently used provincial sediment quality criteria based on Screening Level Concentration (SLC) and laboratory toxicity tests in determining a need for remediation. It was also shown that test communities will fall within, or outside of, a range of reference communities depending upon sediment quality. The model was developed to address sediment quality criteria in the near-shore areas of the Laurentian Great Lakes.

As a result of this study, a large database has been assembled from reference sites in Lakes Ontario, Erie, Michigan, Superior, Huron and St. Clair. It includes information on: the composition of the benthic invertebrate communities; measured environmental variables; and the response of four species of benthic invertebrates exposed in the laboratory to sediment collected from the same sites.

2.1.7 Diagnostic capacity of benthic macroinvertebrate community structure for the assessment of sediment toxicity.

Reynoldson and co-workers (1993, 1995) have shown that the Biological Sediment Guidelines developed, and their predictive modelling approach, can be used in the assessment of sediment quality. Collingwood Harbour, located in Collingwood Bay, Georgian Bay, Lake Huron, was identified as an area of concern by the International Joint Commission (IJC, 1987) partly because of the sediment contamination by various metals and partly due to eutrophication. As part of the remediation program for the harbour, dredging and removal of the sediments was being considered.

On the basis of sediment chemistry, the harbour was heavily contaminated by metals, with some sites within the boat slips exceeding the Ontario Ministry of Energy and Environment's severe effects criteria. All of the sites in the boat slips and outer harbour exceeded the low effects levels. The large area of sediment for removal and the anticipated costs prompted the biological significance of the contamination to be examined.

Using a number of previously selected predictor variables, Reynoldson and co-workers (1995) were able to predict the community assemblage for the test sites in the harbour. The harbour sites were compared with reference sites from the Great Lakes database of the same community type to determine whether the observed community assemblage was similar to that predicted. Of the 24 sites predicted as having the same community type, most fell within the range of variation found in the reference sites of that community type.

From the model, Reynoldson and co-workers were able to conclude that sediment remediation was not warranted at all of the harbour sites in the bay. Based on the results found, removal of contaminated sediment was only necessary in the boat slips. The remainder of Collingwood Harbour was not considered as having a degraded benthic community despite the fact that several of the provincial chemical sediment criteria were

exceeded for metals. Thus, it has been shown that the guidelines described by Reynoldson *et al.* (1995) have the capability to define whether or not a community is impacted (whether it significantly differs from that predicted), and whether there is evidence of sediment toxicity.

While ordination techniques can provide a clear pass or fail criterion, by placing impacted communities either within or outside the reference/expected community boundaries, they can also provide a measure of the extent of failure by the degree to which the test site is outside the reference/predicted community boundaries. The degree by which the site can diverge from the predicted state before it is considered statistically significant, and hence affected, can be addressed as can the significance of such a divergence (i.e. what does it indicate?). When there is divergence from the expected state, as yet it is not possible to define what is/are the causative agent(s). In a site exposed to multiple stressors no clear statement can be made as to which potential sources should be controlled, other than by inference from chemical analysis. Because divergence from an expected biological state is due to a change in species abundance from the predicted community, it is reasonable to hypothesise that communities of organisms will have characteristic responses to certain stressors. The direction in ordination space in which a site diverges from the expected state, together with the supporting toxicity data, may provide useful diagnostic information on the nature of an impact at a site. Thus, the appropriate management action can be made. When there is divergence from an expected state, the question now arises as to whether this change in community composition has any diagnostic capacity to indicate the nature of a stress.

2.2 Site Description

2.2.1 General site description – The Great Lakes

The Great Lakes were formed over a span of 2 million years by glacial and geological action, and took their most recent form almost 10,000 years ago, at the end of the last ice

age. Lakes Superior , Michigan, Huron, Erie and Ontario are joined by rivers and other connecting channels to form the largest surface freshwater system in the world (Bolsenga & Herdendorf, 1993), and account for one-fifth of the world's fresh surface water. The area is heavily used for a variety of water related activities. They provide water for drinking and industrial use, and a valuable fishery is contained in the lakes. They are a source of hydropower, and they serve as a focal point for recreation.

In the early nineteenth century, the whole of the Great Lakes basin supported fewer than 300,000 people. The basin has since been transformed from a hunting and farming ground of the native North Americans to the industrial heartland of North America. Over 30 million people now reside along the Great Lakes, a hundred-fold increase in the population in less than 200 years. Much of the natural character of the region has changed in the last two centuries, and the lower lakes have seen the most change (Bolsenga & Herdendorf, 1993).

The Great Lakes as an environment

Approximately 30 million people depend upon the Great Lakes as a source of Water (IJC, 1991), and the basin's ecosystem has been, and currently is, being seriously altered by the large amounts of pollutants the lakes have been receiving from various sources. For some time now there has been a great deal of research into the many and varied effects of these pollutants on this water system. As a result, there is considerable background information available on the many aspects of the Great Lakes.

Changes in the water quality of the Great Lakes have been fairly well documented. Beeton (1961) noted that, "Changes in the chemical characteristics in Lake Ontario have closely paralleled those of Lake Erie. Prior to 1910 the chemical characteristics of the two lakes were similar, and conditions in Lake Erie were probably the same as those indicated by the 1854 and 1884 analysis of Lake Ontario water". Some of the most dramatic biological changes in the lower Great Lakes appeared to be in the bottom fauna and among certain fishes (Dambach, 1968). Dambach felt that the most significant changes

occurred from 1929 through to 1965. *Hexagenia*, once abundant in the western basin of Lake Erie, was almost locally extinct by the end of this period. With the decline in *Hexagenia* there was a definite increase in the abundance of chironomids. More recent studies have shown a return of the burrowing mayfly to the western basin (Corkum *et al.*, 1995). Many of these changes appear to be intimately related to the eutrophic conditions in the western end of Lake Erie (Brinkhurst, 1968) which, together with toxic contaminants, modifications of fish stocks, and habitat loss, is one the most significant impacts on the Great Lakes from human activity (Reynoldson *et al.*, 1989).

Barton (1989) documented changes in the Great Lakes fauna from the 1950's. For example, in 1931 Lake Michigan benthos consisted mainly of *Diporeia* and a small number of oligochaetes, sphaeriids and a few chironomids. By 1964, all of the major groups had increased in abundance, as would be expected if the lake had become more eutrophic. From the mid 1960's to the early 1980's there was a further increase in the abundance of benthic animals. There were no major changes in the species composition; the increase in standing stock of benthos seemed to reflect the generally increasing nutrient levels in the lake. It was suggested that controls on phosphorus inputs into the lakes in the 1970's were the cause of decreases in the magnitude of spring blooms (Barton, 1989). However, despite this being a major source of input of energy to the benthos, it was not reflected in their abundance.

In the 1960's the Great Lakes fishing industry was in serious decline and the quality of the water was beginning to draw public attention (Barton, 1989). The average abundance of benthic invertebrates in nearshore Lake Ontario decreased since the 1970's (Barton, 1986). This might be expected following the improvements in the sewage treatment facilities around the lake.

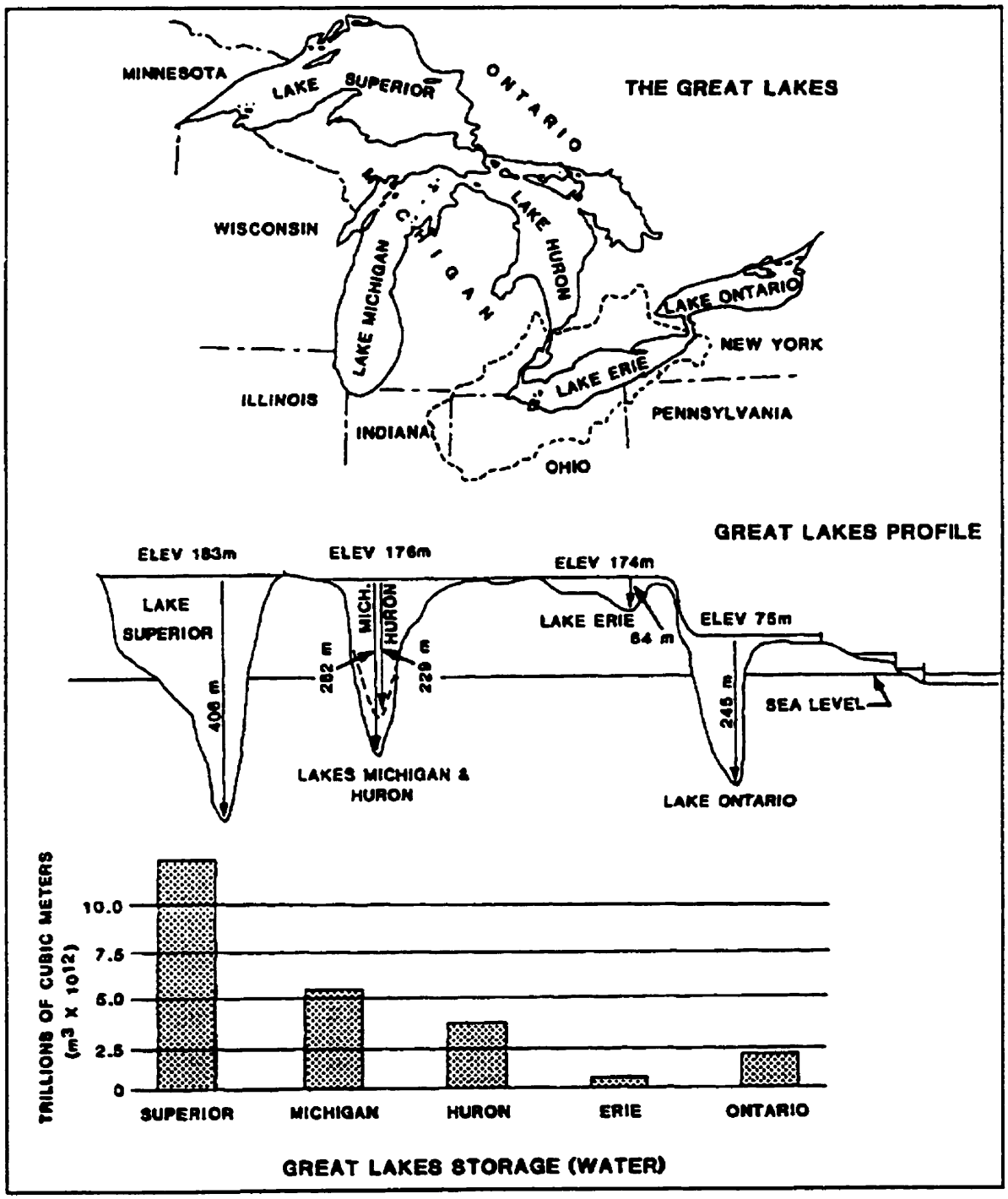

Fig.2.1: Comparison of Great Lakes shapes, depth, and water volume. (Taken from Bolsenga & Herdendorf, 1993).

2.2.2 Specific site description – Lake Erie

Geologically the oldest of the Saint Lawrence Great Lakes, Lake Erie's entire water mass lies above sea level, which is not the case for the other four Great Lakes (fig. 2.1). In comparison to the other Great Lakes, Lake Erie is also the southernmost, warmest, most biologically productive, and most eutrophic (Bolsenga & Herdendorf, 1993). Ninety-five percent of Lake Erie's total inflow of water comes from the Detroit River. Lake Erie lies between 41 21'N and 42 50'N latitude, and 78 50'W to 83 30'W longitude. It is a relatively narrow lake, with its long axis orientated west-southwest to east-northeast. This axis parallels the prevailing wind direction, which causes the lake to react violently to storms, with high waves and wide fluctuations in water level (Bolsenga & Herdendorf, 1993; Hamblin, 1987). Wind, which pushes water from one end of the lake toward the other, usually comes from west to east and can produce large short-term differences in water levels at the eastern and western ends of the lake. These differences have been up to 16ft. (4.88m).

As the shallowest of the Great Lakes, Lake Erie is particularly prone to fluctuating water levels. It has the smallest volume of water and the shortest retention time, yet is the fourth largest of the Great Lakes in surface area. Lake Erie is approximately 388km long and 92km wide with a mean depth of 19m. Its three major physiographic divisions, western, central and eastern basins, and are divided by their depth contours (bathymetry) (fig.2.2) (Bolsenga & Herdendorf, 1993). One of the principal characteristics distinguishing the three basins is mean depth, which in turn affects thermal structure and productivity (Schertzer *et al.*, 1987). The three basins of Lake Erie have sharply defined individual characteristics and have distinct bathymetric, thermal, and trophic characteristics.

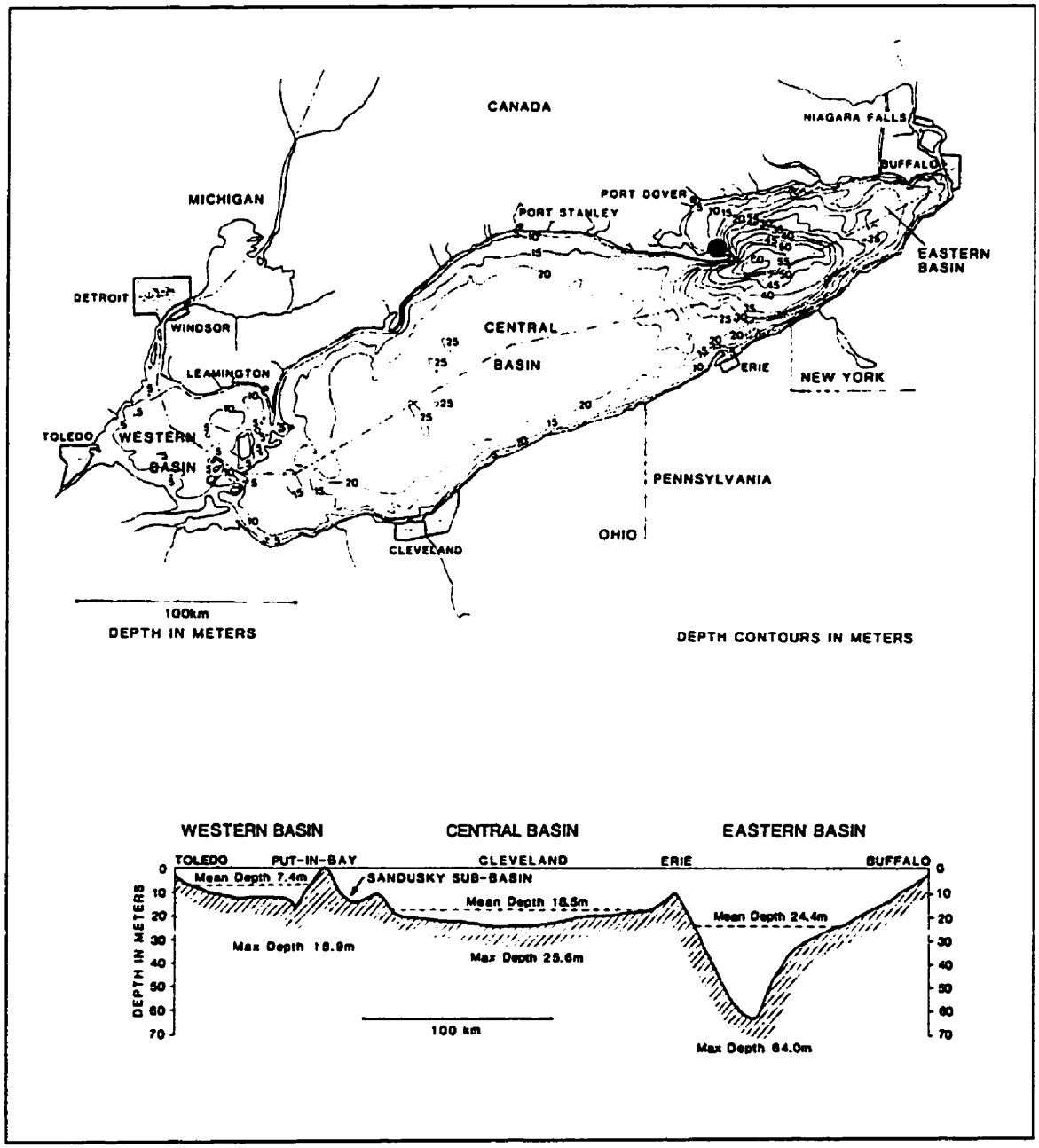

Fig. 2.1: Bathymetric map and longitudinal cross section of Lake Erie. (taken from Bolsenga & Herdendorf, 1993). ● *represents the location of Site 303.*

Seasonal fluctuations of the water temperatures of Lake Erie are the greatest of any of the Great Lakes, and it is the only lake that typically freezes from shore to shore. The thermal bar advance lasts about 5 to 6 weeks from April to mid-May and permanent stratification usually begins in mid-June with maximum heat storage in mid-August and overturn in mid-September. The central basin thermocline position varies significantly from year to year, the variability of the upper and lower mesolimnion boundaries being as large as 10m. Thermocline position shows some dependence on prevailing meteorological conditions and has implications to the development of central basin anoxia (Schertzer *et al.*, 1987). Water exchanges among the basins have both local and basin-wide effects in both the short and long term. The major exchange mechanisms are hydraulic or riverine flow from the mouth of the Detroit River to the entrance of the Niagara River (Bartish, 1987).

A substantial proportion of Lake Erie's drainage area and immediate shoreline is made up of soils capable of sustaining high biological production, rich farmland and industry, but which are readily erodable. The influences of drainage from these soils on biological production in the lake were probably present long before cultural eutrophication. Lake Erie was probably the best-publicised example of eutrophication and severe deterioration of water quality through pollution. It has consequently been the central object of debate on Great Lakes water quality and pollution control (Mortimer, 1987). The seasonal disappearance of oxygen from the lower layers of the central basin, which promoted the pronouncement that Lake Erie as "dead", may have occurred episodically before the onset of human activities (Mortimer,1987).

The water provided by Lake Erie for water-borne commerce, navigation, manufacturing, and power production has led to intensive industrial development along its shore, and more than 11 million people obtain their drinking water from Lake Erie. The basin's moderate temperatures have also encouraged recreation and agriculture (Bolsenga & Herdendorf, 1993).

Lake Erie is also the most sediment-dominated of the Great Lakes. Not only are long stretches of its shoreline subject to episodes of active erosion by storm waters, the lake also receives sediment, via the Detroit river, from Lake St. Claire which in turn is fed from the Thames River, and from actively eroding shores at the south east corner of Lake Huron (Mortimer,1987). Topsoil erosion also contributes sediment to the other rivers, and total sediment (and dissolved nutrient) load profoundly influences the ecology of that basin (Mortimer,1987; Langlois, 1954). The western basin is generally the most turbid of the three basins.

It has long been known that sediment deposits in lakes and rivers exert a significant influence on the cycle of dissolved oxygen in overlying waters (Adams *et al.*, 1982). The oxygen demand of these sediments may account for as much as 50% of the total oxygen consumption in rivers (Hanes & Irvine, 1968). Sediments, especially the clay and silt fractions, are also the dominant pathways by which toxic substances are transported in Lake Erie (Bedford & Abdelrhman, 1987). Resuspension of bottom sediments is the net result of a wide variety of different fluid mechanisms with characteristic time and length scales that extend over six orders of magnitude. The aggregate of these effects is most heavily concentrated in the layer adjacent to the bottom, which is the benthic boundary layer (Bedford & Abdelrhman, 1987).

Lake Erie as an environment

The macroinvertebrate community of Lake Erie, particularly the western basin, has undergone dramatic changes over the past years. In a study carried out on Lake Erie, Barton (1988) noted that in the 1970's water quality declined from east to west. The bottom fauna suggested a west - east gradient from extremely eutrophic to moderately oligotrophic conditions. There is a similar south-north gradient in the lake that is at least partly a reflection of temperature regime (Brinkhurst, 1968). The decline of *Hexagenia* populations in the 1950s and the subsequent increase in the number of oligochaetes, chironomids and sphaeriids in the 1960s demonstrated the negative impact of pollutant loadings into the basin (Carr & Hiltunen, 1965; Britt, 1955). As a result of phosphorus

control measures, there has generally been a improvement in the water quality since the early 1970s, with the re-establishment of *Hexagenia* in certain areas of the western basin (Corkum *et al.*, 1995; Thornley, 1985) and a decrease in abundance of oligochaetes (Nalepa *et al.*, 1991; Britt *et al.*, 1973; Wood, 1973). The most striking differences in the biota of Lake Erie between 1954 and 1974 were: the greatly expanded coverage of solid surfaces by *Cladophora*, which supported larger numbers *of Gammarus fasciatus*; increases in the abundance of the Trichoptera, Hydropsychidae; and the decrease in diversity of other groups such as Ephemeroptera and the Trichoptera *Ceraclea* (Barton, 1989). *Limnodrilus hoffmeisteri* and *Spirosperma ferox* are found almost exclusively in the central basin (Barton, 1988). Barton (1989) described the major species found within each of Lake Erie's three basins. The western basin, having a low diversity, was dominated by the tubificids; the central basin predominantly by *S. ferox* and *Chironomus sp.*; and the eastern basin was dominated by the more oligotrophic indicators such as *Stylodrilus heringanus*, *Diporeia*, and *Heterotrissocladius*. A striking feature of the chironomid fauna of Lake Erie is the marked difference in the species composition that occurs in different parts of the lake; *Pseudochironomus sp.* and *Heterotrissocladius changi* are largely restricted to Long Point Bay area. There are a number of environmental features that contribute to this, but distributions of many of the species appear to be intimately related to the progressively more eutrophic conditions encountered towards the western end of Lake Erie (Brinkhurst, 1968).

Over the past 8 years the Great Lakes have seen the invasion of the zebra mussel (*Dreissena polymorpha*) and the "quagga" mussel (*Dreissena bugensis*) (Rosenberg & Ludyansliy, 1994). The invasion of the Great Lakes by the European bivalves *D. polymorpha* and *D. bugensis* has been associated with subsequent physico-chemical changes in the environment and alteration of the benthic community composition (Howell *et al.*, 1996; Stewart & Haynes, 1994; Griffiths, 1993). Adult *Dreissena* have the ability to colonise and alter the physical structure of hard substrates (Stewart & Haynes, 1994). Studies on the effects of *Dreissena* invasion by Dermott (1993) in the northeastern Lake Erie bedrock substrates, have shown a greater abundance of macroinvertebrates on

colonised substrates. Griffiths (1993) found that species richness and total abundance of benthic macroinvertebrates increased in both northwestern and southwestern regions of Lake St. Clair, following the invasion of *Dreissena*. However, there is evidence that health of other benthic invertebrates can be adversely affected by *Dreissena* due to direct colonisation or crowding (Mackie, 1991).

Changes in water clarity and quality, particularly in Lake Erie and Lake St. Clair, have been attributed to the filtering of large volumes of water by *Dreissena* (Griffiths, 1993; Leach,1993; Nicholls & Hopkins, 1993). Howell and co-workers (1996) showed that at a site in eastern Lake Erie near the Niagara River, *Dreissena* populations were as high as 320,000 individuals/m^2. Increasing secchi disc transparency, from less than 4m to over 6m, and decreasing chlorophyll concentration, together with decreases in the numbers of native bivalves and polychaetes have also been associated with these high densities of *Dreissena,* while gammarid amphipods and tubificid worms remained abundant (Howell *et al.*, 1996). These data also suggest that the large *Dreissena* population has altered the depositional patterns of sediment in this area. The demise of native bivalves has also been noted in other heavily impacted areas of the Great Lakes (Gillis & Mackie, 1994; Mackie, 1991). It has been suggested that other members of the benthic community may actually benefit from changes in conditions mediated by *Dreissena*. For example, gammarid amphipods and some species of oligochaetes have increased in areas colonised by *Dreissena*, presumably as a result of increased availability of particulate organic carbon and through the creation of interstitial habitat (Stewart & Haynes, 1994; Dermott *et al.*, 1993; Griffiths, 1993).

2.2.3 *Lake Erie, Site 303*

In recent years methods have been developed to allow the prediction of biological response (invertebrate assemblage) to 'clean' (or uncontaminated) sites using habitat and water quality parameters (Johnson & Wiederholm, 1989; Armitage *et al.*, 1987; Corkum & Currie, 1987; Ormerod & Edwards, 1987; Wright *et al.*, 1984). Reynoldson and co-

workers (1998; 1995; 1993) have adopted such methods in an attempt to develop biological sediment guidelines based on invertebrate assemblages and benthic invertebrate laboratory tests. As a result of Reynoldson's work, a large database exists of biological and chemical parameters from 271 sites in Lake Ontario, Erie, Michigan, Superior and Huron. The reference sites were organised into groups with similar biological attributes based on the composition of their invertebrate fauna. These reference sites were selected to represent 'unpolluted' conditions, and were defined as sites located in areas that represented normal, minimally impaired conditions. This is based on the premise that sites least affected by human activity will exhibit biological conditions most similar to those at natural, pristine, locations. These sites were located away (>10km) from known discharges (Ontario Ministry of the Environment, 1990), within 2km of the shore, and at a depth of less than 30m (with the exception of Lake Michigan). The sites were also known, or suspected to have, a fine-grained substrate (Reynoldson *et al.*, 1995). Four of these sites were sampled monthly, over a period of 2 years in an attempt to determine the effects on both annual and seasonal variation.

Lake Erie site 303 was selected for use in this study from the Great Lakes reference database, and is located in Lake Erie, just off Long Point (42° latitude and 80° longitude) (Fig. 2.2). This site was designated as a reference site and was one of the four sites to be sampled monthly. It has silty sediment, with a low sand to clay ratio, and a relatively simple community composition. The community is predominant in oligochaetes with few chironomids. The site's relatively simple community composition, dominated by species with completely aquatic life cycles, and their relatively sedentary nature, negates come of the confounding factors that can arise from life-cycle changes, such as emergence. The site's silty sediment composition allowed large numbers of samples to be processed relatively quickly. Monthly data on benthic community assemblages were also available, providing annual and seasonal data for the site. These characteristics, together with the site's relatively close proximity to the Canada Centre for Inland Waters (CCIW) in Burlington, where the laboratory was located, enabled frequent sampling, and made Site 303 an ideal choice of sampling location for the study.

CHAPTER 3

Maintenance of Communities under Laboratory Conditions: Evaluation of community structure for the assessment of sediment toxicity.

3.1 *General Introduction*

A fundamental goal of ecotoxicology and environmental risk assessment is to determine the ecological effect of toxic chemicals on natural communities (Rand, 1993; Petersen, 1985). Although single species toxicity tests show characteristic responses to contaminants (Buikema & Voshell, 1993) and direct effects of contaminants may be determined from laboratory studies (Shaw & Manning, 1996), there are problems when extrapolating response data from single species tests to predict changes in community composition. In most laboratory tests relatively few species are considered and the data available are usually for single species (Maciorowski & Clarke, 1980). Cairns (1984) argued that there is insufficient knowledge to predict toxicological responses from one level of biological organisation on the basis of another. Sheehan (1984) summarised the pitfalls of extrapolating response data from isolated single species tests to predict changes in community and ecosystem functions. Multispecies tests, which can show interspecies effects that are not shown by single species tests (Taub *et al.*, 1986; Cairns, 1985; Sheehan *et al.*, 1985), seem to be the natural progression from the more traditional single species tests. The U.S. Environmental Protection Agency uses benthic community tests in the assessment of toxicant impacts on the estuarine environment. The results have greater environmental relevance, since they consider interspecific interactions and allow more species to be exposed than do single species toxicity tests (Shaw & Manning, 1996; Gray *et al.*, 1988; Tagatz, 1986).

The greatest realism is obtained when ecological studies are conducted on natural, whole ecosystems. Using the whole ecosystem, however, may present some problems; sampling and replication are difficult and experiments are subject to variability in natural conditions and, hence, are difficult to control. Such methods can also be expensive. Multispecies tests mitigate some of these problems (Buikema & Voshell, 1993).

Sheehan and co-workers (1986) have suggested that small aquatic mesocosms, containing natural communities, exhibit typical ecosystem properties. The introduction of toxic substances should alter the community in a statistically significant and ecologically meaningful manner. Measures of the magnitude and duration of the system perturbation enable the potential effects of test chemicals to be evaluated. Mesocosm studies are ecologically more relevant than single species test, yet offer greater control than field studies (Shaw & Manning, 1996). They are as sensitive as single-species tests, and may provide insight not discernible from any combination of single test results (Sheehan, 1984).

The research described in this thesis was designed to determine whether the benthic macroinvertebrate community composition of intact box cores could be used to detect and diagnose the effects of sediment contamination. This chapter aims to investigate the capability of intact box cores, collected from the field and brought back to the laboratory as mesocosms, to assess sediment contamination. In doing this an attempt is made to answer the question:

- *Can intact box cores be brought back from the field and maintained in the laboratory with little change in the resident benthic fauna?* Intact boxes, taken from the field and brought back to the laboratory, were compared to field data; this was done to establish whether there were any changes in the fauna as a result of collection and transportation back to the laboratory. The boxes were maintained in the laboratory and sampled regularly to address whether they could be maintained under laboratory conditions, and how long the communities could be kept. Some of the boxes had nutrients added to them to

determine if the intact box core communities require feeding. The effect of different temperatures was also considered.

3.2 *Summary of Procedures Employed*

Two laboratory experiments were run during the summer field season of 1995. For the first experiment, 9 intact boxes were collected in May; a second experiment used 9 boxes collected in September.

3.2.1 Field procedures

The intact box cores were collected from Site 303, which is located just off Long Point in Lake Erie. The cores were collected using a box corer designed as a combined effort by Precision Enterprises and the Bedford Institute of Oceanography and built by Precision Enterprises in Nova Scotia (Fig. 3.1a), and take a core 25cm by 25cm. The box corer was designed with a false bottom that could be inserted into the base of a detachable liner (Fig. 3.2a). Once the intact box cores were collected, a false bottom was inserted into the liner and the intact core removed from the main apparatus. The boxes were placed into custom-made plastic bins, (Fig. 3.2b), covered with black polythene, and placed in a temperature-controlled walk-in chamber on board ship. The cores were maintained at 4°C and transported back to the laboratory at CCIW.

Collection of the intact box cores was both labour-intensive and time-consuming. As a result field cores were collected from the site using a mini-box core (40cm x 40cm). This sampling apparatus (fig. 3.1b) collects a larger core of sediment than the machinery used to collect the intact cores of sediment, but the overall dimensions of the apparatus is much smaller and can be reset easily to take additional samples. Thus, sediment samples can be collected relatively quickly. Perspex tubes, 10cm in length with an internal diameter of 6.5cm were inserted into the sediment of the mini-box core. Four cores of sediment were taken in the May sampling and five in September, matching the total

Fig. 3.1: Sampling equipment used to collect laboratory (a) and field (b) boxes.

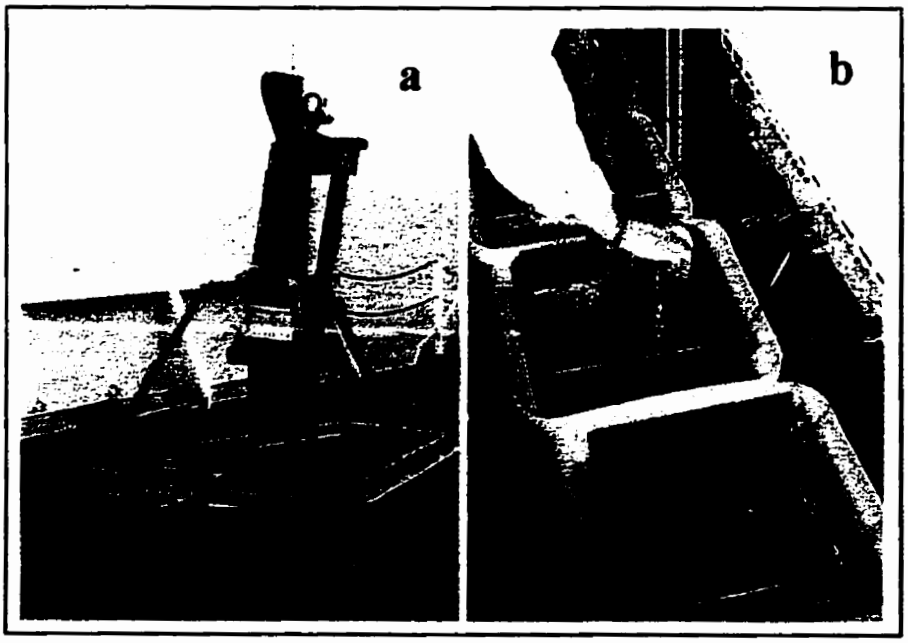

Fig. 3.2: *(a) Intact box cores, removable from the main apparatus. (b) Laboratory boxes, placed into custom-made plastic bins. To sample, tubes were placed into the sediment and the contents removed.*

number of cores to be removed from each of the laboratory boxes. The field boxes were taken to describe the field community with which the laboratory-maintained boxes could be compared. Field samples were collected monthly for the remainder of the field season (May to September). Each core tube was considered to be a replicate sample unit (Reynoldson *et al.*, 1995). The contents of the tubes were placed in plastic bags and kept cool (4°C). These were subsequently sieved through a 250μm mesh sieve and fixed in 4% formalin. After 24-48 hours the formalin was replaced with 70% ethanol, and the sample stored pending identification and analysis.

Oxygen, temperature and pH readings were recorded at 1m from the sediment-water interface. Depth and sampling date (Julian day) were also recorded as environmental parameters at the same time the field boxes and intact cores were collected.

3.2.2 *Laboratory procedures*

The intact boxes were placed in a temperature-controlled chamber upon return to the laboratory at CCIW. The temperature of the chamber was set at 4.5°C, duplicating the temperature at which the cores were taken in May. The box cores collected in September were maintained at 10°C, a temperature higher than that found in the field at this time (7°C), to ensure that any stability noted in the community composition of the May boxes was not due solely to the low temperature. This temperature was chosen as one that would not encourage emergence of chironomids, but would promote reproduction of other macroinvertebrates. Based upon historical data (Reynoldson, Great Lakes database), it is a realistic temperature found at this site. The boxes were gently aerated using standard aquarium air-pumps for the duration of the experiment and topped up with distilled aerated tap water as needed (Reynoldson *et al.*, 1994). The boxes were left for four days to settle before further manipulation. Treatments and sampling strategies for both of the laboratory experiments are summarised in Table 3.1.

Table 3.1: Summary of the sampling design for the boxes collected in May and September. Sampling time is given as number of weeks after set-up the boxes were sampled, hence, 0, 2, 4; etc. represent immediately after set-up, two weeks after, 4 weeks, etc. Additions to the sediment are also outlined.

Treatment	Additions to Sediment	Month	Boxes/ Treatment	Temp (°C)	Sampling Frequency	#Cores /Box	Sampling Times
Control	.	May	3	4.5	1 core fortnightly/ box	4	2-8 weeks
	.	Sept	3	10	1 core fortnightly/ box	5	0-8 weeks
	.	Sept	3	10	1 core monthly/ box	5	0-16 weeks
Enriched	50ml, 5g/L YTC, weekly	May	3	4.5	1 core every 3 weeks/ box	4	2-11 weeks
	50ml, 2.5g/L YTC, fortnightly	Sept	3	10	1 core every 3 weeks/ box	5	0-12 weeks

A set of 3 boxes was enriched (fed) to investigate whether the resident macroinvertebrate communities required feeding. These boxes were enriched with 50ml of a 5g/L mixture of a yeast extract, digested trout chow and cerophyll (YTC) (Reynoldson *et al.*, 1994), added to the surface sediment weekly, for the duration of the experiment (Table 3.1).

The control boxes were sampled fortnightly, while the enriched boxes were sampled every three weeks. In sampling the fed boxes less frequently than the control boxes, the duration of time the communities could be maintained, with additional food being available, could also be investigated. The first cores collected from each of the laboratory boxes, from the May samples, were removed two weeks after the boxes had been set up (week 2).

In September, 3 of the 9 boxes collected were fed with a reduced concentration of the YTC formula used in May, at less frequent intervals (50ml of a 2.5g/L YTC formula, added fortnightly). Feeding commenced 5 days after the boxes were placed in the temperature-controlled chamber, once the boxes had 'settled'. A proliferation of algae

was noted in the May boxes, suggesting overfeeding. The aim was to feed the fauna rather than cause a response due to nutrient enrichment. Thus, the quantity of food was reduced and administered less frequently in the September boxes, in an attempt to more accurately reflect normal sedimentation. The remaining six boxes in September were unmanipulated controls, unfed and unspiked. Three of these boxes were sampled at fortnightly intervals for a duration of eight weeks, The remainder were sampled monthly for a total of 16 weeks, to determine the length of time the boxes could be maintained in the laboratory without being fed. The fed boxes were sampled every three weeks, as with the boxes collected in May. All of the 9 boxes were initially sampled immediately after they had been set up and allowed to settle (week 0).

Dissolved oxygen and pH levels at the sediment-water interface of the boxes were recorded weekly throughout both of the experiments. The oxygen levels were maintained within the normal range found in the field at that temperature (Reynoldson, Great Lakes Database).

The laboratory boxes for both May and September were sampled using 6.5cm diameter Perspex tubes, 18cm in length, inserted into the sediment of each of the boxes. The overlying water within the tubes was siphoned off, and the top 10cm of sediment was removed and placed into plastic bags. This sediment was sieved through a 250μm mesh sieve, fixed and preserved in the same manner as the tubes collected from the field. The sampling core tubes were left inserted into the sediment for the duration of the experiment, to maintain the integrity of the remaining sediment.

The macroinvertebrates in the laboratory and field samples were sorted with the aid of a dissection microscope and identified to species or genus level where possible. For the Chironomidae and Oligochaeta samples, slide mounts were made using polyvinyl lactophenol, for high power microscopic identification. Appropriate identification guides were used (Peckarsky *et al.*, 1990, Brinkhurst, 1986; Merritt & Cummins, 1984; Wiederholm, 1983; Clarke, 1981; Sawyer, 1972; Pennak, 1953). Identification was verified using the Great Lakes reference collection at CCIW.

3.2.3 Data Analysis

The Great Lakes database shows clear changes in the numbers of *Dreissena* in Lake Erie since the start of the sampling program in 1990 (Reynoldson & Day, 1998; Crichton, unpublished). *Dreissena* were left in the intact box cores for the duration of the experiment in May. However, within the first week the *Dreissena* had started to migrate from the sediment to the sides of the metal box liners. Colonisation patterns of this organism, resulting in their patchy distribution, were considered a potential problem when analysing the community data. Discarding *Dreissena* numbers from the analysis was considered. However, *Dreissena* may have an effect on community composition (Dermott, 1993; Griffiths, 1993; Mackie, 1991). It also has been suggested (Howell *et al.*, 1996; Stewart & Haynes, 1994; Griffiths, 1993) that their presence may even be associated with physico-chemical changes in the environment. In light of such confounding problems, *Dreissena* were removed from the September boxes at the beginning of the experiment and their numbers recorded for each of the boxes. These numbers were included with the environmental data.

The relationship between numbers of *Dreissena* and the rest of the community was examined graphically, plotting *Dreissena* numbers against number of taxa, mean density and a diversity measure for the monthly field data. Pearson correlation coefficients (r) were used to estimate the strength of the relationships between *Dreissena* and the diversity and richness measures of the field data for each month.

Multivariate ordination methods allow patterns in the species data to be graphically represented in two or more axis (Faith & Norris, 1989). Non-parametric Multi-Dimensional Scaling (NMDS) was used in the analysis of the effects collection and maintenance of the boxes using the computer software package PATN (Belbin, 1993). NMDS was used as it provides a robust portrayal of ecological distance (Faith *et al.*, 1995; Reynoldson *et al.*, 1995; Faith & Norris, 1989). The majority of ordination techniques assume linear relationships between variables. However, most ecological data behave in a non-linear fashion; NMDS does not assume linearity (Jongman *et al.*, 1995; Faith & Norris, 1989). NMDS calculates a matrix of dissimilarity values from the species

composition data and uses this to create the ordination diagram (Jongman *et al.*, 1995). This is done such that dissimilarities of the samples, analysed in terms of their species composition, are reflected in their distances in ordination space relative to one another. The Bray-Curtis association measure was used in the analysis to express the dissimilarity between the samples, and it is considered to be one of the most reliable coefficients (Faith *et al.*, 1987; Clarke, 1993; Reynoldson *et al.*, 1995). The extent to which NMDS adequately represents the relationships is measured in the stress value (Kruskal & Wish, 1978). Stress values define the amount of scatter around the line of best fit through the NMDS distances and the actual distances (Clarke, 1993). As the number of dimensions increases, a sudden drop in the stress value indicates that a valid configuration has been found. Clarke (1993) suggests the following basic guidelines when selecting an appropriate stress level:

Stress <0.05,	gives an excellent representation with no prospect of misinterpretation
Stress <0.1,	corresponds to a good ordination with no real risk of drawing false inferences. A higher dimensional plot is unlikely to add to the overall picture.
Stress <0.2,	can still lead to a useable picture, although the values at the upper part of this range can potentially be misleading,
Stress >0.2,	likely to yield plots which would be dangerous to interpret.

A low stress value indicates that a configuration has been found that faithfully portrays the real distances. Two-dimensional ordination plots were produced using the average (centroid) values of the replicates taken on each of the sampling dates.

Correlation between the boxes (objects) and the species data (attributes) was established using the Principal Axis Correlation (PCC) option in PATN (Belbin, 1993). The procedure determines how well the attributes can be fitted in ordination space. This is achieved by running a series of multiple regressions of the ordination values for each of the attributes. The direction in which each attribute influences the data in ordination space can then be visually interpreted in the form of ordination vectors. The significance,

or magnitude, of the relationship between each attribute and the observed pattern is tested using a Monte-Carlo permutation procedure. By producing repeated simulations using random permutations of the data set (Faith & Norris, 1989), a list of randomised correlation coefficients is produced. From this, the statistical significance of the treatment effects on the species can be assessed in a non-parametric fashion.

The data were not normalised for use in the multivariate analysis; the raw species scores were used as numeric differences as they are considered important community descriptors (Reynoldson *et al.*, 1995).

To determine whether the macroinvertebrate composition of the box cores differed from that found in the field, the most abundant (common) species in the field cores were first identified, and these species were compared using *t*-tests to the initial cores taken from the laboratory boxes. The abundance data were transformed for the univariate analyses, using the log (x+1) of the species counts. This was done in order to reduce heterogeneity of variance and improve normality. The data were analysed univariately using the SYSTAT statistical software package (Wilkinson, 1997).

As an index of the species composition in the field and intact boxes brought back to the laboratory, richness and diversity measures were considered. Resh & Jackson (1993) consider richness to be the number of distinct, specified taxonomic units (e.g. families, species) in a collection or at a site. However, Hauer & Lamberti (1996) and Mason (1991) define richness as the mean density of individuals (Total number of individuals/ Number of taxa) in a collection or at a site. Both the number of species and mean densities of taxa were used, and are generally expected to decrease with decreasing habitat quality.

Diversity indices are used to measure stress in the environment; the combination of relative abundance and taxa richness summarised in a diversity index gives an indication of the state of the community (Norris & Georges, 1993). It is assumed that an unstressed area is characterised by a large number of species, with no single species making up the

majority of the community. When the community becomes stressed there will be a reduction in the sensitive species and an increase in the abundance of the more tolerant species. The taxa are usually separated at the species level, but the genus or family level are sometimes used (Hughes, 1978). The Shannon-Wiener (Shannon's) diversity index was chosen to measure community diversity as it is the most widely used in both lotic and lentic systems (Resh & McElravy, 1993; Mason, 1991), and was calculated as:

$$\sum_{i=1}^{s} p_i \log_2 p_i$$

The proportion of individuals in the ith species is given as p_i, and the number of species observed by s. The immature tubificids were not included in the calculation of richness and diversity, but their abundance was considered when calculating mean density.

The stability of the communities in the boxes was assessed using a repeated measures analysis of variance (RM-ANOVA). The analysis was run for each of the most abundant species initially identified, as well as for the diversity and richness measures. For both of the experimental runs, treatments were compared to each other and as a function of time. The enriched boxes and monthly sampled boxes collected in September differed from the fortnightly controls in their sampling frequency. Therefore, a RM-ANOVA was run on these treatments individually to determine whether there were any changes in abundance over time. If no time effects were identified for either the fortnightly controls or the treatments, the time series counts were pooled, and a one-way ANOVA was run to identify any significant differences as a result of treatment. A Dunnett's test was then used to identify which treatments differed from the control boxes.

A one-way analysis of variance (ANOVA) of the predominant species and diversity and richness measures was used to compare the field cores and boxes. If a significant difference was noted, a *post-hoc* paired comparison was carried out using a two-sided Dunnett's test to identify the boxes (either enrichment or one of the control groups) that differed significantly from the field data. This test was chosen as it allowed a planned

comparison to be made using unequal sample sizes, comparing each of the boxes to the field data. These comparisons were carried out for both collection dates.

3.3 *Results*

Dreissena

Correlations between the number of *Dreissena* in the field samples collected monthly during 1995, and diversity or richness were mostly non-significant and highly variable (Fig 3.3). No clear conclusions as to the relationship between *Dreissena* and community composition can be drawn from these results, and because of their patchy distribution in the field and their tendency to migrate to the sides of the laboratory boxes, *Dreissena* counts were excluded from the invertebrate community data.

Maintenance of the intact box cores in the laboratory – multivariate analysis

Multivariate analyses were performed using NMDS on both of the experiments started in May and September separately. The results of these analyses are presented in the form of ordination plots (Figs. 3.4 & 3.5) of the first and second axes of variation. Figure 3.4 shows the May field data, with a 95% confidence ellipse plotted about their centroid, and the control and enriched laboratory data from the boxes collected at this time. With the exception of four points, which represent the one control box with lower species abundance, that was eliminated from the univariate analyses, the control and fed (enriched) boxes both lie close to, or within, the confidence ellipse plotted around the field data. Only the taxa identified through Monte-Carlo permutations as having a significant relationship with the ordination pattern have been plotted as species vectors. Based on these results, the outlying control box had a marked reduction in the numbers of immature Tubificidae (Imm_chr, Imm_coh), *Potamothrix moldaviensis* (Pot_mol) and *P. vejdovskyi* (Pot_vej). The remaining control boxes tend to have lower species abundances than the field samples.

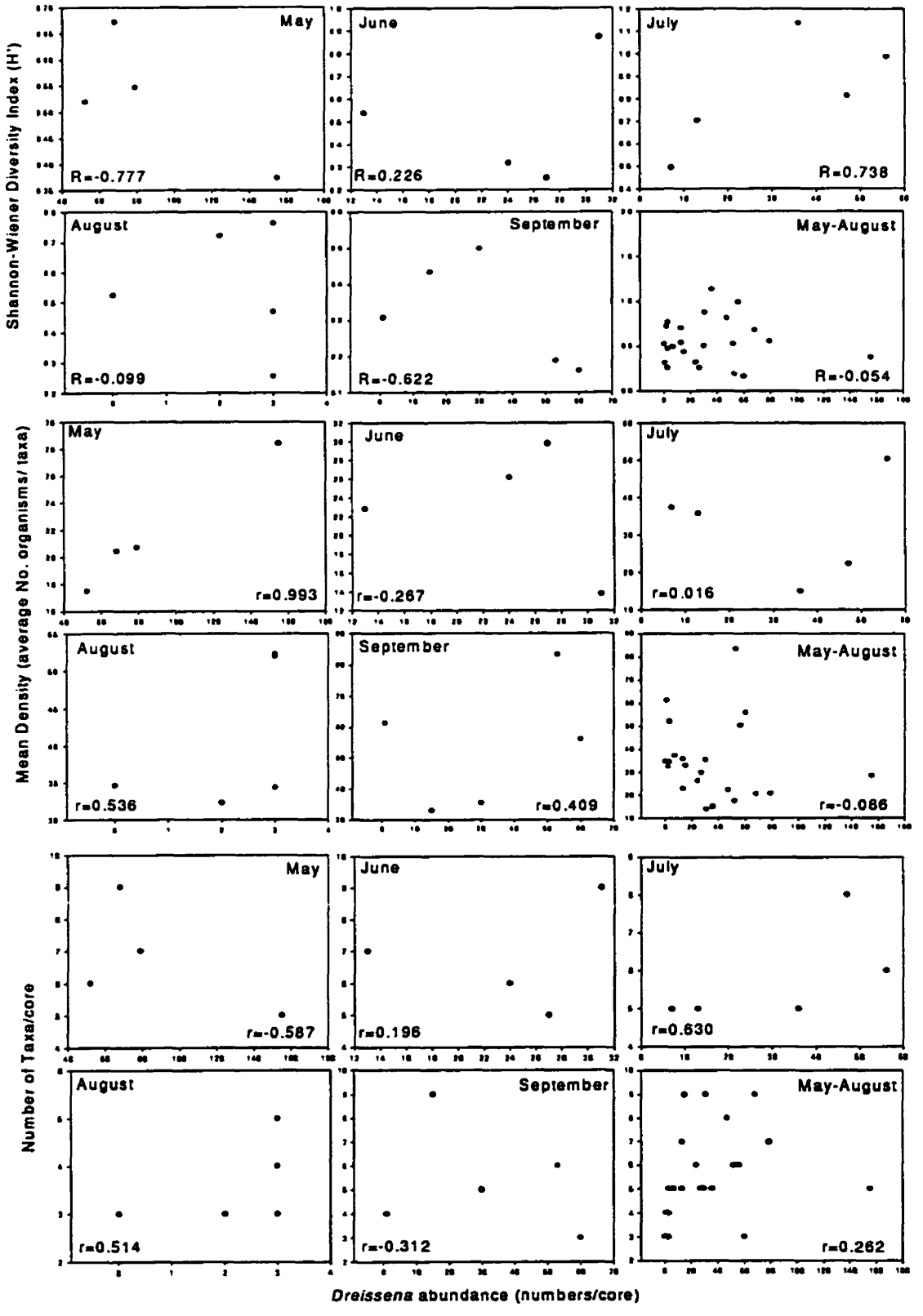

Fig. 3.3: Scatter plots of Dreissena abundance and Shannon-Wiener diversity index, mean density and total number of taxa for monthly samples of 1995. r = Pearson's correlation coefficient.

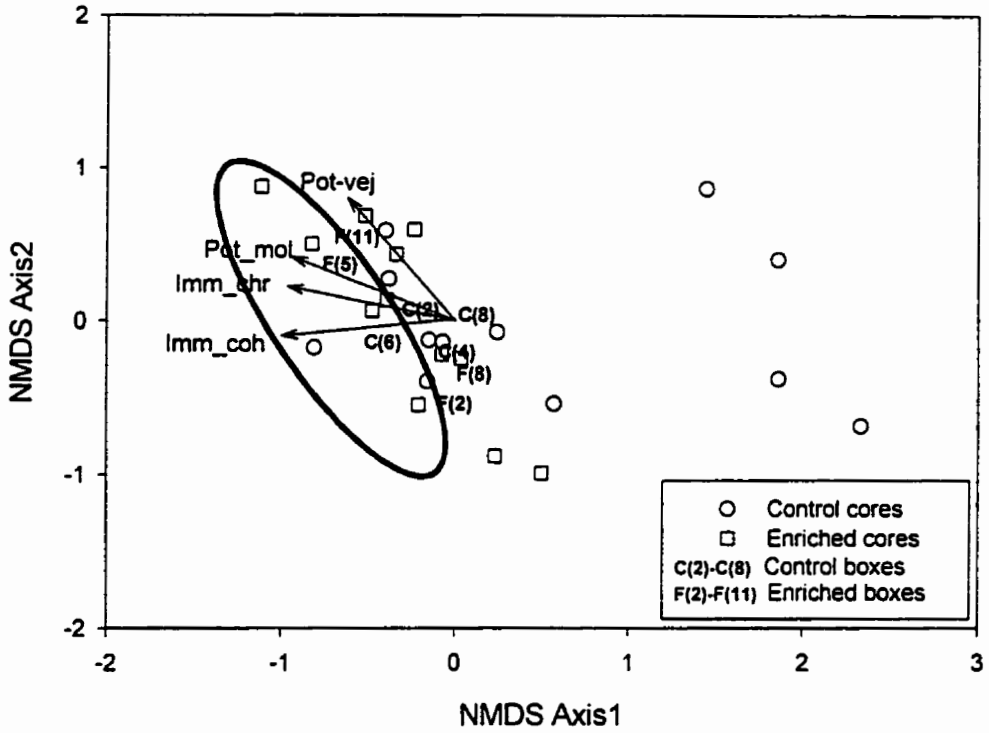

Fig. 3.4: *Field data and laboratory data for May. The ellipse represents a 95% confidence ellipse plotted around the field data (data not shown). Arrows indicate the direction in which the named species influence the data in ordination space. Full species names are given in the text. C(2) to C(8) and F(2) to F(11) show the average values of the cores collected from the control and enriched boxes respectively, sample weeks are given in parentheses. Calculation of the control averages excluded the outlying box (four cores). (Stress=0.099).*

The points labelled C(2) to C(8), in figure 3.4 represent the average values for the remaining two laboratory control boxes. The four sampling times of the cores are given in parentheses; C(2) being collected 2 weeks after the start of the experiment and C(2)-C(8) at two-week intervals after the initial sample was taken. There is movement away from the field ellipse at week eight in the control data, and fluctuation along Axis 2 is seen in the enriched boxes.

The data for the September experiment are given in figure 3.5. Five taxa were identified from the Monte-Carlo analysis as having a significant influence on the position of the data in ordination space; immature tubificids, both with and without hair, *Limnodrilus hoffmeisteri* (Lim_hof), *Potamothrix vejdovskyi*, and the naid *Vejdovskyella intermedia* (Vej_int). All laboratory cores lie outside of September's confidence ellipse.

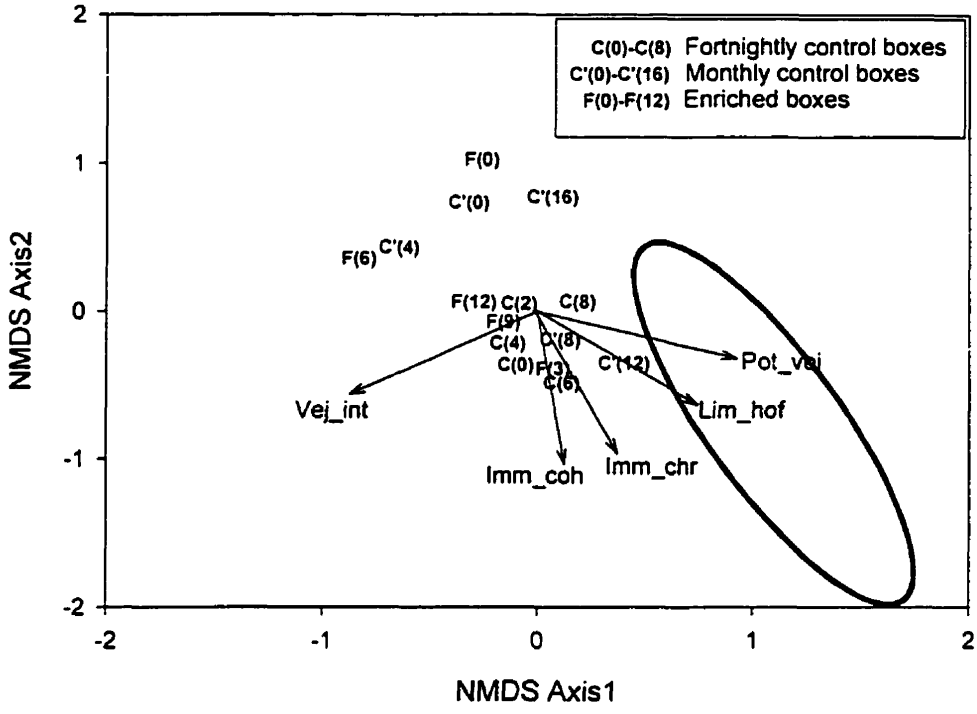

Fig. 3.5: *Field data collected in September and the average values for both the fortnightly(C) and monthly (C') sampled controls and the enriched (F) boxes, sample weeks are indicated in parentheses. A 95% confidence ellipse has been plotted around the field value (data not shown). The arrows indicate the direction in which the named species influence the data in ordination space. Full species names are given in the text. (Stress=0.153).*

Maintenance of intact box cores in the laboratory – univariate analysis

A total of 36 taxa groups were identified and used in the multivariate analyses (Appendix A). Nine of these groups were present with sufficient regularity and numbers to enable univariate analysis. It is these 9 taxa groups which are referred to as the most abundant taxa in the text. The diversity and richness measures consider all of the organisms identified, regardless of rarity.

One of the three control boxes collected in May had consistently low numbers of organisms in it. The mean numbers of organisms per core in the three control boxes were compared using two-sample *t*-tests. The box containing the reduced numbers of organisms differed significantly (p=0.021, 0.011) from the other two control boxes (p=0.332). Considering the outlying box as part of the analyses masks any significant

difference occurring between the boxes, leading to misinterpretation of changes in community composition occurring within the boxes. Thus, this outlying box was removed from further analyses.

Table 3.2 provides *t*-tests comparing total numbers of the most abundant taxa in the field data to the abundances in the first cores taken from the control boxes. Those taxa showing significant differences from the *t*-tests are illustrated in figures 3.6 and 3.7. Most of the tubificids collected were immature and could not be identified beyond family, other than dividing them into those with and without hair setae. The initial cores taken from the boxes (week 2) were compared to the field data. For the May samples there were no significant differences among the field and laboratory boxes, for any of the 9 most abundant taxa selected (immature tubificids without and without hair, the Tubificidae *Limnodrilus hoffmeisteri*, *L. profundicola*, *Potamothrix moldaviensis*, *Potamothrix vejdovskyi* and *Spirosperma ferox*, the naid *Vejdovskyella intermedia*, and the chironomid *Procladius spp.*). In September, all of the immature tubificids and *Potamothrix vejdovskyi* showed significant reductions in numbers in the monthly sampled controls (p=0.003, 0.015, 0.035). *Limnodrilus profundicola* and *Procladius spp.* were absent from the boxes collected in September.

When initial cores from the control boxes were compared to the field data using diversity and richness scores (Tables 3.2, 3.3), the Shannon-Wiener diversity index showed no significant changes for both collection dates. The boxes collected in September tended to have fewer individuals, for all of the taxa, compared to the field samples. However, many of these reductions were not significant (p=0.051-0.793). No differences were noted in mean density or number of taxa for either of the sample times.

Of the most abundant species identified, only three showed statistically significant differences in their numbers (RMANOVA, p=0.005, 0.008, 0.049) with either treatment (fed/unfed) or time, or due to a time and treatment interaction (Table 3.4, Figs. 3.6 & 3.7). For May, the effect of nutrient enrichment was only significant for *Vejdovskyella intermedia* (p=0.023).

Table 3.2: Probability values for t-tests comparing: May to September field data; May field data to control boxes (Con) at their first sampling (2 weeks); and September field data to control boxes sampled at the start of the experiment (week 0), for both the fortnightly and monthly sampled boxes (C and C' respectively). Confidence = 0.95, • = values showing significant differences, ? = insufficient data for test, N/A = no individuals found for that sample season.

Species	May/Sept	May/Con (week 2)	Sept/C (week 0)	Sept/C' (week0)
Immature tubificid:				
with hair setae	0.162	0.137	0.289	0.051
without hair setae	0.023*	0.147	0.764	0.006*
Limnodrilus hoffmeisteri	0.842	0.837	?	0.432
L. profundicola	0.500	0.391	N/A	N/A
Potamothrix moldaviensis	0.951	0.500	0.198	0.198
P. vejdovskyi	0.079	0.559	0.037*	0.192
Spirosperma ferox	0.494	0.500	0.261	0.236
Vejdovskyella intermedia	0.228	?	0.793	0.432
Procladius spp.	0.391	0.500	N/A	N/A
Shannon Index (H')	0.198	0.472	0.836	1.000
Mean Density	0.215	0.073	0.420	0.063
Total Number of Taxa	0.069	0.091	0.997	0.870

Due to the different sample times used for each of the treatment boxes collected in September, the different treatment boxes were first analysed individually over time. The only changes with time noted in the September boxes was the immature tubificidae without hair setae in the monthly sampled controls ($p=0.005$).

Despite the movement of the field data in ordination space in the May ordination plot, no significant differences between the field boxes and the initial cores removed from the laboratory boxes, or across time were observed by the univariate analyses of the most abundant taxa identified (Tables 3.2, 3.3). Diversity and richness measures both consider all of the taxa present, not just the most abundant, and may be more comparable to the

results obtained from the ordination. Diversity and richness measures failed to show any significant changes in the community composition of the boxes compared to that of the field data.

Table 3.3: Mean abundance of the May and September field data, the cores collected for the May control boxes (Con) at their first sampling (2 weeks), and the September control boxes sampled at the start of the experiment (week 0), for both the fortnightly and monthly sampled boxes (C and C' respectively).

Species	Mean Values				
	May	September	Con (May) (week 2)	C (Sept) (week 0)	C' (Sept) (week0)
Immature tubificid:					
with hair setae	26.75	40.20	9.50	29.30	15.00
without hair setae	40.25	74.20	25.00	52.30	30.30
Limnodrilus hoffmeisteri	0.50	1.40	1.00	0.33	0
L. profundicola	0.25	0	0	0	0
Potamothrix moldaviensis	2.25	1.80	2.00	0.33	0.33
P. vejdovskyi	68.25	121.00	62.50	54.00	42.33
Spirosperma ferox	1.75	2.20	0	0.67	0
Vejdovskyella intermedia	0.25	1.40	0	0.67	0.33
Procladius spp.	0.25	0	0	0	0
Shannon Index (H')	1.28	1.16	0.98	1.22	1.15
Mean Density	21.77	54.48	40.08	58.34	25.75
Total Number of Taxa	16	9	9	11	11

Table 3.4: *Probability values from repeated measures ANOVA, comparing treatments against time. * = values showing significant differences, ($p<0.05$), ? = no variation in dependant variables, N/A = no individuals found for that sample season.*

Species	May samples			September samples		
	Treatment	Time	Time x Treatment	Treatment	Time	Time x Treatment
Immature tubificid:						
with hair setae	0.163	0.683	0.555	0.248	0.074	0.210
without hair setae	0.008*	0.502	0.424	0.367	0.005*	0.111
Limnodrilus hoffmeisteri	0.867	0.421	0.743	?	?	?
L. profundicola	0.326	0.398	0.526	N/A	N/A	N/A
Potamothrix moldaviensis	0.069	0.601	0.856	0.231	0.081	0.205
P. vejdovskyi	0.070	0.734	0.319	0.748	0.179	0.364
Spirosperma ferox	0.520	0.341	0.049*	0.573	0.801	0.235
Vejdovskyella intermedia	0.004*	0.238	0.053	0.848	0.056	0.672
Procladius spp.	0.970	0.604	0.198	N/A	N/A	N/A
Shannon Index (H')	0.447	0.526	0.381	0.983	0.167	0.424
Mean Density	0.080	0.714	0.222	0.836	0.443	0.316
Total Number of Taxa	0.137	0.563	0.142	0.337	0.355	0.874

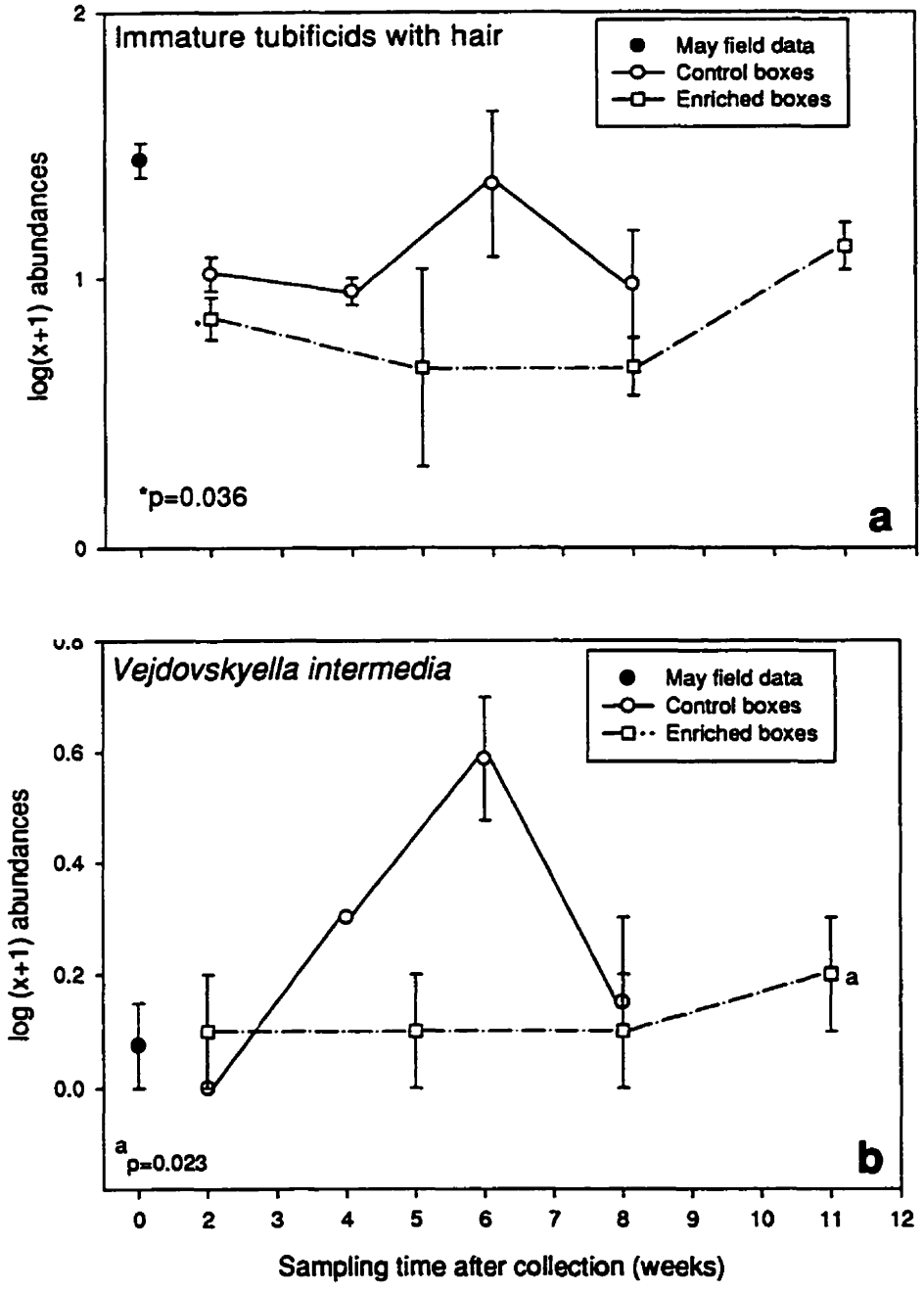

Fig. 3.6 a & b: May samples, scatter plot with standard error bars of the log (x+1) abundance of the predominant taxa identified against sample weeks after the collection of the boxes. • indicates the initial cores taken from the intact boxes that were significantly different in abundance from the corresponding field data (ANOVA, p≤0.05). a, represents the intact boxes which showed significant changes in abundance with enrichment when compared to the controls (RMANOVA, p≤0.05).

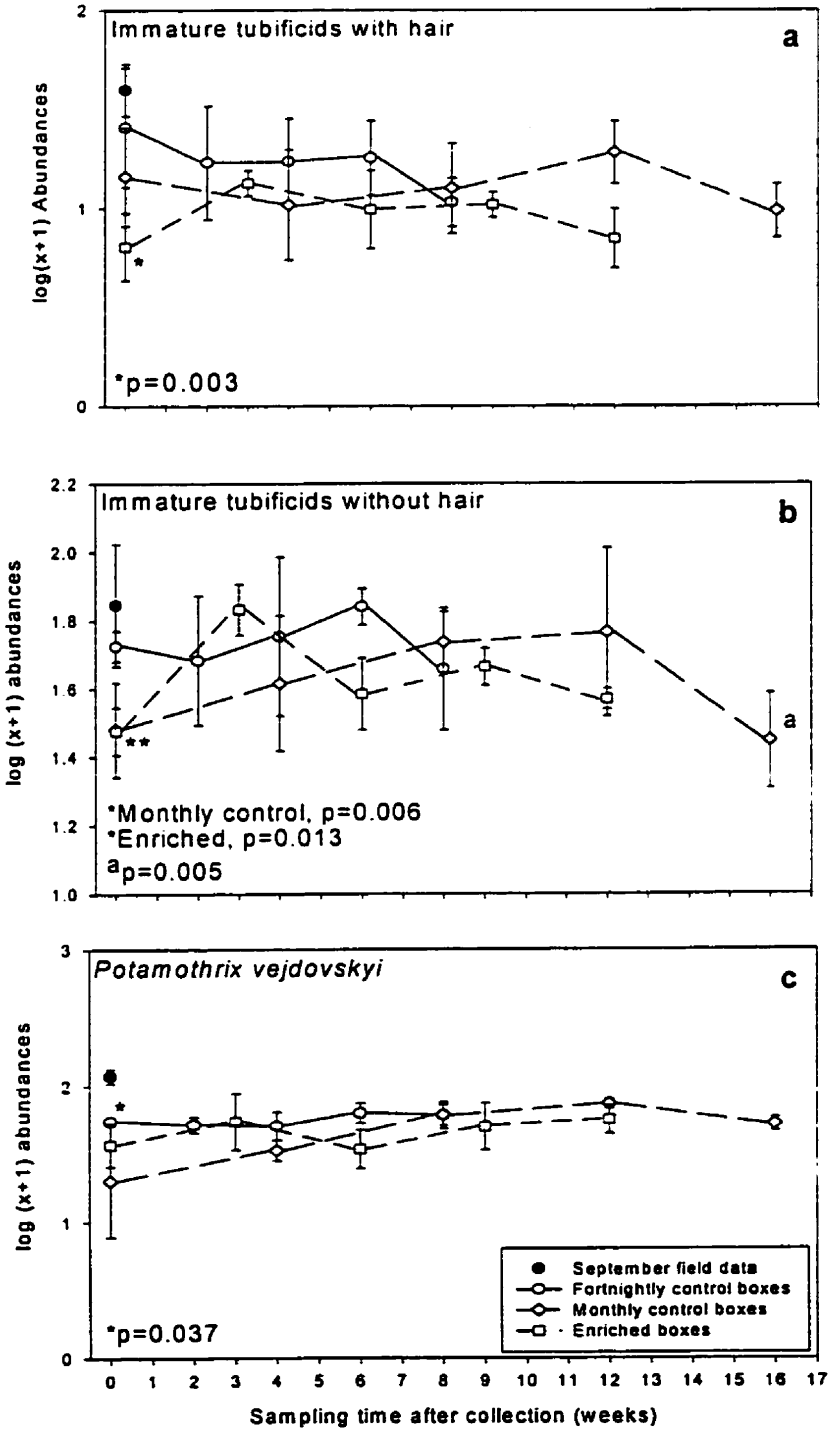

*Fig. 3.7 a - c: September samples; scatter plots with standard error bars of the log (x+1) abundance of the predominant taxa identified against sample weeks after the collection of the boxes. * indicates the initial cores taken from the intact boxes that were significantly different in abundance from the corresponding field data (ANOVA, p≤0.05). a= the intact boxes which showed significant changed in abundance compared to the control boxes (RMANOVA, p<0.05).*

Of the most abundant species used in the univariate analyses, the immature tubificids and *Potamothrix vejdovskyi* were the only taxa from the initial cores taken that differed significantly in numbers from the September field samples (table 3.2). Reductions in these taxa are clearly observed in relation to the position in ordination space of the initial cores taken from the monthly sampled controls and the enriched boxes (C'(0) and F(0) respectively) (fig. 3.5). Despite their position in ordination space, statistically significant changes over time were only noted for the immature tubificids without hair setae in the monthly sampled boxes. Fluctuations in the numbers of this organism were observed in the enriched boxes (fig. 3.7b), and can be observed by the movement of the cores in ordination space (fig. 3.5). The fortnightly sampled controls showed no significant changes with time from univariate analysis of the most abundant taxa, or the diversity and richness measures (Table 3.4).

3.4 *Discussion*

Maintenance of intact box cores in the laboratory

The average values in ordination space of the control boxes for both May and September lay, to varying degrees, outside of the confidence ellipse plotted around the field data. NMDS is designed to represent the distances between samples in two dimensions, positioning those boxes more similar closer together, and those less similar further apart. In doing this, the analysis has a tendency to exaggerate differences in the communities in ordination space (Barry & Logan, 1998), portraying any differences that there are regardless of how small. The ordination plot suggests that the laboratory boxes are more similar to each other than the field data, and vice versa. Univariate analysis of the most abundant taxa, and the diversity and richness measures indicate that, despite the differences in ordination space of the control and field data, very few statistically significant differences occurred between the control and field boxes. This indicates that NMDS tends to be more sensitive to changes in community composition than univariate techniques. Non-significant differences or trends may prove highly significant if analysed by a multivariate procedure (Norris & Norris, 1995). Smith and Morris (1992) noted

similar differences in their studies when they detected pollution effects on fish species using NMDS, which were not detectable using univariate methods.

There are a number of possible explanations that may account for the differences between the field and laboratory boxes. Care was taken to disturb the boxes as little as possible during the collection and set-up of the intact boxes. However, disturbance at the sediment surface may have occurred as a result of water displacement from a 'bow wave', caused by the corer. It is possible that such a displacement may result in a loss of organisms from the sediment surface. The field data was collected using a mini-box corer. Although the dimensions of the actual core taken are larger, the overall size of this apparatus was smaller than the box corer used to collect the intact cores. Differences in sampling methods used for the collection of the laboratory boxes and field data, disturbances and stress as a result of storage and transportation, and the time lapse between collection and the first sample taken, appeared to have a negative of effect on the community abundance of invertebrates in the intact laboratory boxes. Since no cores were collected and sampled on-board the sampling vessel with the larger apparatus used to collect the intact cores, no conclusive explanation can be given for the reductions in taxa abundance noted between the laboratory field and laboratory boxes. Reynoldson and Day (1998) sampled sites using both the box corer and the mini-box corer for a number of sample times. These data confirm that there is an overall reduction, although not statistically significant, in the number of individuals collected using different sized box corers.

No change in community composition was observed over time in the control boxes. The relative stability of the cores collected in May, and their greater similarity to the field cores compared to the September cores, may be due to the lower temperatures at which the boxes were collected and maintained. The boxes collected in September were collected at 7°C, kept at 4°C for transportation back to the laboratory, and maintained at 10°C for the duration of the experiment. The increased temperature would have an effect on the growth and reproduction of the organisms, which may further explain variation in the boxes kept at 10°C.

The predominant species, and the diversity and richness measures, for the enriched (fed) boxes show little change over time or compared to the control boxes. The position of the fed cores in ordination space and the univariate analyses indicate that, although there is some movement toward the field data in May the enriched cores still have a similar community structure to the control cores. The reduced levels of YTC formula used in September show little change in community composition, suggesting that the addition of the YTC mixture to the boxes had little, if any effect on the boxes. Therefore, it can be concluded that feeding the boxes is not necessary.

3.5 *Conclusions*

The effects of contaminants on natural assemblages of organisms have been investigated by numerous techniques (Boyle & Fairchild, 1997; Porcella *et al.*, 1985; Stay *et al.*, 1985) and the results provide useful insights into the ecological mechanisms occurring in the presence of contaminants (Landis *et al.*, 1997). In this study, intact box cores of undisturbed clean sediment were collected from the field and transported back to the laboratory for manipulation. It was important to establish whether or not collection of the boxes and transportation back to the laboratory had an effect on the resident benthic fauna. Univariate analyses of the most abundant species showed that 3 of the 9 common taxa differed significantly between cores collected in the field and those maintained in the laboratory.

In most work on ecological effects of contaminants in nature, the major difficulty is to detect contaminant effects over the normal 'noise' of the system (Giddings, 1986). A major limitation of ecosystem level bioassays is the inherent variability of multi-species biological systems (Barry & Logan, 1998). Indeed, highly variable data are common in aquatic mesocosm studies (Shaw & Manning, 1996). This can be a problem when univariate statistical procedures are used to analyse these data. Replicate variability among replicates can mask the significance of even large differences (Tagatz, 1986). The

data in this study were transformed for the univariate analyses, to alleviate some of the problems caused by variation among the boxes.

When the data were analysed by multivariate techniques, some separation in ordination space was noted between the controls and the treated boxes. Heterogeneity of the communities, together with the presence of rare species, contributed to the position of the cores and their movement in ordination space. The use of multivariate methods to analyse species abundance data, and to summarise their distribution in ordination space, is a useful method in assessing distribution patterns and can be useful in the prediction of anthropogenic disturbances. The main objective of this study was to establish whether intact boxes could be transported back to the laboratory and maintained with little change in the resident benthic fauna. The results from the univariate and multivariate analyses showed little change in the community assemblage within the laboratory boxes, indicating that the intact boxes can be kept in the laboratory for up to 8 weeks without need to feed them.

The study suggests that intact box cores, as laboratory mesocosms, may be useful in the analysis of sediment contamination. However, the results should be viewed with the caveat that only a subset of all of the possible species are represented and there is an inherent loss of abundance of some species due to collection and transportation. Care must be taken extrapolating data obtained from *in vitro* studies to natural communities.

CHAPTER 4

Capability and predictability of benthic macroinvertebrate community structure in the assessment of sediment contamination.

4.1 *Introduction*

Over the past few years, many advances have been made in applying multivariate statistical techniques in the analysis of ecosystems. Methods have been developed that allow biological and environmental data to be related in such a way that the relationships between large data matrices can be described and illustrated graphically. These multivariate statistical techniques have become widely used in biological assessment, both to characterize aquatic communities, and to describe changes in the condition of biological assemblages associated with anthropogenic stresses (Shaw and Manning, 1996; Reynoldson *et al.*, 1995; Wright *et al.*, 1994; Gray *et al.*, 1988; Vollenweider, 1987).

One of the first studies to use such multivariate methods was the British Rivers Study (RIVPACS), where rivers throughout the United Kingdom were surveyed for their benthos (Moss *et al.*, 1987; Wright *et al.*, 1984). The communities identified where then related to the environmental variables recorded for those sites. Similar methods to those used by Wright and co-workers (1984) in the RIVPACS study have been used in the analysis of the Great Lakes (Reynoldson *et al.*, 1995). As a result, a large database exists for 271 reference sites throughout the Great Lakes.

Using such multivariate techniques as cluster analysis and ordination, sites can be assigned to particular community groupings based on either their community composition or the environmental parameters of the site. A site can be placed into a community group

based upon predictions made from its environmental variables (Reynoldson *et al.*, 1997; Reynoldson *et al.*, 1995; Wright, 1995). Should the community not fall within its predicted grouping, it is possible to infer from this that the site may be stressed or impacted in some way. When there is divergence from an expected state, even with extensive chemical data, causation cannot be proven. However, if the deviation were concordant with experimental data, then inference would be stronger.

Fundamental to bioassessment methods is the classification of aquatic systems so that comparisons can be made between the reference areas and areas of concern, or test sites with similar characteristics (Reynoldson *et al.*, 1997). The use of controls or control conditions against which the results of experiments can be compared is fundamental. In laboratory experiments, all of the variables are controlled except for the variables of interest. In field experiments, not all of the variables can be controlled, so an attempt is made to choose test and control conditions that are as similar as possible. Common approaches include choosing adjacent streams (Norris *et al.*, 1982), dividing lakes into halves (Schindler, 1974), or by mesocosms (Shaw and Manning, 1996; Graney *et al.*, 1994, Warwick, 1988). Using such methods only allows one to determine whether a site is impacted (that it differs from the reference sites). If the impacts are complex, the most important impact cannot be directly determined. The use of intact box cores, collected from the field and manipulated in the laboratory, mitigate some of the problems arising from the use of either field experiments or laboratory microcosms. Intact cores are less expensive than field manipulation experiments, and enable numerous replicate experiments to be run simultaneously. Whole community tests can be conducted, while retaining some of the environmental realism lost in laboratory microcosm experiments.

Divergence from a predicted state is due to changes in the abundance of species from the expected species composition, and it is reasonable to assume that the species within a community of organisms will have characteristic responses to certain stressors. Therefore, it is logical to conclude that the direction in which a site diverges from its expected state may provide useful information as to the nature of the impact.

The research presented here was designed to determine whether the community responses to different contaminants are consistent, such that the response can be used to identify the primary contaminant of impact. It has already been shown in the previous chapter that intact box cores can be collected from the field and successfully maintained in the laboratory, with little change in the resident benthic fauna. The diagnostic capability of benthic macroinvertebrate community structure for the assessment of sediment contamination will be addressed in this study using intact box cores taken from the field, brought back to the laboratory, maintained under laboratory conditions and subjected to different stressors.

Three stressors were considered in this study; two different contaminants where used: cadmium and the pesticide Atrazine. Nutrient-enrichment was also applied, as it is perhaps the most common perturbation in the Great Lakes.

Cadmium has no known biological use (Currie *et al.*, 1997) and is thought to be one of the most toxic metals (Dresssing *et al.*, 1982). Because of its severe toxic effects, cadmium has been widely used in toxicological studies of numerous organisms, including macroinvertebrates, and there is an extensive literature on its environmental toxicology.

Atrazine (2-chloro-4-ethyl-amino-6-isopro-pylamino-*s*-triazine) is one of the most common herbicides (Solomon *et al.*, 1996; DeLaune *et al.*, 1977) applied worldwide to control weeds in both agricultural and non-agricultural land. In North America, the greatest quantity is used on corn. In a study conducted by Frank *et al.* (1991) between the years 1986 and 1990, atrazine and its metabolite desethylatrazine were the most frequently found pesticides. They were present at 340 of the 474 (72%) sites sampled from the mouths of 3 major watersheds (Grand River, Saugeen River and Thames River) in the Canadian Great Lakes. The second most frequent pesticide was metolachlor, which was present in only 6.3% of the sites. Between 342 and 2959 Kg/annum of atrazine entered Lakes Erie and St. Clair during the period studied. Atrazine is relatively soluble in water, and because of its wide use there is an abundance of literature as to the toxicological effects and degradation of this compound.

The compositions of the benthic fauna in lakes and rivers have long been considered to be good indicators of water and sediment quality (Wiederholm, 1980; Hynes, 1960). The concept of biological indicators originated with the work of Kolkwitz and Marsson (1908, 1909), who developed the idea of saprobity (the degree of pollution) in rivers as a measure of the amount of contamination by organic matter. Organic contamination of freshwaters was the first type of pollution to be recognized (Mason, 1991). Oligochaetes are considered a valuable group of organisms to use as indicators of pollution or trophic status (Lauritsen *et al.*, 1985; Milbrink, 1973; Brinkhurst & Jamieson, 1971; Hynes, 1960). The site selected in Lake Erie for this study has a benthic fauna made up predominantly of oligochaetes. In light of the faunal composition of the site, the historical concerns of eutrophication in the lake, and literature available, enrichment of the boxes with organic matter was also selected as a treatment.

4.2 *Summary of methods employed*

4.2.1 Field procedures

Intact box cores were collected from Lake Erie, site 303, as previously described. Boxes were collected on four separate collection dates over the sampling season of 1996: 22nd April; 19th June; 16th August; and 25th October. Nine boxes were collected in April and 12 were collected for the remaining sampling dates. Upon collection of the intact boxes, a mini-box core was also collected. The mini-box cores had five replicate benthic cores removed from them, as described in the previous chapter, which were sieved in the field and fixed in formalin. These were later transferred to ethanol, and the organisms in them were enumerated and identified. The cores collected and sieved in the field were used as field controls, providing a reference community for the laboratory boxes. Additional sediment was collected to be sieved in the laboratory and spiked with either cadmium, atrazine or enriched before being added to the intact boxes.

Upon collection of the intact boxes and field cores, environmental parameters such as depth, sediment-surface temperature, dissolved oxygen, and pH were measured. The date on which the samples were collected (Julianday) was also recorded.

4.2.2 Laboratory procedures

The previous chapter examined the impact of the zebra mussel, *Dreissena spp.* Previous studies (Howell *et al.*, 1996; Stewart & Haynes, 1994; Dermott, 1993; Griffiths, 1993; Mackie, 1991) have indicated that the presence of *Dreissena* can have an effect on both community composition and physico-chemical conditions. As the results, from the previous chapter, failed to show any conclusive trends between *Dreissena* numbers and community composition, the *Dreissena* were removed from the intact boxes upon return to the laboratory. Any effect *Dreissena* have on community composition were considered by recording *Dreissena* numbers in each of the boxes and incorporating them into the environmental data-set.

Upon returning to the laboratory, the intact boxes were maintained at 10°C in a temperature-controlled chamber. The overlying water in the boxes was aerated and water lost due to evaporation was replaced with aerated distilled tap water.

To prepare the contaminated sediment, the additional sediment collected from the field was sieved through a 250μm mesh sieve and left for 2 days. At this time the surface water was removed. Based upon the water content of the drained, clean sieved sediment, the volume of sediment required to cover the surface of each box with a 1cm layer of dry sediment was calculated. These volumes of sediment were then spiked with either 250mg Cadmium/kg of dry sediment, in the form of $CdCl_2$, or 4g/L (4ppt) of the pesticide Atrazine. Using the equation outlined in Appendix C, the appropriate quantity of a 5000mg/L $CdCl_2$ stock solution, sufficient to spike the intact boxes with 250mg Cd/Kg dry sediment, was added to three 4L acid-washed glass jars, each containing predetermined quantities of sediment for 1 of the 3 boxes to be spiked. Farm-grade Atrazine pellets were used to spike the sediment with Atrazine; 200mg of the pellets were

dissolved in 50ml distilled water and mixed with enough sediment slurry to cover three of the intact boxes with a 1cm layer of dry sediment. The sediment and contaminants were mixed in the 4L acid-washed jars by an electronic shaker at high speed for four hours; the time and speed used were considered adequate to achieve a homogeneous exposure of the contaminant, and allow it to adsorb to the sediments (Kirkby, CCIW, pers. comm.). The concentrations of contaminants used were based on literature reviews of the LC_{50} and loading values found locally (Appendix D). Those concentrations chosen were considered to affect the whole community and were considered environmentally realistic. An additional set of three boxes was organically enriched with a concentrated YTC formula (10g/500ml of a mixture of yeast extract, digested trout-chow and cerophyll formula, as described in the previous chapter).

Three boxes were randomly allocated for each of the treatments, together with a set of 3 boxes, which were left unmanipulated as control boxes. In April, only 9 boxes were collected; the effects Atrazine were not considered at this time.

Dissolved oxygen levels, pH, and temperature were all monitored biweekly. Oxygen levels within the boxes were maintained in the range of 10.4 and 8.9mg/L, as close to 10mg/L as possible; within the normal range found in the field at a temperature of 10°C. The average readings of the parameters recorded were calculated for each box and used in the analysis.

After a period of seven weeks, the overlying water was removed from the boxes. Five benthic cores, measuring 6.5cm in diameter, were randomly inserted into the each of the boxes, and the top 10-15cm of sediment within each core removed. The contents of each core were sieved through a 250μm mesh sieve, fixed in formalin and preserved in ethanol. Identification and enumeration of the organisms was carried out as outlined in the previous chapter, slide mounts being made where necessary.

A sample of the sediment remaining in the boxes after the cores had been removed was collected, freeze-dried, and sent to Seprotech Laboratories in Ottawa for complete

chemical analysis. Trace metals were determined by hot aqua-regia extraction followed by measurement with an Inductively Coupled Argon Plasma Atomic Emission Spectrometer (ICP-AES). Mercury was assessed by digestion with hot nitric acid and hydrochloric acid, followed by measurement with a Cold Vapour Atomic Absorption Spectrometer. Boron (B) was determined by fusion with sodium hydroxide followed by measurement with a Direct Current Plasma Atomic Emission Spectrometer. Determination of ten major oxides was achieved by mixing the sample with Spectroflux 100 B (4:1 lithium meta- and tetra- borate) in a graphite crucible. Fusion was then carried out and the molten mixture dissolved in nitric acid. Measurements of elemental concentrations are made by ICP-AES with a multi-channel Jarrel-ash AtomComp 1100 and the contents of oxides are calculated. The Seprotech analyses resulted in 46 sediment parameters, and an additional 7 were measured either in the field or the laboratory. The total number of environmental variables used in the analysis was reduced to 42 by eliminating those parameters which provided no additional information to the data set (for example, Al% was discarded as the concentration of Al was also provided).

4.3 *Data Analysis*

4.3.1 Variation among and within samples

The field data for each sampling occasion are from 5 cores taken from one mini-box core. It is important to address the variation within and among boxes across seasons in order to establish that changes noted in the community composition of the laboratory boxes are due to either season or treatment, rather than as a result of large differences between or within the boxes. The community descriptors used in the previous chapter; Shannon-Wiener diversity index, total abundance, average number of species per core, and mean density measurements, and the most abundant taxa, were used in the analysis. The same descriptors were also used by Gray *et al.* (1988) as part of a study carried out to establish cause and effect relationships between measured levels of pollutants and community response in outdoor mesocosms.

Variation seasonally, among boxes, and within the boxes was analysed using a nested ANOVA; season and treatment boxes nested within season, were selected as the independent factors. This analysis was run on the laboratory data only. To investigate the natural variation in the field one-way ANOVAs were run on the field data across season. All analyses were run using the statistical package SYSTAT v.7.0 (Wilkinson, 1997) on the log (x+1) transformed counts of the most abundant species, the Shannon-Wiener diversity index, mean density, total abundance and the average number of species per core. In order to address the differences in seasonal variation of the field and the laboratory samples, an F-ratio was manually calculated by dividing the seasonal mean square value by that given for the laboratory boxes. The same approach was applied to among- and within-box variation.

4.3.2 Responses to contamination

Community response to the different contaminants was analysed by multivariate analysis. According to Clarke (1993), untransformed abundance data will typically lead to a shallow interpretation in which only the pattern of a few very common species is represented. Transformation methods, which tend to make means and variances more similar, may allow a greater contribution from the rarer species. The data were transformed to prevent violation of the assumption of normality required for the univariate analysis techniques used. The untransformed data were used for the multivariate analysis (Reynoldson *et al.*, 1995), as the differences in abundance of each of the individual taxa are considered to be important descriptors of the community. Similarities between every pair of samples in the multivariate analysis were compared using the Bray-Curtis association measure (Bray & Curtis, 1957) as it is a reliable measure of association (Clarke & Green, 1988; Gray *et al.*, 1988; Faith *et al.*, 1987).

All ordination methods are a compromise, where data with an inherently high number of dimensions are viewed in lower dimensional (often 2-dimensional) plots. Non-metric Multi-Dimensional Scaling (NMDS) is one of the most robust ordination techniques available (Gray *et al.*, 1988), and it has been claimed that that NMDS makes the best

possible job of preserving the among-sample relationships accurately in a lower dimensional plot (Clarke, 1993). The relative distance of the samples reflects the relative similarities/ dissimilarities in species composition as measured by the Bray-Curtis metric. Stress values define the amount of scatter around the line of best fit through the NMDS distances and the actual distances. A low stress value indicates that a valid configuration has been found. Where more than 2 axes were considered in the analysis, only the first 2 axes have been presented. Since the first 2 axes account for the first and second most amount of variation in the data set, and represent a major portion of the total variation, it was felt that directional patterns were adequately portrayed in these 2 axes.

The environmental information was linked to the biological analysis by superimposing the values of the abiotic variables onto the respective sample positions in the biotic NMDS (Field *et al.*, 1982). This information was graphically plotted as environmental vectors on the NMDS ordination plot. Only the variables indicating a significant contribution to the observed pattern, tested through Monte-Carlo permutations, were plotted.

4.4 Results

4.4.1 Variation among and within samples

Forty taxonomic groups were used in the analysis (Appendix A); the additional 3 taxa; Porifera, Nematoda and Ostracoda, not considered in the 1995 sample season described in Chapter 3, were consistently present in large numbers and considered to represent important community descriptors. Oligochaete cocoons were also included as it was believed that their numbers reflected reproduction within the boxes. For the 10 most abundant taxa (Porifera (Por), *Vejdovskyella intermedia* (Vej_int), Immature tubificids with and without hair setae (Imm_chr, Imm_coh), *Limnodrilus hoffmeisteri* (Lim_hof), *Potamothrix vejdovskyi* (Pot_vej), *Spirosperma ferox* (Spi_fer), *Tubifex tubifex* (Tub_tub), Nematoda (Nem), and Ostracoda (Ost)), as well as the oligochaete cocoons (Coc), variation seasonally is greater than within the boxes (Table 4.1). This is also true

for diversity (Shannon-Wiener diversity index) and richness measures (mean density, total abundance, and number of species). Variation is greater within the boxes than seasonally if the F-ratio is less than 1. All of the most abundant taxa identified, and the community descriptors, show greater variation seasonally among boxes than within the boxes.

Analysis of the 10 most abundant taxa and oligochaete cocoons for the laboratory control boxes using nested ANOVAs (Table 4.2) show that, of the 11 analyses run, 8 indicate increasing variation from core to box, and from boxes to season. The remaining three taxa; the naid *Vejdovskyella intermedia*, the tubificid *Potamothrix vejdovskyi*, and the immature tubificids without hair setae, all displayed a greater degree of variation between laboratory boxes than across season. These two tubificids (immature tubificids without hair and *Potamothrix vejdovskyi*) are two of the most abundant groups in the data set. One of the three control boxes from both August and October had reduced numbers of *Potamothrix vejdovskyi*, and for August reduced numbers of immature tubificids without hair setae. However, the immature tubificids have an F-ratio close to one (0.892), indicating that the variation between the boxes is only marginally greater than seasonal variation. With the exception of one box collected in October, which showed far greater numbers than the any of the other boxes, very few *Vejdovskyella intermedia* were observed in the control boxes. The diversity and richness measures also give mixed results for the laboratory boxes. The Shannon-Wiener diversity measure and mean density both indicate a greater degree of variation between the boxes than seasonally.

Table 4.1: *F-ratio values for field data comparing the amount of variation within the boxes to seasonal variation. An F-ratio greater than 1 indicated the seasonal variation is greater than that within the boxes. Mean square values are also provided.*

Taxa, diversity & richness scores	F-Ratio (season vs. within-box variation)	Mean Square Values (MS)
Porifera	37.6	0.399
Vejdovskyella intermedia	1.0	0.033
Immature tubificidae		
Without hair	113.3	1.036
With hair	69.5	0.986
Limnodrilus hoffmeisteri	10.0	0.539
Potamothrix vejdovskyi	55.5	1.136
Spirosperma ferox	4.3	0.622
Tubifex tubifex	3.3	0.052
Cocoon	10.6	0.987
Nematoda	23.6	0.164
Ostracoda	13.5	0.136
Shannon-Wiener index, H	4.4	0.116
Mean density	46.2	0.450
Total abundance	52.6	0.463
Average species/core	1.3	0.005

Table 4.2: *F-ratios calculated using a nested ANOVA design, comparing variation within the laboratory boxes, among the laboratory boxes, and across season. Values have been calculated for the most abundant taxa, diversity (Shannon-Wiener diversity index), and richness measures (mean density, total abundance, and average number of species per core).* * *= variation greater between boxes than across season. Mean Square values (MS) are also provided.*

Taxa, diversity & richness scores	Within Box Variation (error)	F-Ratio			
		Among Boxes	(MS)	Across Season	(MS)
Porifera	0.025	3.2	0.081	1.1	0.092
Vejdovskyella intermedia	0.019	9.4	0.178	0.5*	0.098
Immature tubificidae					
Without hair	0.033	5.9	0.295	0.9*	0.174
With hair	0.063	5.1	0.320	1.1	0.335
Limnodrilus hoffmeisteri	0.046	1.2	0.053	8.2	0.433
Potamothrix vejdovskyi	0.135	12.0	1.620	0.7*	1.145
Spirosperma ferox	0.058	3.2	0.185	2.2	0.408
Tubifex tubifex	0.013	3.1	0.039	1.6	0.064
Cocoon	0.034	4.1	0.137	23.4	3.203
Nematoda	0.017	10.3	0.176	2.9	0.519
Ostracoda	0.009	8.0	0.070	4.2	0.294
Shannon-Wiener index, H	0.030	7.8	0.235	0.6*	0.151
Mean density	0.112	9.1	1.014	0.8*	0.858
Total abundance	0.082	12.5	1.023	1.5	1.575
Average species/core	0.034	4.4	0.148	3.0	0.439

4.4.2 Responses to contamination

An ordination of the field data and corresponding laboratory controls is shown in Figure 4.1a. The laboratory control data are represented by 95% confidence ellipses plotted around centroids for each sampling occasion. The field and control data follow the same trajectory through time, and the seven-week lag in the laboratory data is evident; they are just displaced in time. Figure 4.1b shows the species scores for the field and control data, indicating the direction in which the named taxa influence the data in ordination space. Only those taxa identified using Monte-Carlo permutations as having a significant contribution to the position of the box cores in ordination space have been plotted. The

field data differ from the laboratory data by the presence of Platyhelminthes and Hydridae (Hyd), although Platyhelminthes were not considered to be an important descriptor from the Monte-Carlo permutation test. The ordination is dominated by the oligochaetes, which are the predominant group of organisms at this site. Horizontal movement of the laboratory boxes along NMDS axis 1, away from the field data, towards the left, reflects a reduction in the numbers of these organisms. The vertical movement of the boxes, along NMDS axis 2, in ordination space results from changes in numbers of cocoons, ostrocoda, and the gastropod *Fossaria obrussa* (Fos_obr).

Figure 4.2 (a-d) show ordinations of the treatments for each of the sample times, April, June, August and October (a, b, c, and d respectively). As in the previous ordination plot, 95% confidence ellipses have been plotted around the means for each of the different treatments (control, cadmium, enrichment and atrazine). Those species identified as contributing significantly to the ordination pattern have been plotted in the form of vectors. The arrows indicate the direction to which that organism influences the data in ordination space.

The boxes collected in June show little change as a result of the sediment contamination in relation to the controls. The remaining three sample times show similar movements in ordination space of the box scores away from the control condition, as a result of the addition of the different stressors. The addition of cadmium results in an overall reduction in taxa, and move from the control condition in a direction opposite (with the exception of Porifera) to that indicated by the species vectors. Both the enriched and atrazine-spiked treatments show consistent increases in the abundance of some taxa (the immature tubificids, *Spirosperma ferox*, *Potamothrix vejdovskyi*, Nematoda, Ostracoda, and the oligochaete cocoons), and diverge from the control condition in the direction indicated by the species vectors. Although enrichment and atrazine treatments show similar effects, the enrichment boxes generally moved in a direction indicating an increase in the number of immature tubificids with hair setae and *Spirosperma ferox*. The atrazine treatment tends to result in an increase in the number of mature *Limnodrilus spp.*

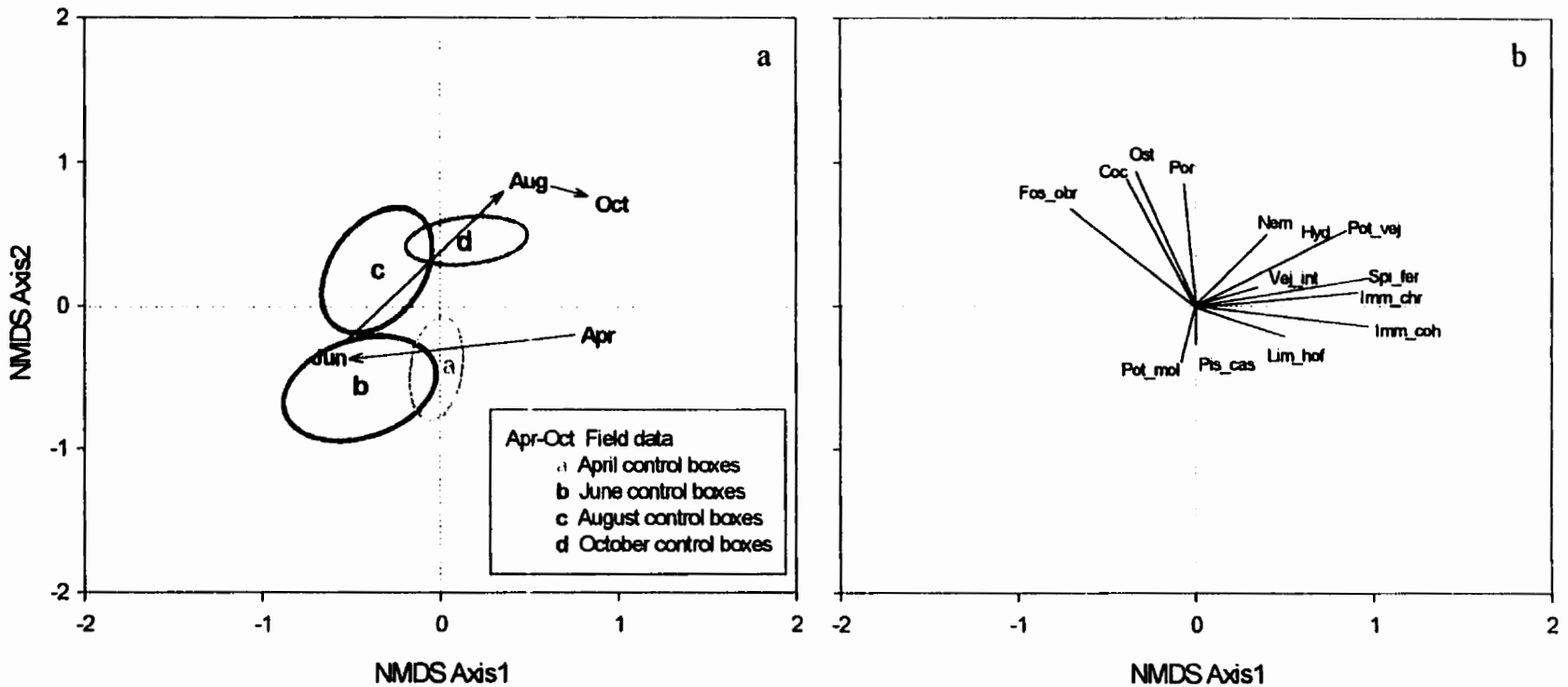

Fig. 4.1: *(a) Ordination plot of field data (Apr-Oct) and control boxes (a-d). 95% confidence ellipses have been plotted around the mean of each of the four monthly control data. Arrows (Apr-Oct) indicate movement of the field data within ordination space. The right hand ordination plot (b) denotes the directional influence of the significant taxa as identified by Monte-Carlo analysis. Full species names are given in the text. (Stress = 0.119).*

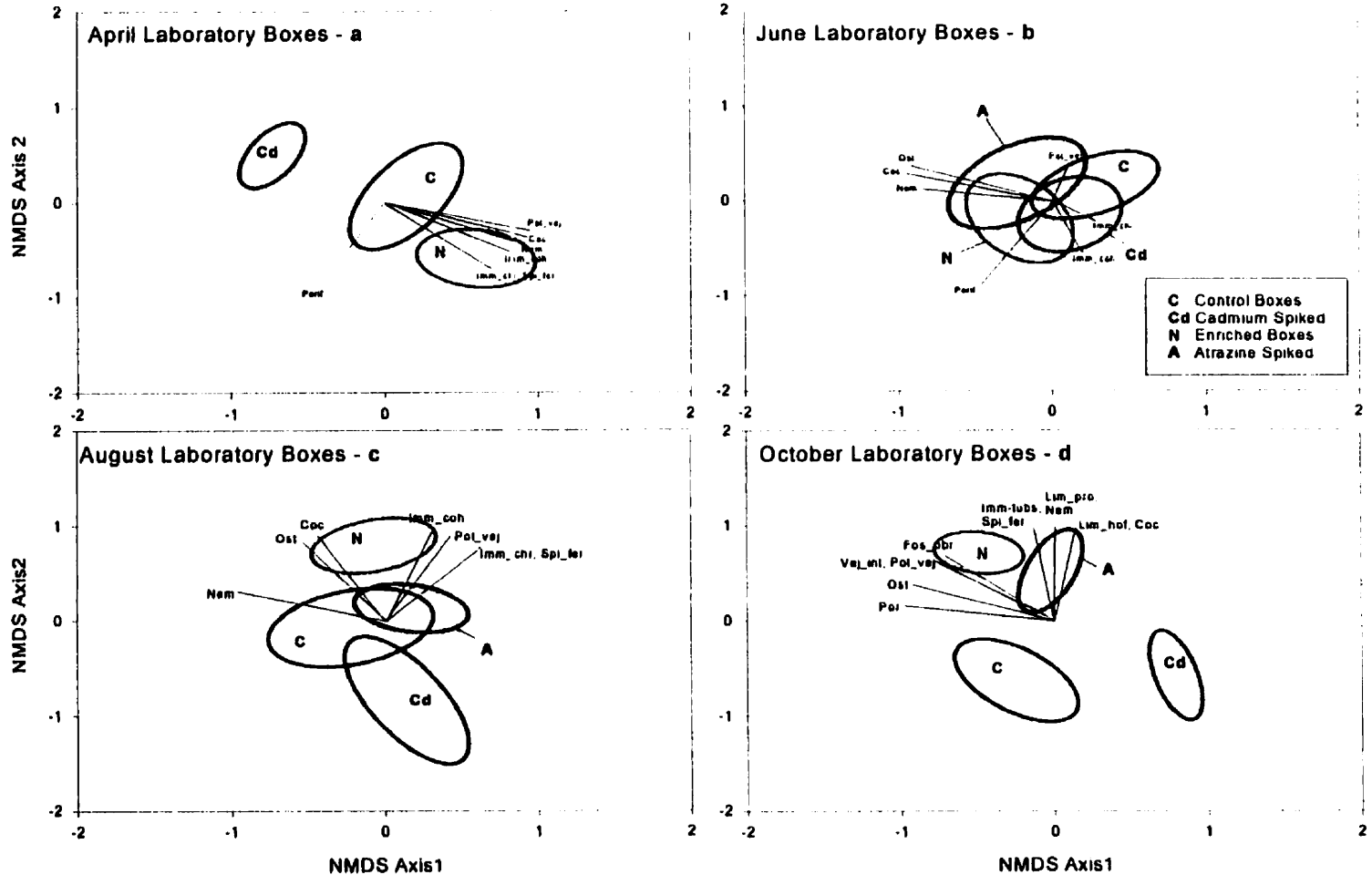

Fig. 4.2 (a-d): *Monthly ordination plots of the laboratory data. 95% confidence ellipses have been plotted around the sample means for each of the treatments. Species vectors of the significant species as identified by Monte-Carlo analysis have been plotted for each month. (Stress = (a) 0.169, (b) 0.12), (c) 0.181, (d) 0.169).*

Treating the seasons separately makes it difficult to assess whether the treatment effects are consistent. Figure 4.3a shows the combined laboratory boxes seasonally, together with the corresponding field data. A consistent pattern of divergence from the control condition, as a result of the different stressors, is observed. Enrichment and atrazine additions cause a movement of the box scores toward the field data. The June data are different from the other months sampled and had a confounding effect, so were eliminated and the analysis was re-run. Upon removing the June boxes from the analysis (Fig. 4.3b) there is still the same directional separation of the treatments, although this separation is more pronounced.

Figure 4.4a illustrates directional changes in the community composition of the laboratory boxes and field data in ordination space. The taxa and environmental variables identified through Monte-Carlo analysis are illustrated in Figure 4.4b. Forty-two environmental variables were measured. Of these, 13 were identified as being significantly correlated to the sample scores; 8 chemical parameters (cadmium (Cd), manganese (Mn), barium oxide (BaO), magnesium oxide (MgO), silicon dioxide (SiO_2), total phosphorous (Ptot), nitrogen (TKN) and dissolved oxygen (O_2), 4 physical parameters (Julian-day, sample week, sediment surface temperature (temp), and depth), and the *Dreissena* counts taken from the boxes (fig. 4.4b). The cadmium treated boxes are positively correlated in ordination space to the cadmium environmental vector and indicated an overall loss of species. The field data are separated from the laboratory data along temperature and 'sample week' gradients. Both these vectors are related to each other, as the laboratory boxes were all held the same temperature (which was marginally higher than that found in the field) and sampled 7 weeks after the return of the boxes to the laboratory (the field samples were collected at 'week 0'). Collection date (Julian-day) is correlated to NMDS axis 1, which is reflected in the horizontal spread of the field data along this axis. MgO, BaO and Mn are also strongly associated with this axis, as well as total phosphorous (Ptot) and total nitrogen (TKN) in the opposing direction. No significant differences between the treatments and the field data are observed for the MgO, BaO, or Mn measurements, although there are generally lower levels of MgO found in the sediments of the enriched and atrazine treated boxes. Levels of Manganese

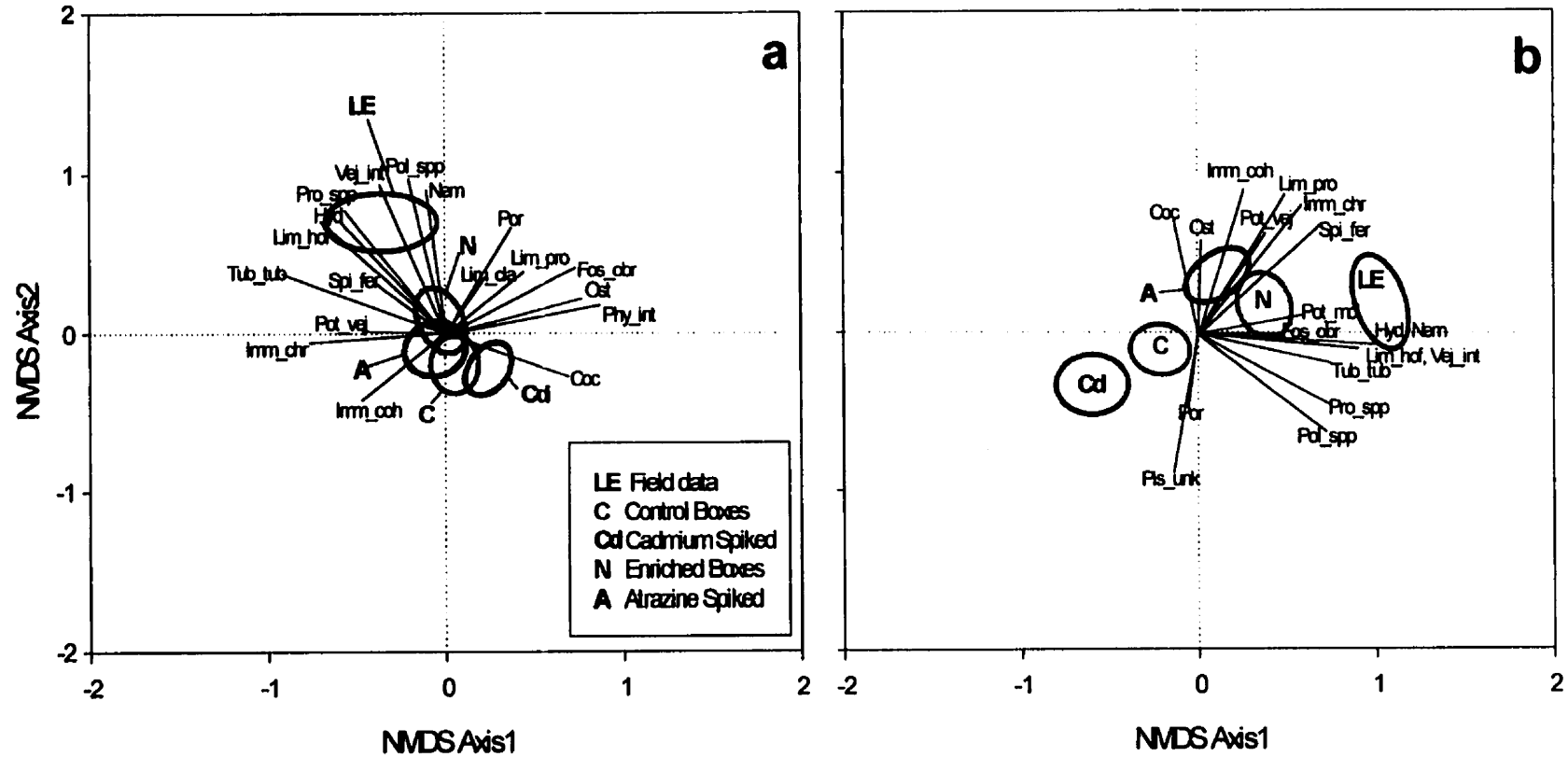

Fig. 4.3: Ordination plots of the combined field and laboratory data. 95% confidence ellipses have been plotted around the sample means for the field and treatment data. Significant species as identified by Monte-Carlo analysis are represented by vectors. (a) represents all of the seasonal data, and (b) shows the combined seasonal data without the June samples. (Stress = (a) 0.134, (b) 0.132).

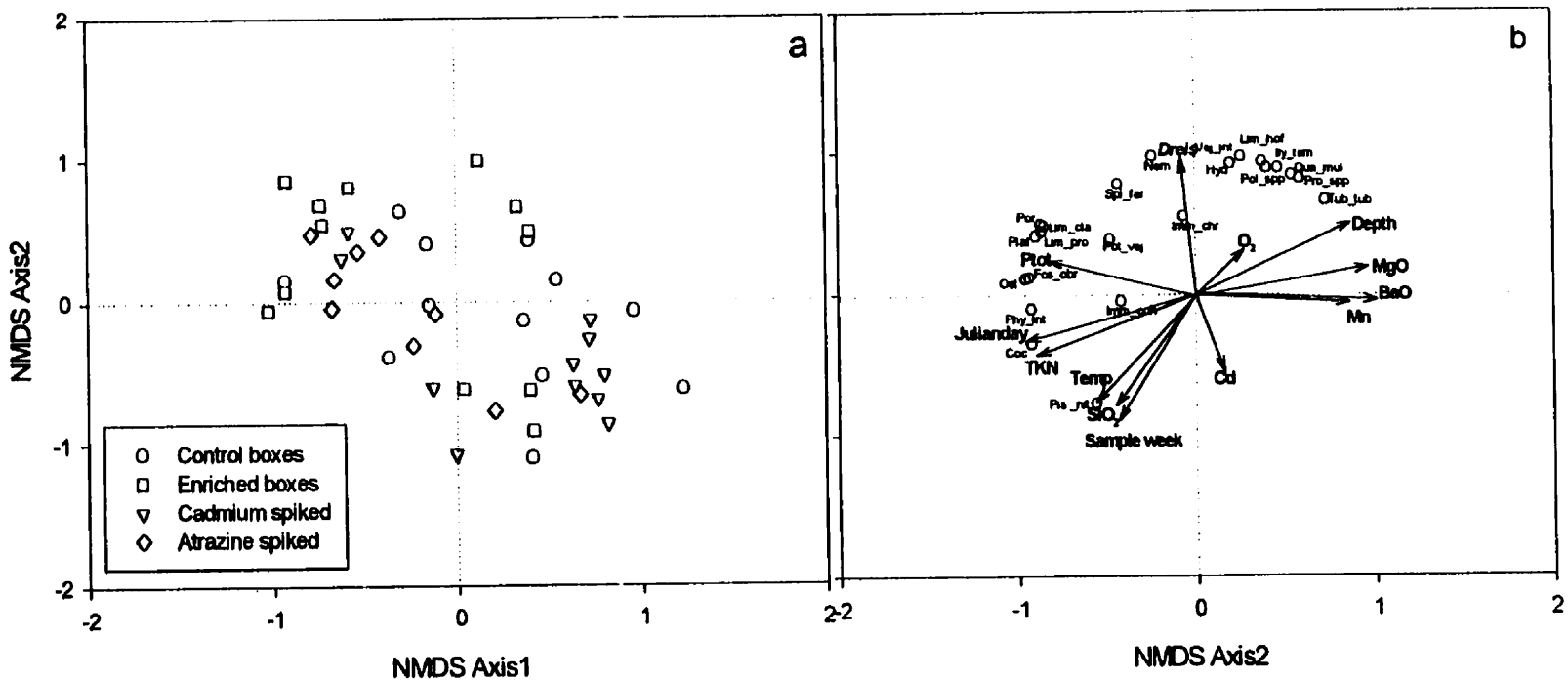

Fig. 4.4: (a) Ordination of the combined seasonal data (June included), each symbol represents the average values for each box. Apr - Oct represent the field data. (b) Ordination plot of significant species scores (identified by Monte-Carlo analysis), significant environmental scores have been plotted in the form of vectors.)Stress = (a) 0.103, (b) 0.05).

(Mn) are lower in the atrazine boxes than in the enriched boxes despite their similar positions in ordination space. Overall decreases in BaO and MgO are noted with season, although only those boxes collected in October differed significantly from the rest (ANOVA, $p< 0.05$). Total nitrogen levels are significantly higher in the enriched and atrazine treated boxes compared to the field and control boxes ($p_{enriched}< 0.001$; $p_{atrazine}< 0.05$). Total phosphorous levels are significantly higher in the enriched boxes compared to the field and control boxes ($p= 0.053, 0.052$). Both total nitrogen and total phosphorous reflect the position of these treatments in ordination space.

The position of dissolved oxygen reflects the dissolved oxygen content recorded from the field. The average laboratory levels of this parameter are significantly lower than the average of those recorded seasonally from the field ($p = 0.002$), and is reflected in the separation the field data from the laboratory data in ordination space.

Significant differences in the average number of *Dreissena* is observed between the laboratory and field boxes ($p< 0.013$), no significant differences were observed seasonally. Treatment effects excluded these organisms, so these data reflect the variable distribution of these organisms between boxes and are related to NMDS axis 2, helping to account for the vertical separation in ordination space of the test boxes from the field boxes.

Total phosphorous and nitrogen are correlated with numbers of the tubificids, immature and the mature *Limnodrilus profundicola*, *L. claparedeanus*, *Potamothrix vejdovskyi* and (to a lesser extent) *Spirosperma ferox*, the gastropod *Fossaria obrussa*, as well as Porifera and cocoon counts. These findings are in agreement with the correlations found between species counts and treatment in the laboratory boxes (figs. 4.2a-d and 4.3).

4.5 *Discussion*

4.5.1 Variation within and among boxes

Spatial and temporal variation in the distribution and abundances of organisms is an inherent property of ecological systems (Anderson & Gribble, 1998). Results from univariate analyses show that variation seasonally is relatively large and is not masked by the variation observed between or within the boxes.

Movement of the communities of the laboratory control boxes in ordination space followed a similar pattern to that noted for the field data. As discussed in the previous chapter, differences between the laboratory boxes and the field boxes may be a result of sampling and collection differences. Disturbance of the overlying sediment, resulting from transportation back to the laboratory, may also be a contributing factor for these differences in the overall composition of the communities. Despite movement of the intact boxes away from the field data, a similar seasonal movement of the field and laboratory data in ordination space indicates that there is little change in the species composition within the boxes, despite the loss in benthic fauna as a result of the move from the field to the laboratory.

4.5.2 Responses to contamination

Cadmium caused changes in species composition, showing an overall reduction in the abundances of species. Wiederholm (1988) suggests that cadmium may retard or inhibit growth, but does not usually affect survivorship over several months. However, there are differing opinions in the literature as to the tolerance of oligochaetes to metals (Wiederholm *et al.*, 1987, Canfield *et al.*, 1994, Poulton *et al.*, 1988). Brinkhurst and Jamieson (1971) as well as Hynes (1960) and others have suggested that an absence or marked reduction in the number of worms may indicate the presence of heavy metals. Later field studies by Cairns (1984) confirmed this coupling between high metal concentration in the sediment and low abundances of oligochaetes. Chapman's (1982) work indicates that *Tubifex tubifex* are significantly more tolerant to cadmium that

Limnodrilus hoffmeisteri. This cannot be confirmed from the data, although reductions of both of these species were observed.

The effects of atrazine are contrary to the results expected. Previous researchers (Forget *et al.*, 1998; Gruessner & Watzin, 1996; Solomon *et al.*, 1996; Huber, 1993) indicated that, although this herbicide does not pose a significant threat to the aquatic environment, higher concentrations of the herbicide (>20μg/L) caused a decline in aquatic organisms. Individual organisms were found to vary in their sensitivity to the compound. However, this study yielded different effects than those expected; an increase in the abundance of the immature tubificids and *Limnodrilus spp.* is indicated by the ordination plots, and is confirmed by inspection of the abundance counts for these species.

Work carried out by Pratt and co-workers (1997) using microcosms consisting of polyurethane artificial substrates to assess the ecological effects of herbicides, reported that atrazine caused significant increases in algal biomass. The concurrent enhancement of nutrient recycling, and increases in the detectable number of heterotrophic microbial species, were also observed. Such effects would result in increased food availability for the benthic fauna, and thus an increase in the aquatic organisms. Gruessner and Watzin (1996) observed in their microcosm studies increases, though not significant, in oligochaete and gastropod numbers and decreases in Nematoda and Sphaeriidae. They also observed significant increases in the emergence of insects. Although emergence was not considered in this study, there was a distinct increase in the numbers of identifiable mature oligochaetes. However, the results were only observed at low levels, 3-100μg/L in Pratt's study, and 1-5μg/L by Gruessner and Watzin. Higher levels produced a general collapse of the laboratory ecosystem. The concentrations applied to the boxes in this study exceed the low concentrations sited by Pratt, Gruessner and Watzin.

Atrazine degradation can occur via biotic and abiotic processes. Biotic degradation is mediated by microorganisms and results in N-dealkylation and cleavage of the nitrogen ring; chemical hydrolysis results in dechlorination of the compound, and is primarily a soil catalysed process (Mersie *et al.*, 1998). Degradation of atrazine occurs readily in the

presence of Manganese (Mn) II and ozone (Ma & Graham, 1997; Rodriguez & Harkin, 1997), or in oxidized conditions (DeLaune *et al.*, 1997). Under favourable conditions it can be degraded completely in a few weeks. The laboratory boxes were aerated for the duration of the experiments and sediment manganese levels are high enough to promote rapid degradation (Ma & Graham, 1977) providing the favourable conditions described above. Atrazine has a low vapour pressure and low Henry's law constant; therefore, volatilization of the compound from the surface water was considered negligible (Solomon *et al.*, 1996). In light of the favourable conditions provided, together with the significantly higher levels of nitrogen found in the atrazine treated boxes, it is likely that degradation of atrazine occurred. However, unrecorded environmental factors in the treatment boxes may co-vary closely with the atrazine signal, and may be themselves the cause of the observed change (Clarke, 1993). Failure to record all of the environmental variables affecting the community composition is inevitable.

The abundance and species composition of oligochaetes have long been associated with the effects of enrichment (Hynes, 1960). The enrichment of sediments is expected to result in an increased abundance of certain oligochaetes, and species have been characterised by their relation to different trophic states (Lauritsen *et al.*, 1985). As observed by others (Lang, 1997; Verdonschot, 1996; Wiederholm *et al.*, 1988; 1987; Lauritsen *et al.*, 1985; Milbrink, 1983; Brinkhurst, 1967; Hynes, 1960), nutrient enrichment of the sediment resulted in increased abundances of oligochaetes. Many oligochaetes can only be identified in their mature form; enrichment of the sediment encourages maturation. The presence of immature tubificids and cocoons indicates reproduction is occurring.

Increases in oligochaete abundance were noted in both the enriched and Atrazine-spiked boxes. It was predominantly the tubificid worms that responded to the enriched boxes. Of the tubificids, the immature tubificids, *Spirosperma ferox*, *Limnodrilus claparedeanus*, *L.profundicola*, and, to a lesser extent, *L. hoffmeisteri* were all identified as being significantly affected by the enrichment treatment. These species favour such conditions, and their relationship to enrichment has been documented by other researchers

(Brinkhurst, 1967; Milbrink, 1983; Lauritsen *et al*, 1985; Wiederholm *et al*., 1987, 1988; Verdonschot, 1996; Lang, 1997). The observed relationship of *Spirosperma ferox* to enrichment may also be associated with changes in sediment structure (Brinkhurst & Jamieson, 1971). Unfortunately this cannot be confirmed, as sediment structure indices, such as particle size, were not recorded. Dissolved oxygen concentrations were correlated negatively to the presence of the pollutants in the enriched and atrazine spiked boxes, which is in keeping with findings from other researchers (Lauritsen *et al*., 1985; Pratt *et al*., 1997) and the assertion that Atrazine acted to 'enrich' the microbial community.

The June data was different from the other months; seasonally, observed from the field data, and upon the addition of contaminants to the laboratory boxes (Fig. 4.1). Stratification of Lake Erie occurs around this time (Schertzer *et al*., 1987) and may contribute to the anomaly noted here. The presence of the June data in the analysis masked some of the overall effects observed in the pooled seasonal data. Excluding these data from the analysis, the impacts of the contaminants were strong, and distinct from each other and from the seasonal changes.

4.6 *Conclusions*

This study set out to establish whether intact box cores could be used in the assessment of sediment toxicity. Benthic animals are not evenly distributed in benthic systems and "communities" can vary a great deal over relatively short distances (Swift *et al*., 1996). Nevertheless, the study design used, with only 3 sets of replicates for each treatment, was able to demonstrate treatment effects. The use of multivariate approaches and replicate samples showed that there was a consistent directional change in the community composition as a result of different stressors. Changes in species composition are comparable to those found in the literature for both single species and community toxicity tests.

Spatial and temporal variation in the distribution and abundances of organisms is an inherent property of ecological systems (Anderson & Gribble, 1998). Barton (1989) emphasises that the timing of fieldwork will reflect life-cycle events such as recruitment, migration and sexual maturation, all of which may influence apparent water quality. He suggests that, in the Great Lakes, sampling in May-June would tend to overestimate the importance of *L. hoffmeisteri* and *Tubifex tubifex*, whereas more *L. cervix* and *P. moldaviensis* would be found later in the season. Combining the seasonal data of this study retained the directional patterns caused by sediment contamination; this indicates that changes in the community composition as a result of sediment contamination are not masked by seasonal changes in species composition. These directional trends can be compared to the environmental variables recorded.

In summary, the methods described here provide a promising use of multivariate methods to identifying the effects of stressors on community composition. The study has shown that the use of intact box cores in the form of laboratory mesocosms show consistent and predictable responses to certain stressors.

CHAPTER 5

Uses as a Diagnostic Tool to Predict Sediment Contamination

5.1 General Introduction

It is difficult to predict the effects of contaminants on an ecosystem based on its individual components (Dewey, 1998; Landis *et al.*, 1997). Indeed, Cairns (1984) argues that there is insufficient knowledge to accurately make predictions of a toxicological response from one level of organisation to another. To be predictive, a test must be environmentally and ecologically realistic (Sheehan, 1984; Buikema, 1993). Microcosm and mesocosm tests, conducted in conjunction with field calibration studies, could greatly enhance our understanding of chemical stress on natural aquatic systems (Sheehan *et al.*, 1986). Results from such tests would have environmental applicability, since they provide for interspecific interactions and allow more species to be exposed than single species tests (Tagatz, 1986).

Numerous studies using multispecies data from microcosms, mesocosms, and field studies have successfully employed multivariate techniques (Kedwards *et al.*, 1999; Sparks *et al.*, 1999; Shaw & Manning, 1997; Reynoldson *et al.*, 1995; Gray *et al.*, 1988; Wright *et al.*, 1984), in an effort to identify the effects of contaminants at a community level. However, no multispecies communities have thus far been used in a standardized bioassay for sediment quality assessments in spite of their greater realism compared to single species tests (Clarke, 1999). An important question, in defining community level responses to contaminants, is whether the type of community response detected by multivariate analysis is specific to particular pollutants, or whether it tends to reflect a general effect of the contaminant impact (Bailey *et al.*, 1995).

This thesis set out to establish whether community structure, observed in intact boxes collected from the field, could be used as a diagnostic tool to identify the principal stressor at sites of unknown contamination. Thus far, the thesis has established that collection of intact sediment cores, and the maintenance of the resident macroinvertebrate communities is possible. To an extent, these communities can be compared to those found in the field, and used for mesocosm sediment bioassays. The diagnostic capacity of the communities within the intact boxes, to assess sediment contamination, will be considered in this chapter.

5.2 Summary of Methods Employed

The data used and described in the previous two chapters were pooled and analysed, using the same multivariate ordination methods already described. As the communities from the boxes collected in 1995 were considered to change negligibly over time, the taxa counts from all of the cores removed from each of the boxes (laboratory and field) from 1995 and 1996 were averaged. This resulted in an average value for each species identified in each of the boxes. The additional taxa enumerated in 1996 (Porifera, Nematoda, Ostracoda) as well as the cocoons were not considered, resulting in a total of forty taxa used in the analysis.

The outlying control box identified in Chapter 3, and the data collected in June 1996 described in Chapter 4, were excluded from the analysis as they were considered anomalous, and would be confounding to the analysis. A total of 58 boxes were considered; 50 laboratory boxes and 8 boxes collected from the field over the two-year sampling period. Of the 50 laboratory boxes, 17 were controls and had been left unmanipulated for the duration of the experiments, 15 were enriched with varying quantities of a yeast, trout-chow and cerophyll formula. The remaining boxes all had spiked sediment additions: 9 boxes had sediment spiked with 250μg/L cadmium, in the form of $CdCl_2$; 6 boxes had sediment spiked with the pesticide Atrazine; and 3 had

copper-spiked sediment applied. The methods used to spike sediment with cadmium were used for the copper-treated sediment (Appendix C); clean sieved sediment from Lake Erie Site 303 was spiked with 1g copper/ Kg dry weight sediment, in the form of $CuCl_2$. The copper-spiked boxes were collected in May 1995, and maintained at 4.5°C.

In setting guidelines for invertebrate community structure at a test site, Reynoldson and Day (1998) adopted a multivariate approach that would aid in defining the degree of impact. The algorithms used in this approach may be complex, but the output is relatively straightforward: the closer together two points are, the closer their community composition. The likelihood of sites being the same as the reference sites described by Reynoldson and Day (1998) was qualified by constructing three probability ellipses (90%, 99% and 99.9%) around the reference data. Data falling within the bounds of the first (90%) ellipse represent those data that would be considered equivalent to the reference data, and therefore unstressed. Sites lying between this ellipse and the next largest (99% probability ellipse) would be given the designation of 'possibly different', and the sites between the 99% and 99.9% ellipses designated as 'different'. Anything outside of the outer (99.9%) ellipse would be considered very different from the reference data. A 90% probability ellipse was used, rather than the more typically used 95% interval, under the premise that a multivariate approach tends to result in noisier relationships than univariate methods (Reynoldson *et al.*, 1998).

Probability ellipses, as described by Reynoldson and Day (1998), were constructed around the field data for 1995 and 1996. These ellipses are different from the confidence ellipses used in the previous chapters. The probability ellipses used here are larger than the confidence ellipses as they are constructed for the total sample rather than for the centroid of the sample. The difference is analogous to the standard deviation verses the standard error of the mean. The effects of the different contaminants are described in relation to the directional movements of the laboratory boxes in ordination space.

The species data (attributes) were first correlated to the boxes (objects) using the principal axis correlation (PCC) option in PATN (Belbin, 1993). This procedure is

designed to interpret how well a set of attributes can be fitted to an ordination space. The program produces a number of multiple regressions of the ordination vectors with each of the attributes. As part of the output, together with a set of ordination values for each object and attribute, multiple correlation coefficients (R^2) are given which indicate how correlated a given attribute is with the variation in community structure as represented by the ordination. The direction in which each attribute influences the data in ordination space can be interpreted by plotting the ordination vectors. The environmental variables were also correlated to the species data using this method.

Once the attributes (species or environmental data) were correlated to the data set, their statistical significance was identified using Monte-Carlo permutation procedures.

5.3 Results

The directional changes in community composition of the laboratory boxes, compared to the field boxes, are graphically illustrated in figure 5.1a. There is a 1 in 10 (10%) chance of sites falling outside the 90% ellipses and a 1 in 100 (1%) chance of them lying outside the 99% ellipses through natural variation (Wright *et al.*, 1995; Clarke *et al.*, 1992). Of the 17 control boxes, 12 (71%) were found to lie within the 90% probability ellipse and be considered equivalent to the field data (Table 5.1). A further 3 (17%) were considered to be only 'possibly different' for the field data, and the remaining 2 different. Copper-spiked sediment resulted in one of the three boxes (33%) lying within the 90% ellipse, and the remaining 2 boxes qualified as being 'possibly different' from reference condition (field data). The effects of cadmium additions to the sediment were observed as an overall movement away from the field sites; only 1 of the 9 boxes (11%) was considered to be equivalent to that of the field data, 1 (11%) were designated as being possibly different. The remaining boxes (78%) were considered to be different. Enrichment of the boxes resulted in an increase, compared to the control boxes, in the number of boxes considered to be equivalent to the field data (87%). All of the boxes treated with Atrazine were considered to be equivalent to the field data.

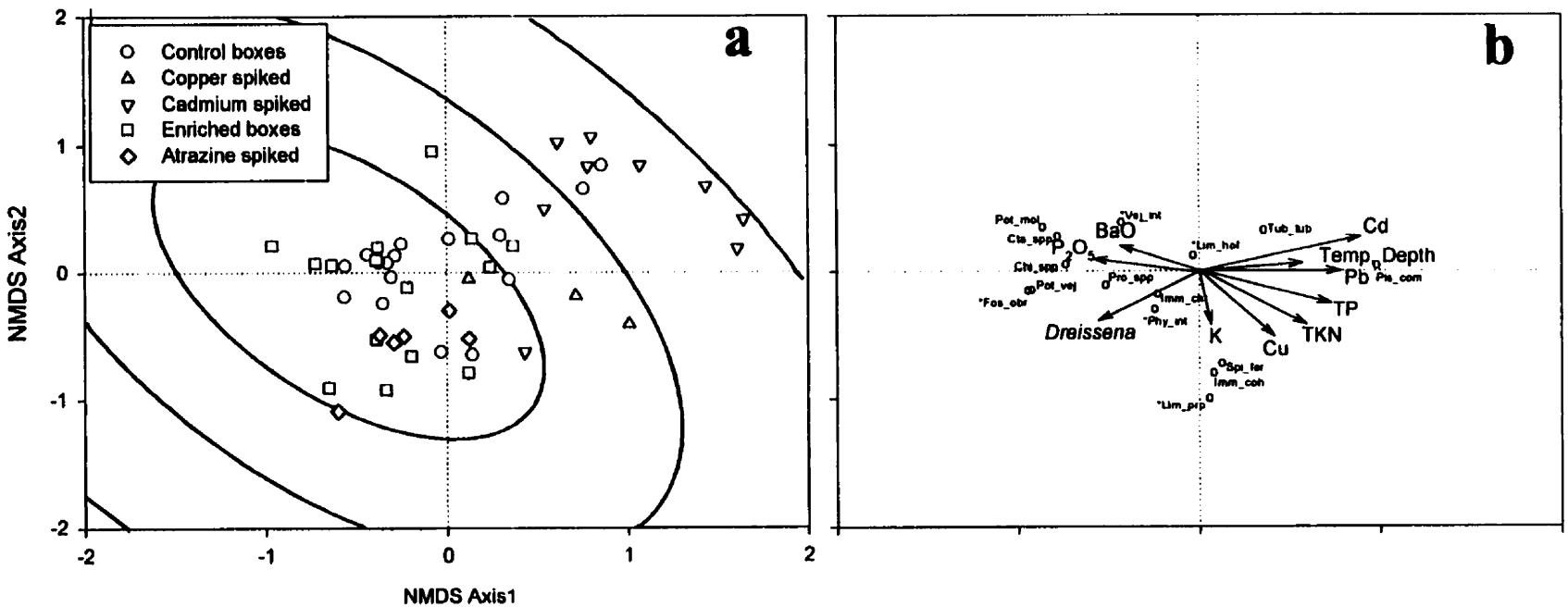

Fig. 5.1: (a) Ordination plot of 1995 and 1996 data (June, 1996 excluded). 90%, 99% and 99.9% probability ellipses have been plotted around the field data (points not shown). (b) Ordination of species scores (indicates those not identified as significant through Monte-Carlo analysis) and the environmental scores (plotted in the form of vectors). Full species and environmental names given in text.*

The environmental and species attributes, which were considered important descriptors by the multivariate analysis, were plotted in the form of bubble plots (figs. 5.2 & 5.3). The values of each of the attributes selected were plotted in ordination space for each of the boxes. Where necessary, these values were scaled down by dividing them by 2, 5, or 10. The cadmium values were log(x+1) transformed as the differences in concentrations of the spiked boxes were a factor of 10 greater than the other boxes. These plots show the relative attribute values rather than the absolute concentrations. The correlation coefficients calculated as part of the PCC analysis are also given.

A total of 40 measured environmental variables were used in the analysis (Appendix B). Of these, 11 were identified as being significantly correlated to the sample scores: 8 chemical parameters (cadmium (Cd), copper (Cu), potassium (K), barium oxide (BaO), phosphate (P_2O_5), total phosphorous (TP), and total Kjeldahl nitrogen (TKN); 2 physical parameters (sediment surface temperature (temp), and depth), and the *Dreissena* counts taken from the boxes (fig. 5.1b). Generally temperature separates the laboratory boxes (fig. 5.2), which were maintained (with the exception of the boxes collected in May 1995) at temperatures higher than that found in the field, along NMDS axis2. Depth, Cd, Pb, TP and TKN are also strongly associated with this axis, as well as P_2O_5 and BaO in the opposing direction. No significant differences between the treatments and the field data are observed for the BaO or depth measurements (ANOVA, $p > 0.05$), although there are generally higher levels of BaO found in the samples collected in 1995 (Appendix A). Levels of P_2O_5 also tend to be higher in the boxes collected in 1995, and are significantly higher in the field boxes than in the laboratory boxes ($p < 0.001$), contribution to the position of the boxes in ordination space in relation to this vector. Cadmium and copper levels are both significantly greater in the boxes treated with these metals ($p < 0.001$) as was expected. The position of their vectors indicates an overall loss of species; these trends are illustrated in figure 5.3. Concentrations of lead, total Kjeldahl nitrogen (TKN) and total phosphorous (TP) are greater in the 1996 samples, and significant increases of TKN and TP were noted in the enriched and Atrazine treated boxes ($p_{TKN} = 0.009_{enrich}$, $0.002_{atrazine}$; $p_{TP} < 0.001$) compared to the field boxes. Both TKN and TP reflect the spread of these treatment boxes along NMDS axis2 in ordination space.

Table 5.1: Summary of the comparison between laboratory boxes and the field data in ordination space using 90%, 99% and 99.9% probability ellipse criteria, as defined by Reynoldson and Day (1998). The percentages of boxes per treatment falling into each criterion are given in parentheses.

		Ellipse Definitions		
Treatment	*n*	'Equivalent'	'Possibly Different'	'Different'
Control	17	12 (71%)	3 (17%)	2 (12%)
Copper	3	1 (33%)	2 (67%)	.
Cadmium	9	1 (11%)	1 (11%)	7 (78%)
Enrichment	15	13 (87%)	2 (13%)	.
Atrazine	6	6 (100%)	.	.

Potassium concentrations are correlated to NMDS axis1 and reflect the position of the enriched boxes in ordination space. Although no significant differences are observed between the laboratory and field boxes ($p>0.05$), an overall increase is noted in the enriched boxes.

Significant differences in the average number of *Dreissena* are observed between the laboratory and field boxes ($p< 0.006$), while no significant differences were observed seasonally. Treatment effects excluded these organisms, so these data reflect the variable and patchy distribution of these organisms between boxes.

Those species marked with an asterisk in figure 5.3 were not identified as having a significant relationship to the boxes in ordination space. However, in the previous two chapters these species were considered important from both the multivariate and univariate analyses, and hence have been included on the plot.

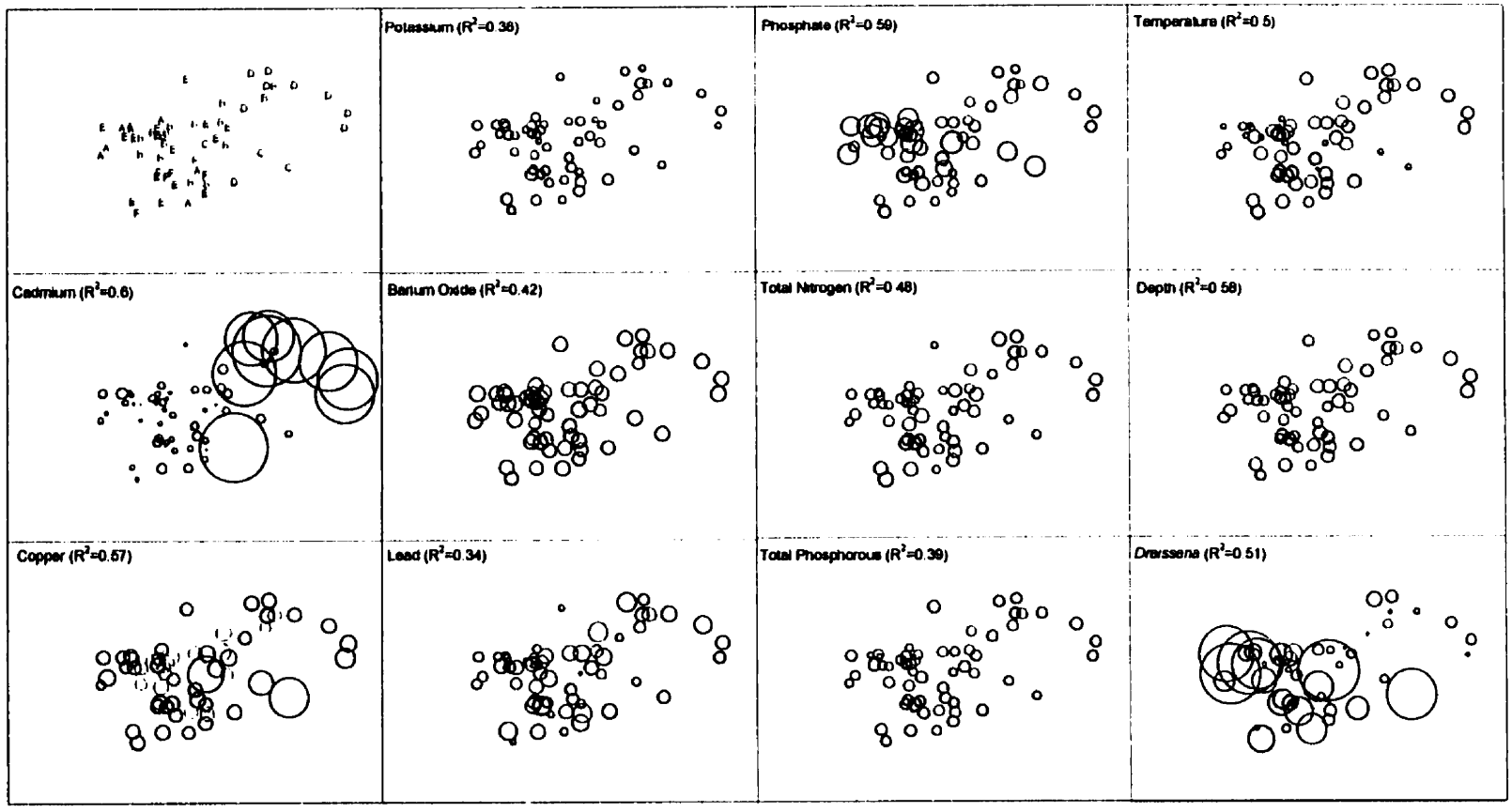

***Fig.5.2**: Bubble plots of environmental variables showing relative concentration for each of the boxes in ordination space. Top left-hand graph represents the ordination plot for the laboratory and field data. A= Field data, B= Control boxes, C= Copper spiked boxes, D= Cadmium spiked boxes, E= Enriched boxes, F= Atrazine spiked boxes. R^2= multiple correlation coefficients calculated from PCC.*

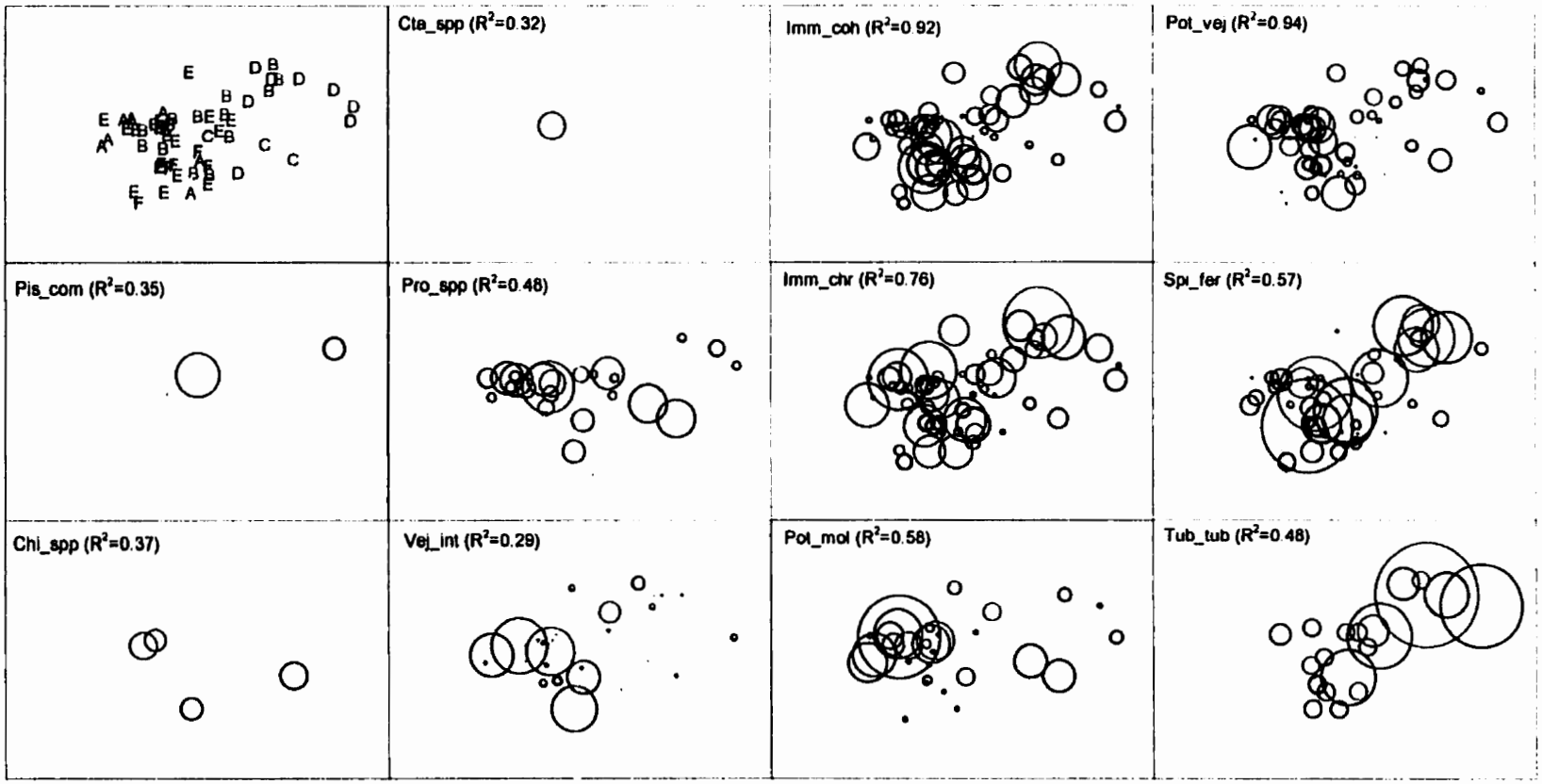

***Fig. 5.3**: Bubble plot of species abundance for each of the boxes in ordination space. The top left-hand graph represents the ordination plot for the field and laboratory boxes. A= Field data, B= Control boxes, C= Copper spiked boxes, D= Cadmium spiked boxes, E= Enriched boxes, F= Atrazine spiked boxes. Full species names are given in the text. R^2= correlation coefficients calculated from PCC.*

The naid *Vejdovskyella intermedia* (Vej_int), the immature tubificids with hair setae (Imm_chr) and the mature tubificids *Limnodrilus hoffmeisteri* (Lim_hof) show increases in numbers unique to the enriched treatments (p= 0.039, 0.001, 0.001, 0.04). The immature tubificids without hair setae (Imm_coh), together with *Spirosperma ferox* (Spi_fer) and *Potamothrix vejdovskyi* (Pot_vej) also increase in numbers in the enriched boxes, although only *S. ferox* were significant (p= 0.001). These increases are more pronounced in the Atrazine treated boxes; the increases in the immature tubificids without hair setae and *S. ferox* were significant (p= 0.006, 0.006) compared to the control boxes. An increase in the number of *Limnodrilus profundicola* is also observed in the Atrazine boxes. Potassium is strongly correlated with numbers of the immature tubificids without hair and the *S. ferox*, it is also correlated with the enriched treated boxes. These results agree with the relationships found between species counts and this treatment in the laboratory boxes described in chapter 4 (figs. 4.4.2a-d). Where a significant reduction in the numbers of a species were observed between the control and field data, the enriched and Atrazine-treated boxes had comparable numbers to the field data. This is observed in the overall movement of these boxes toward the field data in ordination space (fig. 5.2, table 5.1).

Both significant and non-significant decreases in species numbers are noted in the metal-treated boxes, as indicated by the relationship between these vectors and species in ordination space (fig. 5.1b). The immature tubificids (with and without hair setae) decreased significantly with both the copper and cadmium treatments (p_{Cu}= 0.001, 0.004; p_{Cd}= 0.001, 0.022). *Potamothrix vejdovskyi* and *Spirosperma ferox* showed significant decreases in the cadmium treated boxes (p= 0.001, 0.049) and non-significant reductions in the copper treatments (p= 0.053, 0.072). Slight decreases in numbers of *Vejdovskyella intermedia* and *Potamothrix moldaviensis* where observed only in the cadmium treatments, and *Tubifex tubifex* in the copper treatments.

5.4 Conclusions

The previous chapters have indicated an overall loss of organisms from the laboratory boxes compared to the field data. It was felt that these reductions could, in part, be due to the different sampling strategies used to collect the field and laboratory boxes. This is reflected in the overall percentage of control boxes falling outside of the confidence ellipses constructed around the field data, which exceeds the 10% expected to occur as a result of natural variation (Wright *et al.*, 1995). The effects of enrichment of the boxes and Atrazine additions are different, both in regards to their community composition and divergences in ordination space (Chapter 4). However, both treatments result in increases in the number of organisms collected from the boxes and an overall movement of the boxes back towards the field data. This is reflected in the percentage of boxes considered 'equivalent' to the field data.

Distinct movements away from the field data are observed in the metal-treated boxes. The effects of cadmium additions are more pronounced that that of copper. Differences in the species effected by these two contaminants are reflected in their different movements in ordination space away from the field data.

Of the forty species were used in the analysis, 10 of these were identified as having a significant relationship to the ordination plot. Only three of these, the most abundant taxa (the immature tubificids and *Potamothrix vejdovskyi*), showed any reasonable correlation, to the ordination pattern, from the principal axis correlation. A reasonable argument can thus be made that using univariate statistical methods on these three species would be more appropriate. However, although these species are certainly important in regard to their response to the contaminants and their effect on community composition, many of the other species identified through the Monte-Carlo analysis showed responses to the treatments. The changes in the abundance of these other species cause the more subtle differences between treatments in ordination space. For example, copper and cadmium treatments both resulted in an overall reduction in abundance; their separation in ordination space from each other was a result of changes in the abundance of different species. Although significant decreases in the numbers of *Potamothrix vejdovskyi* and

Spirosperma ferox were observed in both the cadmium and copper treatments, non-significant decreases in *Vejdovskyella intermedia* and *Potamothrix moldaviensis* were only observed in the cadmium treatments, and *Tubifex tubifex* in the copper treatments. The overall effects of the enrichment and Atrazine treatments were very similar, with an overall increase in species abundance. Increases in the immature tubificids without hair setae, *P. vejdovskyi* and *S. ferox* were observed in both of these treatments, although the increases in these species were more pronounced in the Atrazine-treated boxes. *V.intermedia* and *Limnodrilus hoffmeisteri* also increased in the enriched boxes, whereas *L. profundicola* increased with Atrazine-treatment.

The changes in community composition of the boxes as a result of the different treatments can be related to changes in the sediment chemistry. Although there is some relationship between community composition and sediment chemistry, the correlation coefficients linking the environmental variables to the ordination plot are not strong. This indicates that the attributes identified only weakly explained the objects' position in ordination space. Bioturbation of the sediment, as well as the removal of the top 10-15cm of sediment from the laboratory boxes for sediment analysis resulted in a dilution of the original spiked sediment by a factor of about ten. Consideration of these factors negates some of the differences in sediment chemistry between the boxes. Despite this, patterns were seen in the concentrations of certain environmental parameters and the treatments. These trends can also be related changes of community composition within these boxes.

From these analyses it is clear that changes in the community composition of benthic fauna can be related to changes in sediment chemistry, and other environmental parameters. The introduction of the contaminated sediment induced a consistent and observable response. This can be qualified using such descriptive techniques as those described by Reynoldson and Day (1998), in a way that the divergences in ordination space away from the expected community state (the field data) has a diagnostic capacity and can be used to suggest the nature of a stress.

CHAPTER 6

Summary and Recommendations

6.1 Realisation of objectives

This thesis set out to investigate the potential of benthic macroinvertebrate community structure to suggest the nature of an impact. Intact box cores were collected from the field for manipulation in the laboratory. To assess the diagnostic capability of the benthic community structure, from intact box cores, to predict sediment contamination it was necessary to consider whether the boxes could be collected from the field, transported back to the laboratory, and successfully maintained. This was achieved by addressing a number of hypotheses posed in Chapter 1. The next step was then to consider whether the changes observed in the community composition upon the addition of contaminants was consistent and predictable.

6.1.1 Maintenance of box cores under laboratory conditions

To overcome the inherent problems faced when working with natural community assemblages, multiple intact cores were collected from one site in Lake Erie and transported back to the laboratory for further manipulation. Before the effects of contaminants on the resident communities could be investigated, it had to be established that the intact boxes could in fact be collected and maintained with little change to the resident benthic fauna. The potential use of the methods proposed in assessing sediment contamination were addressed through four hypotheses:

- *Collection and transportation of the boxes to the laboratory do not significantly alter the community composition, compared to that found in the field.*

An overall reduction in abundance of the taxa was observed in the laboratory boxes compared to the field boxes. This was evident at both a community and species level, and has been observed in other studies (Gruessner & Watzin, 1996). Reductions in the abundance of individual species were primarily seen in the immature tubificids and the smaller oligochaetes, which would tend to inhabit an area close to the sediment surface. One possible explanation for the observed trends may be the effect of bow wave displacement due to different sized boxes used to collect the field and laboratory boxes. Transportation from the field and the set-up of the boxes in the laboratory inevitably result in some disturbance of the overlying sediment. This, together with the time lag between collecting the intact boxes and sampling them, may also contribute to the differences observed between the field and control boxes. However, despite these differences, univariate analysis of the predominant species showed that only the immature tubificids and *Potamothrix vejdovskyi* varied significantly from the field data. Diversity and richness measures failed to show any significant differences from the field data.

- *The communities within the laboratory boxes do not change significantly over time.*

Multivariate analysis found little change in ordination space of the laboratory communities compared to the field data. Of the 9 predominant species, and 3 diversity and richness measures analysed univariately, only the immature tubificids without hair setae in the monthly sampled boxes at 10°C showed any significant change over time. Thus, the boxes can be maintained in the laboratory, with no external inputs, for up to 8 weeks.

- *The community does not require the addition of low levels of nutrients to survive in the laboratory.*

The additions of low levels of nutrients to the intact boxes resulted in negligible changes in the community composition, either multivariately or univariately. Enrichment of the laboratory boxes was unnecessary. Diversity and richness measures indicated that although there was some change in the communities over time, these changes were also not statistically significant either over time or compared to the field boxes. These data

support the findings that the boxes can be maintained for as long as 8 weeks. With the exception of the immature tubificids without hair setae, which showed significant changes over time in the controls sampled monthly, there is little evidence to suggest that the boxes cannot be maintained for longer.

- *There is no significant change in the communities of the laboratory boxes compared to that found in the field when held at elevated temperatures to those found in the field.*

The communities kept at 4.5°C were relatively more stable than those maintained at 10°C; the communities held at higher temperatures tended to vary more in their abundance. Despite this, the communities held at higher temperatures were not identified as changing significantly over time or compared to the field data. Thus, the increase in temperature was not considered to alter the community in a statistically significant way.

6.1.2 Community structure for the assessment of sediment contamination

Based on the premise that different species will have different tolerances to the stressors, the benthic macroinvertebrate communities should have characteristic responses to these stressors. These changes in community composition may be useful in the identification of an unknown stress. Using multivariate analysis, such as Non-metric Multi-Dimensional Scaling (NMDS), it is possible to illustrate these changes in the community graphically, in the form of ordination plots. Such plots would allow the changes in communities due to contamination to be reflected as a divergence away from an expected or predicted state. The direction in which these divergences occur can then be related to environmental parameters, such that the effect of a contaminant on a community is consistent in the direction in which it moves the community in ordination space. It then stands to reason that changes in the community assemblage, specifically those species which are effected, can be used diagnostically to suggest a type of impact at a site where the nature of the contaminant is unknown.

Changes in community composition, as a response to certain stressors (cadmium, atrazine and nutrient enrichment), and the consistency and predictability of such responses were evaluated through several hypotheses.

- *Seasonal changes in the community composition of the intact boxes are greater than the between- and within-box variation.*

A major limitation of ecosystem level bioassays includes the inherent variability of multispecies biological systems (Barry & Logan, 1998). Indeed, highly variable data are common in aquatic mesocosm studies (Shaw & Manning, 1996). This is a problem when using univariate statistical techniques to analyse these data. Replicate variability, which can be due to specific and identifiable natural factors, could mask the significance of even large differences (Tagatz, 1986). Spatial and temporal variation in the distribution and abundances of organisms is an inherent property of ecological systems (Anderson & Gribble, 1998).

Variation of the boxes seasonally was relatively large but was not masked by the variation within and between the boxes. Barton (1989) emphasises that the timing of the fieldwork will reflect characteristics of life-cycle events such as recruitment, migration and sexual maturation. Combining the seasonal data of this study retained the directional patterns caused by sediment contamination. Thus, changes in community structure were not masked by seasonal changes in species composition.

There was an observed reduction in the numbers of organisms between the field and laboratory data. However, movement of the community scores for the field and laboratory data within ordination space followed a similar seasonal pattern. Although there is a loss of benthic fauna in the laboratory boxes there is little loss in the species composition within these boxes.

- *Changes in the community composition caused by the addition of the stressors are greater than the between- and within-box variation.*

Spatial and temporal variation in the distribution and abundance of organisms is an inherent property of ecological systems. The results from both the univariate and multivariate analyses showed that although seasonal variation was large, it was not masked by any variation found between or within the boxes.

Combining the seasonal data retained the directional pattern caused by the additions to the sediment, illustrating that divergences in ordination space of the treated boxes from the control data are not masked by any seasonal effects on community composition. The June data behaved differently from the other months, both seasonally and upon the addition of contaminants, possibly as a result of stratification occurring in the lake when the samples were taken. Excluding these data showed that the impacts of the contaminants were strong, and distinct from each other and from the seasonal changes.

- *A consistent pattern is observed in the changes in community composition in ordination space as a result of the different stressors.*

Observations recorded for each of the sampling times indicated that the addition of the different contaminants to the intact boxes produced distinct directional changes in ordination space; cadmium resulted in an overall reduction in species counts, and enrichment and Atrazine-treatments caused an increase in species counts. The boxes collected in June differed from both the field data and the laboratory data with respect to the community response to contaminants. However, even when these data were included in the overall analysis of the effects of the contaminants, the multivariate approach and replicate samples showed a consistent directional change in community composition as a result of the addition of different stressors.

- *Directional changes in ordination space of the intact boxes can be related to changes in specific species.*

Generally, the changes in species composition were found to be similar to those described in the literature, for both single species and community toxicity tests.

Addition of cadmium to the laboratory boxes in this study caused consistent reductions in overall abundance, and as a result changes in the species assemblage, and therefore position in ordination space, of the laboratory boxes. Atrazine effects were unexpected and differed somewhat to those recorded by previous researchers (Forget *et al.*, 1998; Gruessner & Watzin, 1996; Solomon *et al.*, 1996; Huber, 1993), who documented declines in aquatic organisms. An increase in emergence of insects has also been recorded as an affect of atrazine (Gruessner & Watzin, 1996). Although emergence was not assessed in this study, a consistent increase in the numbers of mature tubificids was observed. As observed by others (Lang, 1997; Verdonschot, 1996; Wiederholm *et al.*, 1988; 1987; Lauritsen *et al.*, 1985; Milbrink, 1983; Brinkhurst, 1967; Hynes, 1960), nutrient enrichment of the sediment resulted in increased abundances of oligochaetes.

- *Directional changes in ordination space of the contaminated boxes can be related to changes in sediment chemistry.*

Many of the environmental variables that were identified as having a significant relationship to the field and laboratory boxes in ordination space gave no significant results from univariate analysis. The physical parameters identified, together with *Dreissena* counts and dissolved oxygen, explain some differences between the field and laboratory data. The remaining chemical parameters account for much of the movement in ordination space of the boxes as a result of the added contaminants. With the exception of the cadmium vector, these parameters are related to NMDS axis1.

Total phosphorous and nitrogen could be correlated with numbers of the tubificids, immature and the mature *Limnodrilus profundicola*, *L. claparedeanus*, *Potamothrix vejdovskyi* and (to a lesser extent) *Spirosperma ferox*, the gastropod *Fossaria obrussa*, as well as Porifera and cocoon counts. Cadmium was correlated to reductions in the numbers of all of the species identified. Similar trends to cadmium have been noted by Shaw and Manning (1996) in their evaluation of community level effects of copper in outdoor mesocosms. The findings are in agreement with the correlations found between species counts and treatment in the laboratory boxes.

6.1.3 Uses as a diagnostic tool:

Construction of probability ellipses, described by Reynoldson and Day (1998) was successful as a descriptive tool to identify impacts on the intact boxes in reference to the field data. Directional changes were observed in the cadmium and copper treated boxes, and a movement back toward the field condition was observed in the enriched and Atrazine-treated boxes. Subtle changes between contaminants with similar overall effects could also be observed. These changes can be related to changes in sediment chemistry and species composition.

6.2 Assessment of methods employed and recommendations

6.2.1 Sediment and Water Quality

Ideally, sediment chemistry measurements should be taken before any addition to the boxes to identify the actual increase in concentration of contaminant to which the biota is subjected. Chemical analysis of the sediment at the end of each experiment, taking into account dilution effects resulting from bioturbation and sampling methods, would portray loss of contaminant to the water column or anoxic layer.

Since only sediment chemistry was considered in the analysis, movement of contaminants back into the water-column was not considered. Metals tend to be more toxic in solution than when combined to sediments (Giesy & Hoke, 1989) and should therefore be considered.

6.2.2 Dreissena

Over the past 8 years the Great Lakes have seen the invasion of both the zebra mussel (*Dreissena polymorpha*) and the "quagga" mussel (*D. bugensis)*, (Reynoldson & Day, 1998; Reynoldson, 1995; Rosenberg & Ludyansliy, 1994; Dermott *et al.*, 1993; Griffiths, 1993; Mackie, 1991). Many researchers (Howell *et al.*, 1996; Stewart & Haynes, 1994) have noted that the invasion of the Great Lakes by these European bivalves has been

associated with subsequent physico-chemical changes in the environment and of the benthic community composition of some organisms. Correlations between the number of *Dreissena* in the field boxes and diversity and richness values were highly variable and mostly non-significant. Despite failure to identify any consistent correlation between *Dreissena* numbers and diversity and richness scores, multivariate analysis of the data and subsequent correlation of the environmental data to the taxa indicated that *Dreissena* numbers did have an initial influence on the species composition.

Dreissena were removed from the laboratory boxes at the beginning of each experiment, thus their response to sediment contamination not considered. Since *Dreissena* are considered important community descriptors (Reynoldson & Day, 1998), their removal from the boxes poses a problem when extrapolating the laboratory data back to the field.

6.2.3 Taxonomic resolution

There are conflicting views as to the level at which identification should be taken in the analysis of community composition. Some researchers (Resh & Unzicker, 1975) advocate species level identification, while others (Bowman & Bailey, 1997; Warwick, 1993; 1988; Furse *et al.*, 1984) show that lower levels of identifications have been successful in detecting human impacts. Warwick (1988) and Furse (1984) concluded that aggregation to family level showed no less information than species level data, and when considered in relation to the extra effort and costs involved in identification to higher levels, any loss in information was negligible. Indeed, human perturbations may be more easily detected against a background of natural variability in factors such as water depths and sediment size by looking at higher taxonomic levels (Bowman & Bailey, 1997; Warwick, 1993).

Marchant (1990) found that when qualitative data were aggregated to a family level, or when fewer than four replicates were taken, disturbances in ordinations occurred. In the study described in this thesis, the site used has very few families. Exclusion of *Dreissena* from the analyses described in this thesis, resulted in the data being dominated by only one family. Variation within this dominant family could be expressed in the first axis, and

as a result no meaningful interpretation of the analysis could be achieved. Any advantages gained from using higher taxonomic levels can only be achieved when the data are diverse, with no single family being dominant.

6.2.4 Field validation

Although bioassay tests can provide quantifiable relationships between contaminants and organisms, and can isolate contributing factors, most of these biological tests of sediment toxicity are performed using single species. There are several pitfalls when extrapolating response data from isolated single species tests to predict changes in the community and ecosystem (Sheehan, 1984). To be predictive, tests must be environmentally realistic (Buikema & Voshella, 1993). The field data were collected simultaneously with the intact boxes. Various structural attributes among the laboratory and field communities were therefore considered similar. Data derived from the laboratory tests will therefore have good environmental applicability. The laboratory boxes, although not differing significantly from the field data, showed some reductions in species abundances. These differences were clearly illustrated by the separation of the lab data from the field data in ordination space. It was suggested that these differences could be the result of differing sampling equipment for the laboratory and field boxes. External migration, recruitment, emergence and predation were not accounted for in the laboratory boxes. The effects of these external influences could be addressed by comparing the laboratory results to field sites that are contaminated with similar stressors. However, there may be problems finding such sites, that have comparable physical, chemical and biological conditions to the site used. Transplant experiments, whereby contaminated sediment is placed at clean sites and left to be colonised naturally could also be used.

6.2.5 Multivariate techniques

Ecological data, by its nature, are multivariate. Although univariate methods are often favoured, only those species present in sufficient numbers and with adequate frequency can be analysed by these methods. Unless presence/ absence data are used rare species

are not considered. There is an inherent loss of information when the data are analysed univariately. Diversity and the biotic indices reduce the information from the samples to a single value; however, multivariate techniques retain information on the taxonomic composition of a sample and display 'real' effects of water and sediment quality on the community. Multivariate analysis of the communities using NMDS will reflect the slight reductions in taxa abundance observed in the univariate analyses. However, multivariate analysis tends to exaggerate differences in the communities in ordination space. Thus the ordination plots presented suggest that the laboratory boxes are more similar to each other than the field data, and vice versa. Data should therefore be viewed with the caveat that non-significant trends, identified through univariate analysis, may prove highly significant if analysed multivariately. If the significant attributes, identified through multivariate means, were to be re-analysed by univariate methods, this may alleviate some of the problems inherent in interpreting multivariate data.

6.3 Conclusions

Not all of the contaminants were identified as having an overall impact on the field data, but this study did succeed in establishing that intact box cores collected from the field can be maintained as laboratory mesocosms with little change in the resident benthic fauna. Despite some unexpected results, the response of intact box cores to different contaminants showed consistent and predictable changes in community composition. These changes can also be related to environmental conditions. Intact box cores of naturally co-adapted species show potential for use as a diagnostic tool and can provide a useful technique in the analysis and identification of sediment contamination. However, the results should be viewed with the caveat that only a subset of species is represented and there is an inevitable loss of some species due to collection and transportation. Care must be taken when extrapolation data obtained from *in vitro* studies to natural communities.

Supplemented by field validation, using transplant experiments or by comparing the laboratory data to sites of comparable loadings, the techniques described in this thesis have the potential to provide a useful diagnostic tool in the evaluation of sediment contamination, at sites where the nature of the impacts are unknown. Further identification of the effects of different contaminants, as well as complex stressors (e.g. pulp-mill effluents) on community composition can allow the primary source of impact to be identified. At sites exposed to multiple stressors, valuable information can be provided and clear statements made on which potential sources should be controlled, thus reducing the need for extensive and expensive chemical analyses.

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Appendix A

Table of species counts for field and laboratory data
May 1995-October 1996

Sample	Treatment	Aul_lim	Aul_plu	Chl_spp	Erpob	Fos_obr	Gyr_cir	Har_spp	Hel_sta	Het_spp
303May95	Field	0	1	0	0.5	0	0	0	0	0
303Jun95	Field	0	0.5	0	0	0	0	0	0.25	0
303Jul95	Field	0.2	0	0	0	0	0	0	0	0
303Aug95	Field	0	0	0	0	0	0	0	0	0
303Sept95	Field	0	0	0	0	0.6	0	0	0	0
303Apr96	Field	0	0	0	0	0	0	0	0	0
303Jun96	Field	0	0	0	0	1.4	0	0	0	0
303Aug96	Field	0	0	0.2	0	0.4	0	0	0	0
303Oct96	Field	0	0	0	0	0.8	0	0	0	0
Cp1a	Control	0	0	0.25	0	0	0	0	0	0
Cp1a	Control	0	0	0.25	0	0	0	0	0	0
Cup1a	Copper	0	0	0	0	0	0	0	0	0
Cup1a	Copper	0	0	0	0	0	0	0	0	0
Cup1a	Copper	0	0	0	0	0	0	0	0	0
Fp1a	Feed	0	0	0	0	0	0	0	0	0
Fp1a	Feed	0	0.25	0	0	0	0	0	0	0
Fp1a	Feed	0	0	0	0	0	0	0	0	0
Cp1b	Control	0	0.2	0	0	0.8	0	0	0	0
Cp1b	Control	0	0	0	0	2.6	0	0	0	0.2
Cp1b	Control	0	0	0	0	1.4	0	0	0	0
Fp1b	Feed	0	0	0	0	1	0	0	0	0
F1b	Feed	0	0	0.2	0	0.6	0	0	0	0
F1b	Feed	0	0	0	0	2	0	0	0	0
C'1b	Control	0	0	0	0	0.4	0	0	0	0
C'1b	Control	0	0	0	0	3	0	0	0	0
C'1b	Control	0	0	0	0	0.6	0	0	0	0
AA	Cadmium	0	0	0	0	0	0	0	0	0
AB	Control	0	0	0	0	0	0	0.2	0	0
AC	Cadmium	0	0	0	0	0	0	0	0	0
AD	Cadmium	0	0	0	0	0	0	0	0	0
AE	Control	0	0	0	0	0	0	0	0	0
AF	Enrich	0	0	0	0	0	0	0	0	0
AG	Enrich	0	0	0	0	0	0	0	0	0
AH	Enrich	0	0	0	0	0	0	0	0	0
AI	Control	0	0	0	0	0	0	0	0	0
BA	Atrazine	0	0	0	0	0.2	0	0	0	0
BB	Enrich	0	0	0	0	0	0	0	0	0
BC	Atrazine	0	0	0	0	0	0	0	0	0
BD	Control	0	0	0	0	1.4	0	0	0	0
BE	Enrich	0	0	0	0	0.4	0	0	0	0
BF	Enrich	0	0	0	0	0	0	0	0	0
BG	Cadmium	0	0	0	0	0	0	0	0	0
BH	Cadmium	0	0	0	0	0.6	0	0	0	0
BI	Cadmium	0	0	0	0	0.2	0.6	0	0	0
BJ	Control	0	0	0	0	0	0	0	0	0
BK	Atrazine	0	0	0	0	0.2	0	0	0	0
BL	Control	0	0	0	0	0.4	0	0	0	0
CA	Control	0	0	0	0	1.2	0.2	0	0	0
CB	Control	0	0	0	0	0	0	0	0	0
CC	Cadmium	0	0	0	0	0	0	0	0	0
CD	Atrazine	0	0	0	0	0.6	0	0	0	0
CE	Atrazine	0	0	0	0	0	0	0	0	0
CF	Enrich	0	0	0	0	0.2	0	0	0	0
CG	Cadmium	0	0	0	0	0	0	0	0	0
CH	Atrazine	0	0	0	0	0.6	0	0	0	0
CI	Control	0	0	0	0	0	0	0	0	0
CJ	Cadmium	0	0	0	0	0	0	0	0	0
CK	Enrich	0	0	0	0	0.2	0	0	0	0
CL	Enrich	0	0	0	0	0.2	0.4	0	0	0
DA	Cadmium	0	0	0	0	0.8	0	0	0	0
DB	Enrich	0	0	0	0	1.4	0.2	0	0	0
DC	Enrich	0	0	0	0	2.4	0.6	0	0	0
DD	Cadmium	0	0	0	0	0	0	0	0	0
DE	Control	0	2.8	0	0	2	0	0	0	0
DF	Atrazine	0	0.2	0	0	1.6	0	0	0	0
DG	Atrazine	0	0	0	0	0.8	0.2	0	0	0
DH	Enrich	0	0	0	0	1.6	0	0	0	0
DI	Atrazine	0	0	0	0	0.4	0	0	0	0
DJ	Cadmium	0	0	0	0	0.2	0.2	0	0	0
DK	Control	0	0	0	0	0.2	0.2	0	0	0
DL	Control	0	0	0	0	0.4	0	0	0	0

Sample	Hyd ame	lly tem	Lim cer	Lim cla	Lim hof	Lim pro	Mon spp	Nai var	Par spp	Phy int
303May95	0	0	0.25	0	0.5	0.25	0	0	0	0.25
303Jun95	0	0	0	0.25	1	0.5	0	0	0	0.5
303Jul95	0	0	0	0.2	8	0.6	0	0	0	0
303Aug95	0	0	0	0.2	5.6	0	0	0	0	0
303Sept95	0	0	0	0	1.4	0	0	0	0	0.2
303Apr96	0	0	0	0	5.2	1	0	0	0	0
303Jun96	0	0	0	0	1.8	0.6	0	0	0	1.4
303Aug96	9.6	0.2	0.2	0	5.4	0.2	0.2	0	0	0.6
303Oct96	8.8	0	0	0	0.2	0	0	0	0	0.4
Cp1a	0	0	0	0	0.75	0	0	0	0	0
Cp1a	0	0	0	0	1	0	0	0	0	0
Cup1a	0	0	0	0	1.25	1.75	0	0	0	0
Cup1a	0	0	0	0.25	1	0.5	0	0	0	0
Cup1a	0	0	0	0	0.5	0	0	0	0	0
Fp1a	0	0	0	0	0.5	0.25	0	0	0	0
Fp1a	0	0	0	0.25	2.25	0.25	0	0	0	0
Fp1a	0	0	0	0.25	0.75	0.25	0	0	0	0
Cp1b	0	0	0	0	0.2	0	0	0	0	0.6
Cp1b	0	0	0	0	0.2	0	0	0	0	1.2
Cp1b	0	0	0	0	0	0	0	0	0	1.2
Fp1b	0	0	0	0	0	0	0	0	0	0.2
F1b	0	0	0	0	0	0	0	0	0	0
F1b	0	0	0	0	0	0	0	0	0	0.2
C'1b	0	0	0	0	0	0	0	0	0	0.8
C'1b	0	0	0	0	0	0	0	0	0	0.4
C'1b	0	0	0	0	0.4	0	0	0	0	0.2
AA	0	0	0	0	3.2	0	0	0	0	0
AB	0	0	0	0	1.8	0	0	0	0	0
AC	0	0	0	0	0.6	0	0	0	0	0
AD	0	0	0	0	2.6	0	0	0	0	0
AE	0	0	0	0	4.6	0	0	0	0	0
AF	0	0.2	0	0	4.8	0	0	0	0	0
AG	0	0	0	0	2.6	0.2	0	0.2	0	0
AH	0	0	0	0	3.2	0	0	0	0	0
AI	0	0	0	0	1.2	0.2	0	0	0	0
BA	0.4	0	0	0	1.4	0	0	0	0	0
BB	0	0	0	0	1.4	0	0	0	0	0.2
BC	0	0	0	0	0	0	0	0	0	0.2
BD	0	0	0	0	0.2	0	0	0	0	0.2
BE	0	0	0	0	2.6	0.4	0	0	0	0.4
BF	0	0	0	0	4.4	0.2	0	0	0	0.4
BG	0	0	0	0	0.8	0	0	0	0	0
BH	0	0	0	0	0.6	0	0	0	0	0.2
BI	0	0	0	0	1.2	0	0	0	0	0
BJ	0	0	0	0	0.4	0	0	0	0	0
BK	0	0	0	0	0.2	0	0	0	0	0.8
BL	0	0	0.2	0	0.4	0	0	0	0	0.4
CA	0	0	0	0	0.4	0.2	0	0	0.2	0.4
CB	0.4	0	0	0	0	0	0	0	0	0
CC	0	0	0	0	0.2	0	0	0	0	0.2
CD	0	0	0	0	0	0	0	0	0	0.2
CE	0	0	0	0	0	0	0	0	0	0.8
CF	0	0	0	0.4	1	0.4	0	0	0	0.6
CG	0.2	0	0	0	0.4	0	0	0	0	0.6
CH	0	0	0	0	0.2	0	0	0	0	0.4
CI	0.8	0	0	0	0.2	0	0	0	0	0.4
CJ	0	0	0	0	0.4	0	0	0	0	0
CK	0	0	0	0	3.2	0.2	0	0	0	1
CL	0	0	0	0	1.6	0.2	0	0	0	0
DA	0	0	0.2	0	0.8	0.8	0	0	0	1.2
DB	0.4	0	0	0.4	2.4	1.8	0	0	0	1.8
DC	0	0	0	0.8	5	3	0	0	0.2	1.4
DD	0	0	0	0	1.4	1	0	0	0	1.8
DE	0.6	0	0	0	0.4	0.6	0	0	0	1.4
DF	0	0	0	0.2	4.4	1.8	0	0	0	1.4
DG	0.4	0	0	0.2	1.6	0.2	0	0	0	1
DH	0.8	0	0	0.8	2.8	2	0	0	0.2	1.4
DI	0.4	0	0	0	2	0	0	0	0	0.4
DJ	0	0	0	0	0	1	0	0	0	1.4
DK	0	0	0	0.2	0.6	0.2	0	0	0	0.2
DL	0.2	0	0	0	1.2	0.8	0	0	0	0.2

Sample	Pis_cas	Pis_com	Pis_nit	Pol_spp	Pot_mol	Pot_vej	Pot-bed	Pot_spp	Pro_spp	Qua_mul
303May95	0	0	0.25	0	2.25	68.25	0	0	0.25	0.5
303Jun95	0.25	0	0	0	1.25	80	0	0	1	1
303Jul95	0.2	0	0	0	3.8	56.2	0	0	1	0.2
303Aug95	0.4	0	0	0	0.4	37.6	0	0	0	0
303Sept95	0.4	0	0	0	1.8	121	0	0	0	0.2
303Apr96	0	0	0	0	0.8	25	0	0	0.6	0.2
303Jun96	0	0	0	0.2	2	5.8	0	0	0.2	0
303Aug96	0	0	0	1	0.2	92.2	0	0	0.6	0
303Oct96	0	0	0	0.2	0.2	37	0	0	0.4	0
Cp1a	0	0	0.25	0	1.25	49.25	0	0	0.5	0
Cp1a	0	0	0	0	1.5	61.75	0	0	1.25	0
Cup1a	0	0	0	0.25	1.5	33.25	0	0	1.25	0
Cup1a	0	0	0	0	0	16.25	0	0	1	0
Cup1a	0	0	0	0	0.25	21	0	0.25	0.5	0
Fp1a	0	0	0	0	1	56.75	0	0	0.25	0
Fp1a	0	0	0.25	0	1.75	99.25	0	0	0.75	0
Fp1a	0	0	0.25	0	1.75	74	0	0	2	0
Cp1b	0.6	0	0.4	0	0.2	53.2	0	0	0	0
Cp1b	1.4	0	0	0	0.6	47.6	0	0	0	0
Cp1b	1.2	0	0.2	0	0	70.4	0	0	0	0
Fp1b	1.4	0	0	0	0	22.8	0	0	0	0
F1b	1.2	0	0.2	0	0	57.6	0	0	0	0
F1b	1	0	0.2	0	0.4	75.2	0	0	0	0
C'1b	0	0	0	0	0.2	58.6	0	0	0	0
C'1b	1.2	0	0.4	0	0.6	53	0	0	0	0
C'1b	1.2	0	0.2	0	0.8	46.2	0	0	0	0
AA	1	0	0	0.2	0	1	0	0	0.2	0
AB	1.4	0.2	0	0	0.2	17.8	0	0	0.4	0
AC	1.2	0.4	0	0.2	0	1	0	0	0.4	0
AD	1.2	0	0.2	0	0	0.6	0	0	0	0
AE	1.4	0	0	0	0.2	36.2	0	0	0.2	0
AF	1.6	0	0	0.2	0	14	0	0	0.2	0
AG	1	0	0	0	0	24.8	0.2	0	1	0
AH	0.6	0	0	0	0	11.6	0	0	0	0
AI	0.6	0	0	0	0	21.2	0	0	0	0
BA	0.6	0	0.2	0	0	34.8	0	0	0	0
BB	1	0	0.4	0	0	0.4	0	0	0	0
BC	1.2	0	0	0	0	0.2	0	0	0	0
BD	0.8	0	1	0	0	0	0	0	0	0
BE	0.6	0	0.4	0	0	0	0	0	0	0
BF	1	0	0.4	0	0	4.6	0	0	0	0
BG	0.4	0	0	0	0.2	0.4	0	0	0	0
BH	0.4	0	0.2	0	0	8	0	0	0.2	0
BI	0.4	0	0.6	0	0	10.2	0	0	0	0
BJ	0.4	0	0	0	0	8.4	0	0	0	0
BK	1.2	0	0.6	0	0	50.8	0	0	0	0
BL	1.8	0	0	0	0	24.4	0	0	0	0
CA	0	0	0	0	0	1.8	0	0	0	0.2
CB	0	0	0	0	0	32.4	0	0	0	0
CC	0	0	0	0	0	6.2	0	0	0	0
CD	0	0	0	0	0	55	0	0	0	0
CE	0.6	0	0.2	0	0	31.2	0	0	0	0
CF	1.6	0	0.4	0	0.4	34.2	0	0	0	0
CG	0.6	0	0.6	0	0	15.8	0	0	0	0
CH	0.2	0	0	0	0	60	0	0	0	0
CI	0.8	0	0.4	0	0	37.6	0	0	0	0
CJ	0.2	0	0.8	0	0	4.6	0	0	0	0
CK	0.2	0	0.8	0	0	42.8	0	0	0	0
CL	0	0	0	0	0	78.4	0	0	0	0
DA	0.6	0	0	0	0.2	4.4	0	0	0	0
DB	1.8	0	0.4	0	0	41.8	0	0	0	0
DC	1	0	1	0	0.2	51.4	0	0	0.4	0
DD	0.2	0	0.8	0	0.2	3.8	0	0	0	0
DE	0.4	0	0.2	0	0	54	0	0	0	0
DF	1	0	0.2	0	0.2	63.8	0	0	0	0
DG	2	0	0.4	0	0	52.2	0	0	0	0
DH	2.2	0	0.2	0	0.6	52.6	0	0	0.2	0
DI	2	0	0.4	0	0	37.2	0	0.2	0	0
DJ	1.2	0	0	0	0	3.2	0	0	0.2	0
DK	1.2	0	0	0	0	9.4	0	0	0	0
DL	1.4	0	0.4	0	0.8	23.8	0	0	0	0

Sample	Spe_jos	Spi_fer	Sty_lac	Sty_her	Tas_kes	Tub_tub	Unc_unc	Val_lew	Vej_int
303May95	0.25	1.75	0.25	0	0	0.25	0	0	0.25
303Jun95	0	1.5	0	0	0	0	0	0	0
303Jul95	0	2.6	0	0.2	0	0	0	0	0
303Aug95	0	0	0	0	0	0.2	0	0	0.4
303Sept95	0	2.2	0	0	0	0	0	0	1.4
303Apr96	0	7.2	0	0	0	0.8	0.2	0	15.2
303Jun96	0	1.8	0	0	0	0	0	0	20.4
303Aug96	0	1.8	0	0	0	0.2	0	0	21.4
303Oct96	0	12	0	0	0	0	0.2	0	22.8
Cp1a	0	0	0	0.25	0	0	0	0	1
Cp1a	0	1.75	0	0	0	0	0	0	1.25
Cup1a	0	1	0	0	0	0	0	0	0.25
Cup1a	0	0.5	0	0	0	0	0	0	0
Cup1a	0	0.25	0	0	0	0	0	0	0
Fp1a	0	1.5	0	0	0	0	0	0	0.25
Fp1a	0	1.75	0	0.25	0	0	0	0	0.5
Fp1a	0	0.25	0	0	0	0	0	0	0.5
Cp1b	0	0.8	0	0	0	0	0	0	0.4
Cp1b	0	0.4	0	0.4	0	0	0	0.4	2.8
Cp1b	0	0.2	0	0	0	0	0	0	1.6
Fp1b	0	0.2	0	0	0	0	0	0	26.8
F1b	0	3.8	0	0	0	0	0	0	1
F1b	0	0.6	0	0	0	0	0	0	0.8
C'1b	0	0.6	0	0.2	0	0	0	0	0.4
C'1b	0	0	0	0	0	0	0	0	2.8
C'1b	0	1.4	0	0	0	0	0	0	9.2
AA	0	0	0	0	0	0	0	0	0
AB	0	1.4	0	0	0	1.4	0	0	0.2
AC	0	0.2	0	0	0	0.2	0	0	0
AD	0	0.2	0	0	0	0.2	0	0	0
AE	0	0.2	0	0.4	0	0.2	0	0	0
AF	0	8.4	0	0	0	1	0.4	0	0.2
AG	0	2.6	0	0	0.2	0.4	2.8	0	1.6
AH	0	8.4	0	0	0.2	2	2.4	0	1.2
AI	0	0.2	0	0	0	0.2	0	0	1
BA	0	0.2	0	0	0	0	0	0	0.8
BB	0	0.6	0	0	0	0	0	0.2	0
BC	0	0.6	0	0	0	0	0	0	0
BD	0	0	0	0	0	0	0	0	0
BE	0	0.2	0	0	0	0	0	0.2	0
BF	0	0.2	0	0	0	0.8	0	0	0.8
BG	0	0.2	0	0	0	0.2	0	0	0
BH	0	0	0	0.2	0	0	0	0	0
BI	0	0.6	0	0.4	0	0.6	0	0	0
BJ	0	1.2	0	0	0	0	0	0	0
BK	0	0.4	0	0	0	0.2	0	0	0
BL	0	1.8	0	0	0	0.4	0	0	0.2
CA	0	0.2	0	0	0	0	0	0.2	0.2
CB	0	2.2	0	0	0	0.2	0	0	0
CC	0	1	0	0	0	0	0	0	0
CD	0	1.4	0	0	0	0	0	0	0
CE	0	0.2	0	0.2	0	0	0	0	0
CF	0	1.2	0	0	0	0	0	0	0
CG	0	0.4	0	0	0	0	0	0	0
CH	0	2	0	0	0	0	0	0	0.2
CI	0	2.6	0	0	0	0.2	0	0	0.2
CJ	0	0	0	0	0	0	0	0	0
CK	0	4.8	0	0	0	0.2	0	0	0
CL	0	1.8	0	0	0	0.2	0	0	0
DA	0	0.8	0	0	0	0.2	0	0	0
DB	0	8.8	0	0.2	0	0.4	0	0	5.6
DC	0	2.4	0	0	0	0.2	0	0	1.8
DD	0	2	0	0	0	0	0	0	0
DE	0	5.2	0	0	0	0	0	0	3.8
DF	0	16.6	0	0.2	0	0	0	0	3
DG	0	9.6	0	0	0	0	0	0	1
DH	0	7.2	0	0	0	0.6	9.4	0	1
DI	0	6	0	0	0	0	0	0	2.6
DJ	0	1	0	0	0	0	0	0	0
DK	0	2.6	0	0	0	0	0	0	0
DL	0	1.4	0	0.2	0	0	0	0	0

Appendix B

Table of measured environmental parameters for field and laboratory data
May 1995-October 1996

Sample ID	Treatment	Al	Ba	Be	Ca2	Cd	Co	Cr	
May-95	Field	7872.948	55	0.4	91487	0.25	9	14	
Jun-95	Field	6921.289	54	0.5	94319	1.25	9.5	14	
Jul-95	Field	7516.142	41	0.4	91857	0.4	9.5	16	
Aug-95	Field	8629.397	52	0.4	89971	0.65	9	13	
Sep-95	Field	7893.647	42	0.5	92887	0.55	7	16	
Apr-96	Field	9274.056	55	0.6	95392	0.97	8	18	
Aug-96	Field	7601.717	46	0.5	88563	0.361	8	15	
Oct-96	Field	6473.709	36	0.4	95679	0.834	5	13	
1A1	Control	7774.478	47	0.5	93009	0.397	10	15	
1A3	Control	8689.959	49	0.5	97057	0.113	9	17	
1A4	Copper	9304.539	55	0.6	96206	0.411	9	18	
1A5	Copper	8056.482	47	0.5	96275	0.652	7	15	
1A6	Copper	8993.475	51	0.6	97500	0.926	9	17	
1A7	Feed	8938.464	53	0.6	94371	0.466	6	17	
1A8	Feed	9167.308	53	0.6	97628	0.825	7	18	
1A9	Feed	8199.548	48	0.5	94535	0.513	6	15	
1BA	Control	7395.519	42	0.5	93298	0.274	7	14	
1BB	Control	7995.962	44	0.5	93851	0.643	6	16	
1BC	Control	7777.203	44	0.5	89566	0.307	5	15	
1BD	Feed	7686.327	42	0.5	97066	0.327	6	15	
1BE	Feed	7920.505	43	0.5	96186	0.636	5	15	
1BF	Feed	8508.379	47	0.5	96408	0.149	6	16	
1BG	Control	7790.628	43	0.5	97047	0.658	5	15	
1BH	Control	8971.188	51	0.5	99074	0.319	8	17	
1BI	Control	8745.971	49	0.5	99816	0.365	6	17	
2AA	Cadmium	8021.754	46	0.5	95985	72	5	15	
2AB	Control	8766.267	50	0.5	98785	1	9	18	
2AC	Cadmium	9424.517	55	0.6	95856	80	9	19	
2AD	Cadmium	8021.095	46	0.5	91923	68	6	16	
2AE	Control	6799.729	39	0.5	89439	0.692	6	14	
2AF	Enrich	9376.132	54	0.6	96376	0.138	9	19	
2AG	Enrich	9390.283	53	0.6	97847	0.763	8	17	
2AH	Enrich	8323.195	49	0.6	91750	0.897	8	17	
2AI	Control	7777.029	45	0.5	96872	0.863	9	15	
2CA	Control	8909.979	50	0.5	93803	0.714	8	16	
2CB	Control	9663.534	55	0.6	90569	0.159	7	18	
2CC	Cadmium	9633.58	54	0.6	94553	140	8	17	
2CD	Atrazine	8083.005	50	0.5	84546	0.465	6	16	
2CE	Atrazine	7106.867	44	0.5	83098	0.51	6	14	
2CF	Enrich	8690.999	52	0.5	91622	0.592	8	17	
2CG	Cadmium	7505.299	44	0.5	91327	99	5	15	
2CH	Atrazine	9174.567	53	0.6	96220	0.754	10	18	
2CI	Control	8020.989	47	0.5	97519	0.525	7	15	
2CJ	Cadmium	9369.25	54	0.6	97368	154	8	18	
2CK	Enrich	9967.092	56	0.6	98061	0.953	8	19	
2CL	Enrich	9090.903	52	0.6	92030	0.46	7	17	
2DA	Cadmium	7762.336	47	0.5	91958	39	7	16	
2DB	Enrich	7951.218	48	0.5	88860	0.554	6	16	
2DC	Enrich	7427.508	44	0.5	89126	0.48	7	15	
2DD	Cadmium	9051.124	54	0.6	92318	44	7	18	
2DE	Control	9081.055	54	0.6	91984	0.429	8	18	
2DF	Atrazine	9567.263	55	0.6	92861	0.27	10	18	
2DG	Atrazine	9525.987	55	0.6	95416	0.459	8	18	
2DH	Enrich	9205.178	53	0.6	94907	0.845	7	18	
2DI	Atrazine	8869.872	51	0.6	96372	0.765	10	17	
2DJ	Cadmium	7919.186	47	0.5	98707	103	8	16	
2DK	Control	6694.571	41	0.5	83914	0.677	6	14	
2DL	Control	8172.624	49	0.5	92323	0.695	7	16	

Sample ID	Cu	Fe	K	Li	Mg	Mn	Mo	Na
May-95	21	15993	1552.086	13	24168.85	642	0.714	245.983
Jun-95	21	16245	1536.266	10	23190.6	669	0.635	247.52
Jul-95	22	18024	1525.503	10	24133.91	631	0.73	808.88
Aug-95	20	15008	1522.418	13	22746.49	702	0.923	350.936
Sep-95	14.5	18412	1561.991	14	23624.25	620	0.882	567.276
Apr-96	24	19684	1599.572	18	24117.715	701	0.507	262.389
Aug-96	26	16507	1261.57	14	22611.338	611	4	953.483
Oct-96	20	14848	1182.529	10	24824.697	564	0.157	243.352
1A1	20	17491	872.828	16	23438.465	652	0.409	236.531
1A3	21	18287	1291.353	17	24215.494	640	0.133	256.364
1A4	56	19349	1346.012	19	23421.539	668	0.272	236.65
1A5	62	17374	1235.558	17	23793.301	631	1	220.212
1A6	38	18662	1473.991	17	24133.805	652	0.614	249.078
1A7	19	18575	1613.334	18	22550.725	648	0.681	273.219
1A8	21	19063	1605.167	18	23995.529	655	0.118	253.543
1A9	19	17402	1197.912	16	23274.354	635	0.243	224.794
1BA	20	16288	1276.937	15	23497.127	603	0.12	229.789
1BB	20	17026	1411.912	17	23423.795	618	0.555	258.419
1BC	19	16614	1365.615	15	22107.438	606	0.347	246.139
1BD	20	16549	1252.675	15	24560.055	596	0.282	247.487
1BE	22	16974	1294.292	16	23960.379	615	1	251.723
1BF	21	17813	1466.549	17	24169.033	622	0.922	270.294
1BG	22	17021	1254.422	16	24588.172	631	0.696	274.311
1BH	23	19014	1477.467	18	24489.775	668	0.219	277.547
1BI	28	18784	1276.114	18	25020.805	675	0.172	275.138
2AA	31	17562	1049.029	16	25305.996	668	0.265	257.205
2AB	24	18773	1111.68	18	26061.051	687	0.656	304.685
2AC	26	19774	1572.337	20	25154.105	707	0.52	401.241
2AD	23	17360	1412.517	16	23805.338	646	2	274.136
2AE	22	15043	1244.699	15	22950.359	571	3	272.413
2AF	26	19507	1462.338	18	25306.957	716	0.727	312.424
2AG	26	19485	1506.297	18	25890.381	697	0.675	365.294
2AH	25	18151	1265.539	14	23992.91	668	0.348	260.357
2AI	28	17209	965.898	13	25210.543	655	0.573	266.582
2CA	21	17952	1379.761	16	24217.834	656	0.846	269.485
2CB	22	18985	1685.609	17	22964.395	659	0.652	265.566
2CC	22	19092	1775.849	17	24250.326	661	0.222	259.298
2CD	20	17292	1319.294	16	21025.373	600	2	231.667
2CE	20	15879	1223.059	13	20889.652	583	6	233.663
2CF	21	18380	1782.198	17	22940.277	628	0.838	253.146
2CG	20	16597	1455.75	14	23450.105	615	0.592	240.175
2CH	23	19178	1506.829	17	24877.678	676	0.679	269.923
2CI	21	17401	1339.211	13	25551.58	641	0.266	249.953
2CJ	23	19132	1765.838	17	25205.35	684	0.261	286.49
2CK	23	19873	1602.451	19	25293.164	691	0.581	290.472
2CL	22	18344	1745.609	16	23817.865	645	0.194	256.593
2DA	23	17311	1093.625	14	24075.316	634	0.167	224.427
2DB	20	17189	1351.33	15	22892.027	618	0.244	250.718
2DC	20	16355	1437.834	13	22984.475	582	1	247.436
2DD	22	19005	1410.64	19	23475.973	647	0.397	271.884
2DE	24	18935	1354.71	17	23860.523	657	0.113	261.092
2DF	22	19592	1298.634	18	23698.029	660	0.411	267.349
2DG	23	19411	1448.16	17	24648.943	659	0.652	252.356
2DH	21	18772	1853.587	17	24735.787	651	0.926	263.203
2DI	21	18199	1460.39	15	25153.328	653	0.466	255.776
2DJ	22	17532	1223.672	13	26034.887	662	0.825	250.454
2DK	18	14914	1456.004	11	21686.807	558	6	218.921
2DL	20	17667	1494.841	14	24643.652	651	0.834	261.526

Sample ID	Ni	Pb	Sr	Ti	V	Y	Zn	Al2O3
May-95	22	6	133	256	24.5	8	62	9.4
Jun-95	23.5	7	125	253	41	8	63	9.39
Jul-95	23.5	6	127	286	42.5	9	65.5	8.815
Aug-95	23.5	7	125	274	40	8	56.5	9.275
Sep-95	17	8	132	249	35.5	9	43.5	8.855
Apr-96	23	9	138	291	24	9	60	10.21
Aug-96	18	8	127	254	20	8	103	10.11
Oct-96	16	6	132	243	18	8	45	9.22
1A1	18	10	131	250	20	8	59	9.41
1A3	17	11	137	281	22	9	56	9.94
1A4	22	3	138	294	23	9	56	10.28
1A5	18	10	135	266	21	8	52	9.62
1A6	21	7	138	291	23	9	58	10.18
1A7	19	7	135	286	22	8	58	10.19
1A8	19	8	139	287	23	9	59	10.13
1A9	19	11	134	266	21	8	56	9.81
1BA	18	10	130	248	20	8	50	9.62
1BB	19	6	132	265	21	8	52	10.18
1BC	18	9	126	256	20	8	51	10.06
1BD	18	5	136	266	20	8	50	9.44
1BE	18	11	135	272	21	8	52	9.61
1BF	18	9	136	287	22	9	55	9.65
1BG	17	7	135	272	21	8	49	9.59
1BH	20	5	141	287	23	9	57	9.69
1BI	19	12	141	287	22	9	54	9.54
2AA	18	9	133	268	21	9	54	9.42
2AB	21	15	139	286	23	9	59	9.59
2AC	21	11	136	293	24	9	62	9.75
2AD	19	14	129	265	21	8	54	9.77
2AE	16	12	123	247	19	8	49	9.57
2AF	19	11	136	307	24	9	63	9.98
2AG	20	13	138	306	24	9	61	9.63
2AH	20	13	129	267	22	9	57	9.68
2AI	18	10	135	266	21	9	54	9.51
2CA	19	11	132	332	24	9	54	9.26
2CB	19	15	130	328	25	9	56	9.62
2CC	20	12	135	331	24	9	60	9.51
2CD	19	14	121	257	22	8	54	10.01
2CE	16	7	117	236	20	7	52	9.73
2CF	21	13	130	280	23	9	56	9.59
2CG	18	6	128	262	20	8	50	9.48
2CH	21	11	137	302	24	9	58	9.75
2CI	17	11	136	286	21	9	53	9.05
2CJ	21	11	138	314	24	9	59	9.89
2CK	21	12	140	333	25	10	61	9.53
2CL	19	14	131	313	24	9	57	9.84
2DA	16	9	129	258	21	8	54	9.58
2DB	19	13	126	254	21	8	56	9.64
2DC	17	5	125	251	20	8	53	9.56
2DD	21	15	131	289	23	9	58	10.11
2DE	20	11	132	285	23	9	61	10.01
2DF	20	4	133	297	24	10	61	10.18
2DG	20	8	137	314	24	10	59	9.83
2DH	19	12	135	311	24	9	58	10.07
2DI	20	9	136	309	23	9	54	9.57
2DJ	18	12	138	268	21	9	58	9.64
2DK	18	9	117	228	19	8	47	9.61
2DL	19	8	130	267	21	9	54	9.83

Sample ID	BaO	CaO	Fe2O3	K2O	MgO	MnO	Na2O	P2O5
May-95	0.046	14.11	3.825	2.075	3.88	0.115	1.27	0.2
Jun-95	0.045	13.41	3.91	2.17	4.02	0.12	1.25	0.185
Jul-95	0.046	13.255	3.595	2.34	3.78	0.11	1.31	0.15
Aug-95	0.046	13.43	3.77	1.96	3.79	0.11	1.295	0.16
Sep-95	0.046	12.28	3.185	1.825	3.8	0.085	1.6	0.16
Apr-96	0.044	13.45	4.18	2.39	4.06	0.11	1.53	0.13
Aug-96	0.045	12.51	3.97	2.39	4.09	0.11	1.58	0.08
Oct-96	0.047	13.41	3.45	2.21	4.03	0.1	1.72	0.07
1A1	0.045	13.39	3.98	2.29	4.21	0.11	1.31	0.17
1A3	0.048	13.47	3.97	2.46	4.02	0.09	1.36	0.15
1A4	0.048	13.47	4.05	2.46	3.83	0.09	1.34	0.17
1A5	0.047	13.48	3.68	2.34	3.78	0.09	1.42	0.16
1A6	0.047	13.51	3.88	2.45	3.91	0.11	1.39	0.15
1A7	0.047	13.18	4.03	2.49	3.85	0.09	1.35	0.14
1A8	0.048	13.21	3.99	2.46	3.86	0.09	1.36	0.15
1A9	0.047	12.97	3.92	2.32	3.99	0.09	1.36	0.17
1BA	0.046	13.06	3.81	2.18	4.19	0.09	1.32	0.16
1BB	0.047	13.15	3.97	2.35	4.18	0.11	1.41	0.11
1BC	0.047	12.54	3.99	2.34	4.15	0.11	1.37	0.1
1BD	0.044	13.59	3.69	2.23	4.13	0.09	1.43	0.09
1BE	0.045	13.66	3.92	2.26	4.23	0.1	1.41	0.09
1BF	0.048	13.59	4.01	2.33	4.21	0.1	1.41	0.07
1BG	0.047	13.77	3.96	2.22	4.27	0.11	1.37	0.08
1BH	0.047	13.79	4.13	2.29	4.09	0.11	1.29	0.1
1BI	0.047	14.07	3.97	2.29	4.15	0.11	1.34	0.09
2AA	0.048	13.54	3.81	2.21	4.15	0.11	1.41	0.09
2AB	0.048	13.79	4.03	2.21	4.21	0.11	1.36	0.08
2AC	0.047	13.52	4.19	2.37	4.11	0.11	1.31	0.1
2AD	0.046	12.78	3.83	2.24	4.04	0.1	1.37	0.09
2AE	0.046	12.61	3.54	2.18	4.08	0.09	1.55	0.09
2AF	0.045	13.43	3.98	2.29	4.04	0.11	1.31	0.08
2AG	0.044	13.71	3.92	2.24	4.05	0.11	1.33	0.09
2AH	0.044	12.81	3.91	2.29	4.02	0.11	1.38	0.09
2AI	0.047	13.52	3.74	2.23	4.13	0.11	1.51	0.08
2CA	0.045	13.14	3.87	2.27	4.13	0.11	1.47	0.07
2CB	0.043	12.72	4.18	2.35	4.03	0.11	1.31	0.08
2CC	0.043	13.24	4.02	2.36	3.94	0.1	1.36	0.09
2CD	0.044	12.04	4.21	2.44	3.98	0.11	1.35	0.07
2CE	0.045	11.82	4.21	2.44	4.26	0.11	1.45	0.06
2CF	0.043	12.82	4.02	2.37	3.93	0.1	1.31	0.07
2CG	0.047	12.78	3.91	2.37	4.15	0.11	1.52	0.08
2CH	0.046	13.47	4.07	2.39	4.08	0.11	1.43	0.07
2CI	0.046	13.65	3.73	2.25	4.06	0.1	1.49	0.08
2CJ	0.044	13.64	3.93	2.34	3.94	0.1	1.39	0.09
2CK	0.042	13.73	3.92	2.35	3.84	0.1	1.35	0.08
2CL	0.045	12.88	4.04	2.41	4.02	0.11	1.39	0.08
2DA	0.043	14.16	4.02	2.39	3.93	0.11	1.23	0.07
2DB	0.043	12.45	3.86	2.35	3.86	0.1	1.4	0.11
2DC	0.042	12.91	3.86	2.37	4.07	0.1	1.51	0.11
2DD	0.046	12.74	4.13	2.46	3.95	0.11	1.41	0.09
2DE	0.037	13.82	4.07	2.09	3.96	0.11	1.32	0.11
2DF	0.044	13.02	4.17	2.47	3.87	0.11	1.34	0.1
2DG	0.044	13.36	3.98	2.41	3.91	0.1	1.38	0.09
2DH	0.045	13.31	3.91	2.37	3.89	0.1	1.4	0.11
2DI	0.043	13.52	3.71	2.34	3.88	0.1	1.48	0.1
2DJ	0.046	13.71	3.75	2.31	3.99	0.1	1.51	0.11
2DK	0.038	14.09	3.69	2.37	3.87	0.11	1.12	0.11
2DL	0.038	13.69	3.89	2.09	3.89	0.11	1.39	0.1

Sample ID	SiO2	Ti02	TKN	Ptot	julianday	temp	depth	o2	Dreissena
May-95	48.42	0.585	528	654	145	4.35	24	13.32	26
Jun-95	47.555	0.57	656	401	178	6.56	25	15.21	24
Jul-95	48.79	0.55	605	366	191	5.07	26	21.01	18
Aug-95	48.74	0.575	623	518.5	234	4.79	29	9.65	2
Sep-95	52.19	0.535	538	540	255	7.08	26	7.96	32
Apr-96	46.12	0.6	630	718	107	2.12	32	10.88	12
Aug-96	47.61	0.55	691	625	228	6.33	30	10.7	95
Oct-96	47.63	0.52	503	617	297	8.1	24	10.1	47
1A1	47.04	0.58	593	560	145	4.5	24	9.2	9
1A3	47.97	0.57	569	554	145	4.5	24	9.9	7
1A4	47.52	0.56	528	546	145	4.5	24	9.4	96
1A5	47.21	0.54	576	564	145	4.5	24	10.2	80
1A6	48.48	0.57	575	588	145	4.5	24	10.1	14
1A7	47.82	0.56	645	666	145	4.5	24	9	35
1A8	47.86	0.57	637	714	145	4.5	24	10	87
1A9	47.33	0.56	602	721	145	4.5	24	9.1	99
1BA	47.42	0.56	537	644	255	10	26	9.6	22
1BB	49.74	0.59	563	651	255	10	26	8.5	7
1BC	48.26	0.58	673	690	255	10	26	9.1	39
1BD	47.31	0.53	450	782	255	10	26	9.6	1
1BE	48.19	0.56	475	581	255	10	26	10.2	7
1BF	47.76	0.58	573	581	255	10	26	9.2	80
1BG	47.62	0.59	649	624	255	10	26	9.9	7
1BH	47.03	0.59	601	700	255	10	26	9	2
1BI	47.04	0.59	657	660	255	10	26	9.6	28
2AA	48.01	0.57	739	672	107	10	32	9.9	7
2AB	47.82	0.58	749	652	107	10	32	10	1
2AC	47.33	0.61	813	700	107	10	32	10	16
2AD	47.53	0.55	703	797	107	10	32	10	18
2AE	49.58	0.51	557	673	107	10	32	9.8	18
2AF	47.31	0.58	749	755	107	10	32	8.5	10
2AG	46.64	0.55	828	623	107	10	32	9.2	12
2AH	46.61	0.54	857	686	107	10	32	9.2	15
2AI	49.28	0.55	660	659	107	10	32	9.9	5
2CA	48.18	0.56	653	679	228	10	30	8.9	2
2CB	47.04	0.59	827	686	228	10	30	9	19
2CC	47.71	0.56	709	727	228	10	30	8.8	35
2CD	47.82	0.58	838	818	228	10	30	9.5	24
2CE	49.12	0.59	749	658	228	10	30	8.9	5
2CF	47.53	0.57	823	707	228	10	30	8.6	18
2CG	49.11	0.56	602	672	228	10	30	9.5	4
2CH	48.88	0.58	757	676	228	10	30	9.2	31
2CI	48.18	0.54	592	676	228	10	30	9.4	2
2CJ	47.52	0.55	749	672	228	10	30	9.4	6
2CK	47.02	0.54	856	748	228	10	30	9.1	13
2CL	47.42	0.57	848	731	228	10	30	8.3	8
2DA	47.72	0.57	791	686	297	10	24	10.3	19
2DB	46.94	0.54	921	770	297	10	24	10	17
2DC	48.33	0.53	698	696	297	10	24	10.1	44
2DD	48.87	0.58	855	721	297	10	24	10.3	24
2DE	49.03	0.57	838	665	297	10	24	10.3	2
2DF	47.5	0.57	931	672	297	10	24	9.6	41
2DG	47.98	0.56	733	735	297	10	24	10.3	10
2DH	47.79	0.55	851	700	297	10	24	9.7	40
2DI	47.63	0.53	700	700	297	10	24	10.1	47
2DJ	49.05	0.54	698	696	297	10	24	10.2	9
2DK	48.89	0.56	696	676	297	10	24	10.4	14
2DL	49.03	0.57	749	648	297	10	24	10.3	1

Appendix C

Equation to calculate the appropriate quantity of contaminant to spike the intact laboratory boxes.

To make stock solution:

i) Calculate the molecular weight of the compound used to spike the sediment and the molecular weight of the metal:

e.g. $CdCl_2.2^1/_2H_2O$ = 228.35
Cd = 112.40

ii) Calculate the amount of compound required for a given 1g metal:

e.g. $\frac{112.40}{228.35}$ = 0.4922,

for 1g Cd, 1/0.4922 = 2.03158g $CdCl_2.2^1/_2H_2O$ required.

iii) Calculate the amount of compound required for a given stock solution, e.g. 5000ppm (5g Cd/L):

e.g. 5 x 2.03158 = 10.1579 $CdCl^*$ /L H_2O

To calculate the amount of stock solution required to spike the sediment:

i) Calculate the water content the sediment.

ii) Calculate the amount of wet sediment required to spike the desired amount of dry sediment

iii) For the desired concentration of stock solution to spike the wet sediment:

e.g. $\frac{5000mg\ Cd}{1000ml\ H_2O}$ x $\frac{desired\ concentration\ (mg\ Cd/L)}{ml\ stock\ solution\ required}$

Appendix D

LC50 concentrations and loading values for the contaminants used.

Contaminant	Concentration	Response/Endpoint	Reference
Atrazine	450µg/L	Phytoplankton, bacteria, zooplankton & benthic inverts.	Sheehan et al. 1986
	1-5000µg/L	Community level response	Kennedy et al. 1995
	3.2-337µg/L	Community level response	Pratt et al. 1988
	1->100,000µg/L 360-30,000µg/L 220-100,000µg/L	Algae Invertebrates Fish	Day, 1991
	5µg/L	Multispecies	Gruessner & Watzin, 1996
	>20µg/L	Multispecies	Huber, 1993
	3-300µg/L	Multispecies	Pratt, 1997
	<5-20µg/L	Natural surface waters	Solomon et al. 1996
	0.15-8mg/L (LC_{50})	9 Oligochaete spp.	Giesy & Hoke, 1989
	0.4-9.5µg/L	Community level	Niederlehner et al. 1985
	1.2-9.6ppm	Bulk chemical concentrations	Long et al. 1995
Cadmium	5mg/Kg (marine)	3 benthic invertebrates	Skei et al. 1996
	0.17-12mg/L (LC_{50}s for sediment & no-sediment tests)	Oligochaete species	Chapman & Brinkhurst, 1985
	0.17-12mg/L (LC_{50}s for sediment & no-sediment tests)	Oligochaete species	Chapman et al. 1982
	1µg/L 3.2µg/L	*H. azteca* *G. fasciatus*	Borgman et al. 1989
	1.1µg/L	*H. azteca*	Kemble et al. 1994
	749-1088ppb	Oligochaete species	Long & Long-Dobler, 1979
	0.68µg/g	Natural sediment concentration in Hamilton Harbour	Poulton et al. 1988
	0.4-9.8µg/L (LC_{50})	*Tubifex tubifex*	Reyoldson et al. 1996
	0.7-1.2 mg/L	*Tubifex tubifex*	Brkovic-Popovic, 1977
	0.32 mg/L	*Tubifex tubifex*	Chapman et al. 1982
	0.65-29,560µg/L	5 Invertebrates	Suedel et al. 1997

Contaminant	Concentration	Response/Endpoint	Reference
Enrichment	12mg C/Kg (marine)	3 benthic invertebrates	Skei et al. 1996
	1.1-12% (LC_{50}s for sediment & no-sediment tests)	Oligochaete species	Chapman et al. 1982
	1.73-2.36 % C 862-1297 ppm totP	Oligochaete species	Long & Long-Dobler, 1979