

# **MODELLING WATER AND SEDIMENT CHEMISTRY IN URBAN CANALS USING CHIRONOMID PUPAL EXUVIAE**

By

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# ABSTRACT

This study has four principal aims. The first was to classify chironomid assemblages in canals using pupal exuviae. The second was to understand the distribution of species, their ecology and function. The third was to find indicator taxa which were suitable to classify canals in terms of water and sediment chemistry. The fourth was to analyze potential boundaries between WFD classes and develop a method to calculate ecological quality ratios that will support the objectives of the WFD.

The water and sediment chemistry of the canal sites were first studied. The heavy metal concentrations were characterised by applying a Critical Criterion Unit (CCU) and for sediments, spatial trends were analysed by using a metal pollution index (MPI).

Species data were associated with environmental data. Canonical Correspondence Analysis (CCA) was carried out on the whole dataset as well as separate analyses for water and sediment chemistry data using epi- and enbenthic species data. Biological classifications were constrained by each of the significant variables from the CCA analyses and were used to calculate indicator species scores and classify species assemblages. Body size distributions were presented for the chironomid species. It was found that there were significant differences in body length within species at different sites. Ordination of size classes against environmental variables found that metals were significant in determining distribution of body size. When functional feeding groups were investigated it was found that predators were positively correlated to zinc and negatively correlated to CCU, whereas grazers were negatively correlated with lead. Calibrated chironomid-based inference models were constructed and these were used successfully to predict water and sediment chemistry parameters.

Finally, this study found that there was potential to apply this tool to the requirements of the Water Framework Directive (WFD) and define ecological potential of canals through comparison of observed to reference EQRs (Ecological Quality Ratio). This was despite the fact that the study was conducted within a small geographical area.

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## ACRONYMS AND ABBREVIATIONS

AWB	Artificial Water Bodies
ASPT	Average Score Per Taxa
BMWP	Biological Monitoring Working Party
BOD	biological oxygen demand
CCA	canonical correspondence analysis
CCC	criterion continuous concentration (“chronic criterion”)
CCU	cumulative criteria unit exceedance
CF	contamination factor
COINSPAN	Constrained Indicator Species Analysis
CPET	Chironomid Pupal Exuvial Technique
DCA	detrended correspondence analysis
DO	dissolved oxygen
EQI	Ecological Quality Index
EQR	Ecological Quality Ratio
ERL	effects range low
ERM	effects range median
EU	European Union
FA	Factor Analysis
GEP	good ecological potential
GES	good ecological status
GQA	General Quality Assessment
HMWB	Heavily Modified Water Bodies
IMS	industrial methylated spirit
INDVAL	Indicator Value
MEP	maximum ecological potential
PSYM	Predictive System for Multimetrics
RIVPACS	River Invertebrate Prediction and Classification System
SD	standard deviation
SE	standard error of the mean
SEPA	Scottish Environment Protection Agency
TON	total oxidised nitrogen
USEPA	United States Environmental Protection Agency
WFD	Water Framework Directive

# **CHAPTER 1**

## **INTRODUCTION**

### **1.1 ECOLOGICAL MONITORING IN THE ENVIRONMENT AGENCIES OF THE UK**

Ecological assessment using macroinvertebrates is still the backbone of monitoring in the Environment Agency (EA). The majority of site appraisal carried out by the EA has concentrated on organic pollution problems and methods now exist for this type of pollution, such as the Biological Monitoring Working Party (BMWP) method using macroinvertebrates (Environment Agency, 2003). Unfortunately, other forms of pollution, such as metal, have been all but ignored and the time has now come to examine these in more detail (WFD, 2000). Indeed, the EC Water Framework Directive (WFD), emphasises the need to classify water bodies on the basis of their ecological status (WFD, 2000), a criterion that must include all forms of pollution. The complexity of this issue is perhaps best typified within the urban environment, where pollution includes metals, nutrients, as well as organic compounds (Petts et al., 2002), which are difficult to monitor using biological metrics. The detection and assessment of these non-organic water quality parameters will therefore be of utmost importance. Large cities such as Birmingham have the added issue of large stretches of canal within their environs. Rivers and streams and to some extent standing waters are the focus of local EA management plans and Biodiversity Action Plan but canals fall outside of these protective management systems. This is a problem in large conurbations, such as the West Midlands, where there are approximately 1000 km of canals that are variously used for recreation and nature conservation and as wildlife corridors.

## **1.2 WATER FRAMEWORK DIRECTIVE (WFD)**

### **1.2.1 BACKGROUND TO WFD**

The European Water Framework Directive (WFD) (European Union, 2000) states that member states should achieve a target of ‘good ecological status’ in all waterbodies by 2015. One of the challenges for the implementation of this directive is how to define and determine the ecological status of a specific waterbody type. This should be defined relative to its deviation from a reference condition, free from anthropogenic influence. Reference condition under the WFD (2000) must be minimally impaired. The definition is as follows:

*“The values of the relevant biological quality elements reflect, as far as possible, those associated with closest comparable surface water body type, given the physical conditions which result from the artificial or heavily modified characteristics of the water body”.*

It is debatable whether even the best available contemporary site data on canals can be considered to represent true reference conditions, especially in lowland catchments where the majority of feeder streams are likely to be impacted (e.g. by diffuse pollution). However, little or no comparable historical data are available for invertebrates on canals. Additionally, in line with DEFRA’s recommendation (2007), classification of ecological potential of canals should not be to the obvious detriment of navigation.

Under the Water Framework Directive, waters will be assigned to a new classification system. Surface waters will be classified using both biology (ecological classification) and compliance with chemical environment quality standards for pollutants (chemical classification). The new Ecological Classification system for surface waters will be based on five quality classes. This classification system is based on biological, hydromorphological and physio-chemical elements. The overall status of a waterbody will be determined by the worst element for a particular site.

Some water bodies may not achieve good status and under certain circumstances the WFD permits Member States to identify and designate Heavily Modified Water Bodies (HMWB) and Artificial Water Bodies (AWB). HMWB are bodies of water

which as a result of physical alteration by human activity are substantially changed in character and cannot, therefore, meet the “good ecological status”. AWBs are surface water bodies that have been created in a location where no water body existed before and not created by the direct physical alteration, movement or realignment of an existing water body (WFD, 2000). The WFD places canals and reservoirs into this category.

### **1.3 CANALS**

Within the Environment Agency, canals have not been included in biological surveys of water quality to any great extent. This is generally because sampling canal habitats is not easy. The paucity of sites available for sampling and the artificial nature of many of the banks make obtaining a representative sample difficult. The sampling issue is exacerbated by the difficulties of interpreting data derived from current biomonitoring tools to assess water. Water quality is often influenced by a large number of diffuse and point source inputs, with a wide range of contaminants present. Organic and inorganic pollutants are frequently present together. In addition, the physical characteristics and habitat of urban watercourses can make sampling and data interpretation difficult. This is especially apparent in the case of canals, which are often incorrectly graded, especially when chemical monitoring is used on its own. This is because only three determinands, Biological Oxygen Demand (BOD), Dissolved Oxygen (DO) and Ammonia, (NH<sub>3</sub>) are used to set quality grades and within canals DO can be naturally low increasing the likelihood of an inappropriate classification grade.

As a result of low flows in canals and incoming land run-off, there is generally a predominance of nutrient-bound clay and silt sediments setting on the channel bed. Dispersion processes are slow; therefore residence times for pollutants are long and sudden mixing of sediments into the water column from navigation causes high turbidity and a further influx of nutrients into the water column. Lack of flood events to remove sediment build-up and clear channels worsens the sedimentation problems in canals. This produces a continuous rise in the channel bed and promotes eutrophication via nutrient resuspension. Canals generally pass through a range of

land use (urban, agricultural and industrial), from which originate a range of pollutants, such as heavy metals and nutrients (Swanson et al., 2004).

### 1. *Nutrient enrichment*

This can be from diffuse sources such as agricultural run-off or from point sources such as sewage discharges to canal feeders and ditches draining into the canals as well as directly into the canal itself. Slow water movement coupled with warmer weather in the summer months provide conditions for algal blooms.

### 2. *Chemical pollution*

Chemicals can enter the canal via discharge pipes or seep from factories. Storm overflows can bring in sewage or chemicals. Diffuse sources can also be considerable. These sources drain slowly from residential, industrial or derelict land bordering the canals, reservoirs or their tributaries.

The WFD develops the concept of ecological potential in artificial water bodies, as opposed to ecological status in natural water bodies to allow their continued beneficial uses such as navigation and recreation. AWBs should attain Good Ecological Potential (GEP) by 2015 rather than Good Ecological Status (GES) for natural water bodies. Sites will be classified under the WFD using both biological and physiochemical determinants. Currently the only adequate biological data available for the construction of tools are based on macrophytes and macroinvertebrates, but there are problems in using these as bioassessment tools.

Mitigation measures in relation to canals will be needed in order to achieve good ecological potential. It is also necessary to identify those measures that do not have a significant adverse effect on the sustainable use of canals. A series of steps need to be developed in order to achieve this, as follows:

**Step 1:** Define driving forces, pressures and impacts

**Step 2:** Define a set of mitigation measures

**Step 3:** Exclude measures that have an 'adverse impact on use'

**Step 4:** Exclude measures that deliver only slight ecological improvement

**Step 5:** Identification of where mitigation measures are already in place, and what measures still need to be implemented to achieve GEP

There are a number of mitigation measures that can be implemented, as follows:

- (i) Naturalisation – The removal of hard engineering structures, or replacing with a soft engineering solution. This can involve installing vegetative fibre such as coconut matting. Emergent plant specie can then be planted to help protect the bank from water-wash.
- (ii) Preserving and where possible enhancing the ecological value of marginal aquatic habitat, banks and riparian zone.
- (iii) Selective vegetaion control regime by appropriate control techniquess and/or appropriate timing.
- (iv) Traffic management – encouraging the reduction of boat wash impacts through traffic management in sensitive areas.
- (v) Increasing boat traffic from zero to low levels can be ecologically beneficial by controlling the excessive growth of dominant species.
- (vi) Where practicable keeping a shallow margin and reed fringe on the off side during sediment management. This requires complying with width and depth standards for each waterway.

## **1.4 BIOASSESSMENT**

A fundamental requirement of any bioassessment technique that is used for water quality monitoring is the ability to distinguish natural variability from induced changes (Resh et al., 1995). In developing bioassessment indicators a number of factors need to be considered: (i) number of species in the taxonomic group (ii) reliability of species (iii) survey methodology (iv) how representative the species is in all the available habitats in the ecosystem to be assessed (Dodkins et al., 2005). To be of diagnostic value, the species or groups should be both sensitive- and stressor-specific (Hämäläinen, 2000). Current approaches in the assessment of waterbodies for systems that use specific biotic indices or scoring systems (Armitage et al., 1983; De Pauw and Vanhooren, 1983) measure either water or habitat quality. In these cases the

quality assessed is more general (mainly organic) pollution and so indicator values of the taxa are more subjective and the resulting index values are also subjective. In the USA, a metrics-based system is used. Metrics are measures (taxa richness, functional groups) that are used to develop multi-metric systems (Simon and Lyons, 1995). Using a metrics-based system can be problematic in that the most commonly used metrics can be highly variable and so would be insensitive to subtle changes in water quality (Resh et al., 1995).

#### **1.4.1 PREVIOUS APPROACHES TO BIOASSESSMENT IN CANALS**

Currently, there are two methods that are used to biologically assess canals. The most common approach is the Environment Agency's General Quality Assessment (GQA), where macroinvertebrate data are used to grade sites using the BMWP scoring system (Armitage et al., 1983). This assesses water quality using family level data assigning scores to each taxon in accordance to their (organic) pollution tolerances. A weakness of the BMWP system, in common with many other score systems, is the effect of sampling effort. A prolonged sampling period can be expected, under most circumstance, to produce a higher final score than a sample taken quickly. To overcome this inherent weakness of the BMWP system, it became common practice to calculate the Average Score Per Taxa (ASPT) by dividing the BMWP Score by the number of taxa (Wright et al., 1984) and to standardise to a 3-minute kick sample. The most useful way of summarising the biological data was found to be one that combined the number of taxa and the ASPT. The best quality is indicated by a diverse variety of taxa, especially those that are sensitive to pollution. Poorer quality is indicated by a smaller than expected number of taxa, particularly those that are sensitive to pollution, while organic pollution sometimes encourages an increased abundance of the few taxa that can tolerate it (Wright et al., 2000).

The data obtained from this scoring system are used in the software package RIVPACS (River InVertebrate Prediction And Classification System). This was developed by the Institute of Freshwater Ecology (IFE) as an application to assess the biological quality of rivers (Armitage et al., 1983). It offers site-specific predictions of the macroinvertebrate fauna to be expected in the absence of major environmental

stress. The expected fauna is derived by RIVPACS using a small suite of environmental characteristics derived from maps or measured at the site. The biological evaluation is then obtained by comparing the fauna observed at each site with the expected fauna. Using this system, it would be difficult to properly assess the impact of toxic, metal or inorganic types of pollution without proper expert interpretation. A lay person unaware of this shortcoming would therefore be likely to misinterpret the results. The results from samples collected in spring and autumn are combined to take account of seasonal variations. Both ASPT and number of taxa in the samples are divided by the equivalent values predicted by RIVPACS so that they are expressed as the proportion of their value when environmental quality is good. These proportional values are called Ecological Quality Indices (EQIs).

BMWP was developed to be used in rivers but has no real use to assess canals. There are problems in obtaining representative samples, in that sites are deep and especially in urban areas very little habitat is present with mainly reinforced sides. Perhaps more significantly, chemical factors other than organic, such as metals or nutrients may be more important than organic pollution and will not be picked up by the BMWP method.

The second approach, PSYM (Predictive System for Multimetrics) is a recent system (Pond Conservation Trust, 2002) aimed at better assessing still and standing waters (canals and ponds). This still uses BMWP as its basis but also possesses a component that aims to assess both water quality and habitat quality using a metric based on the number of Coleoptera, Ephemeroptera, Plecoptera and Trichoptera taxa. PSYM combines the predictive approach of RIVPACS with mutimetric-based methods used for ecological quality assessment in the United States. In multimetric analyses, values from individual metrics are combined to give a single measure that aims to provide an overall ecological quality of the canal. This involves the following steps:

- Predicting the fauna likely to be present, with no ecological impairment. This uses only physico-chemical variables;

- Using metrics to assess how much deviation there is from the minimally impaired state;
- A four point scale is used (0-3) to score individual metrics – where 0= poor quality and 3= good quality (no deviation from baseline);
- An overall integrity score is given to a site by combining individual metric scores.

The assessment is based upon samples taken in the spring, using combined edge (net-sweep) and bottom (dredge) samples. The method comprises:

A one-minute search.

A two-minute semi-continuous hand-net sampling of the canal margin, shallows and any emergent plant habitats present. This sample typically covers a bank length of 5 m to 15 m.

Four net hauls from deeper bottom sediments along a canal length of approximately 10 m, elutriated on site to wash out the bulk of muds and fine sands. These are taken at c. 3 m intervals along the canal sample length.

Family level data are used to calculate four metrics:

- Average Score Per Taxon (ASPT)
- Number of Ephemeroptera, Plecoptera and Trichoptera families (EPT)
- Number of beetle families (COL)
- Number of invertebrate families

It was shown that ASPT and EPT correlate significantly with water quality. In contrast, the number of invertebrate families and number of beetle families strongly correlate with habitat (bank) quality and boat traffic (Williams et al., 1996, 1998; Biggs et al., 2000).

Canals, especially in urban locations are steep sided and generally deeper (greater than one metre) water bodies, so the hand-net sampling methodologies appropriate for rivers cannot be directly applied to canals. Because hand-net methods are difficult, if

not impossible to apply to deeper water, dredges are sometimes used. Unfortunately, these only sample a small area (along a canal length of about 10 m) and unless multiple hauls are used (c. 10+ per sample site) there are problems with sampling sufficiency.

The emphasis on organic pollution is one problems of using PSYM, in that it makes assessment of potential other pollutants difficult, and there is also considerable data redundancy as the system categorizes species within each family as having the same (pollution) tolerances. As a result, chironomids all have a low tolerance score, indicating that they are tolerant to pollution. As will be seen in the following section, this is not the case.

## **1.5 CHIRONOMIDS AS OBJECTS OF STUDY**

Chandler (1998) lists 588 species of chironomids, but in fact there are now over 600 species present in UK aquatic habitats (Ruse, personal communication). They are more species rich and abundant than, Trichoptera, Ephemeroptera and Plecoptera combined and they occur in most types of freshwater both within the water column and in the sediment (Coffman, 1995). Chironomids comprise all six functional feeding groups exhibited by macroinvertebrates (Berg, 1995) and for most species their habitat and water quality preferences are well documented (Moller-Pillot, 1984a,b). The larvae and pupae are mostly aquatic and throughout the course of their development are subjected to the prevalent conditions in the habitat and thus reflect the quality of both water and sediment.

### **1.5.1 USE OF CHIRONOMIDS FOR BIOMONITORING**

Chironomid larvae have been used to study pollution either as individual species (Gower and Buckland, 1978; Surber, 1959) or species assemblages (Armitage and Blackburn, 1985; Yasuno et al., 1985). A common use of larvae has been their application in water quality assessments using the incidence of antennal (Warwick, 1985, 1989) and mouthpart (Janssens de Biisthoven et al., 1992; Lenat 1993; Madden

et al., 1992) deformities and fluctuating asymmetry in these areas to construct indices to typify different types and amounts of pollutants (Clarke et al., 1995).

Larvae have also been used in lake classification studies. A review by Brinkhurst (1974) of the early work up to the 1960s concluded that chironomids were not a useful organism in the classification of lakes because (i) they fluctuated too widely in terms of seasonal variation, depending on the success of the breeding swarm, (ii) there was a lack of knowledge about the relative abundance of accurately identified species, (iii) the reasons behind the associations of organisms to sediments was poorly understood. The debate continued into the late 1970s when Saether (1979) proposed improvements to indices formulated to describe lake typology. These improvements considered the trophic range of each indicator species. Saether developed a model for predicting Holarctic lake trophic classification by grouping holarctic chironomid species (131 in all, 88 of which are found in North America) into 15 communities representing different lake trophic conditions. Six communities were characteristic of oligotrophic lakes, three of mesotrophic lakes and six of eutrophic lakes. From this classification, Saether concluded that chironomid communities are distributed across a curve of total phosphorus/mean lake depth and chlorophyll *a*/mean lake depth. Ruse (2002a) improved this further when using exuviae to classify lakes. A biological classification, constrained by conductivity, was used to calculate indicator species scores. A conductivity gradient of indicator species assemblages was produced to demonstrate how temporal or spatial reference conditions could be inferred.

Pupal exuviae were first shown to be an effective technique for establishing lake species composition in 1910 (Thienemann). The use of exuviae has also been shown to successfully characterise two small streams (Ruse, 1995; Wilson, 1977). Wilson (1988) used pupal exuviae to assess metal-pollution from mine drainage. He was unable to identify any characteristic zinc tolerant species in three English streams although he found lower chironomid diversity at the zinc-loaded sites. Large rivers have also been assessed using pupal exuviae. The River Rhine (Wilson and Wilson, 1983) was sampled from the Swiss Alps to the North Sea. This river was selected as a large river suitable for test out the exuvial technique and to see the extent to which

sample might reflect the changes in quality between different river regions. 62 sites along the river, its tributaries and associated lakes were sampled. The pattern of water quality was derived from consideration of both the sample diversity and the pollution tolerance of taxa. The results matched well with the saprobic system of classification and followed the main trends of chemical water quality. The River Meuse system was studied (Frantzen, 1992) in order to determine spatial water quality variations. It was found that the most upstream sites were relatively unpolluted but deteriorated dramatically as a result of untreated industrial and municipal wastewater discharges. Apart from organic and inorganic pollution other environmental factors such as flow velocity and the nature of the riverbed also played a significant role in the occurrence of the organisms. The River Thames was surveyed over a period of 20 years (1977-1997) to assess the suitability of chironomid data for detecting significant changes in biological structure and functioning and substratum composition (Ruse and Davison, 2000). It was found that indicator taxa could be identified which were used as a simple monitoring tool to track the effects of varying flow and nitrogen flux between surveys.

To date, little work has been carried out on the assessment of canals using chironomids. Possibly the first study carried out was by Wilson (1993) on the Staffordshire & Worcestershire Canal. He interpreted data using CPET (Chironomid Pupal Exuviae Technique) initially developed for use in running waters and looked for assemblages indicating organic pollution. This contrasts to a CPET index based merely on presence/absence of taxa, which only indicates the potential total community of a site. He also found evidence that enbenthic forms (tube dwelling) were more affected by conditions in the substrate itself, whereas epibenthic (attached to hard surfaces such as macrophytes, stones etc) were affected by water quality. Wilson (1994) also collected exuviae on the Oxford Canal at stations above and below a sewage effluent discharge. He found clear evidence that chironomid exuvial assemblages showed clear changes reflecting the effects of the sewage, notably the increased dominance of pollution-tolerant species downstream from the effluent inflow. The only attempt to classify canals on a wider scale was by Ruse (1998) in which he successfully discriminated true canal sites from more riverine watercourses using CPET. Chironomid data were related directly to associate physical and chemical

characteristics of each site. He also proposed a scoring system and dichotomous key using indicator taxa.

To date, very little work has been carried out on urban canals specifically using chironomids and none specifically using pupal exuviae. Rather, in urban areas previous research has generally concentrated on nuisance chironomids (Romeo and Massimiliano, 1996) or substrate preferences, for example in urban flood relief channels (Ali and Mulla, 1976).

## **1.6 SUMMARY AND RESEARCH GAP**

Clearly, there is a gap in our understanding of ecological functioning in canals, especially within urban environments. Not only are they difficult to sample but they have complex water quality issues (e.g. eutrophication and heavy metal pollution) which make their characterisation especially difficult. Although canals form a large proportion of water bodies in urban areas there are no current management tools to monitor them effectively. The limited use chironomid exuviae in previous studies has shown that there is the potential to develop a robust tool for the monitoring of canals to conform to the requirements of the WFD. It has been shown that chironomid exuviae are effective in monitoring organic pollution in canals and what will be required is a more national appraisal of CPET across a wider environmental gradient. This tool would need to be easy to use, be able to sample all available habitats and provide information on the quality of the canal to be investigated. In order to be used to build a classification system biological data needs an explicit link to environmental data covering the geographical range over which the tool is to be applied. The data on which the tool is based should contain a sufficient pool of sites from which minimally impaired sites can be drawn. As well as this, the sites used for tool construction need to cover a gradient of impact across a range of pressures for useful diagnostic metrics to be developed and tested.

## **1.7 PROJECT AIMS AND OUTLINE**

This project develops a tool that will enable the various regulatory bodies in the UK to classify canals in accordance with the requirements of the WFD and which will support operational and surveillance monitoring.

Its four key aims are to:

- I        classify chironomid assemblages in canals using pupal exuviae;
- II       establish species distributions, ecology and function;
- III      determine indicator taxa suitable for classifying canal water and sediment;
- IV      analyse potential boundaries between the WFDs five ecological classes and develop a method to calculate an ecological quality ratio (EQR).

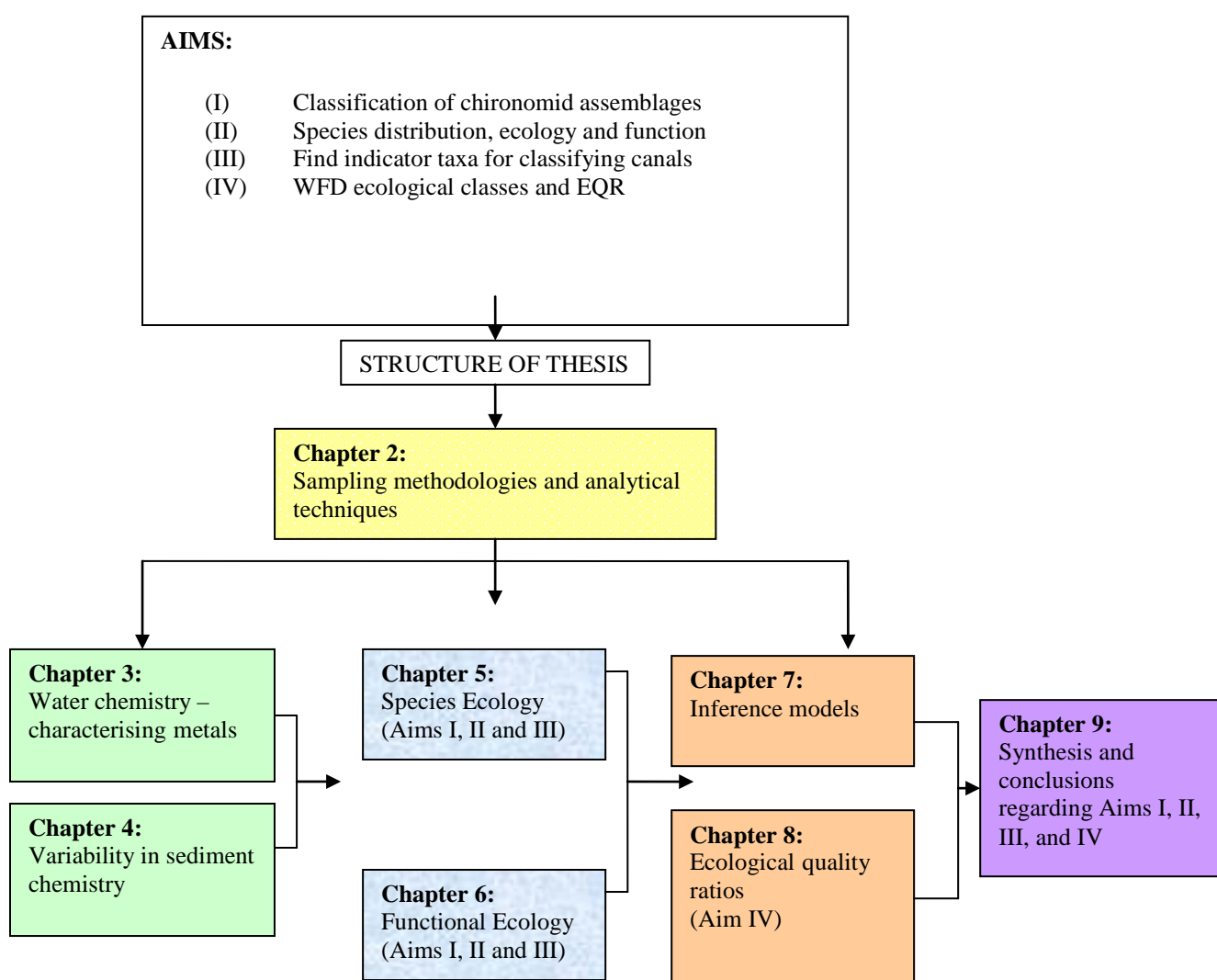
The structure of the thesis and its relation to the projects' key aims is shown in Figure 1.1. The thesis chapters are written as standalone papers with some limited and inescapable repetition in the methods sections.

Chapter 2 presents sampling methodologies and analytical techniques that are used for the rest of the thesis. The remaining chapters then focus on three main areas of study: chemistry, ecology and the calibration, testing and application of models.

Chapter 3 reports on characterising heavy metal chemistry in the water associated with the study sites. Chapter 4 investigates the variability in sediment chemistry associated with urban canals of the West Midlands.

Chapter 5 reports on aspects of bioassessment and community ecology, discussing features such as species and taxonomic distinctness and multimetric analysis. Chapter 6 presents findings on the species ecology of urban canals and the relationship to environmental variables. Indicator species assemblages were also determined and the response of chironomid species to metal pollution was also investigated. Chapter 7 discusses functional ecology in terms of functional groups and body size.

Chapter 8 reports on the development of chironomid-based inference models and the testing of models on an independent data set. Chapter 9 presented findings on how the data can be applied to classify canal quality by means of ecological quality ratios (EQR) necessary for the Water Framework Directive (WFD).



**Figure 1.1** Flow diagram outlining structure of the research project

## **CHAPTER 2**

# **SAMPLING METHODOLOGIES, SITE SELECTION AND STRUCTURE OF DATA ANALYSIS**

### **2.1 RESEARCH DESIGN**

#### **2.1.1 GEOGRAPHICAL BACKGROUND**

Located at the heart of England, the West Midlands conurbation is one of the most highly developed areas in the UK, with a rich industrial past (Gerrard and Slater, 1996). It was the combination of natural resources – coal, iron ore and limestone – which provided the raw material for these industries. Iron forge and furnaces were in operation from the middle of the 17<sup>th</sup> century and after the 1870s, especially in the military arms industries, that the factory system underwent massive growth. The beginning of the 20<sup>th</sup> Century saw new industries being developed, namely electrical apparatus and motor cars and this increased further with the advent of World War 1. During this period there was also a growth in light and medium engineering industries (e.g. metal processing and precision tool manufacturing). In the period after the Second World War there was a massive increase in manufacturing especially in vehicles, metal goods, metals, mechanical engineering and electrical engineering (Gerard and Slater, 1996).

It was the transport system that tied the region together and permitted the large growth in manufacturing. The earliest canal opened in 1769, connecting Birmingham to the Hill Top collieries in West Bromwich, with, immediately cutting the cost of coal by 50%. Within the next 30 years, over 200 miles of canal had been constructed across the West Midlands. The stimulus for building the canals came with the recognition that raw materials and finished products had to be moved from where they were made

to where they were sold. The capital for canal projects came from industrialists and landowners, who saw the opportunity to increase the flow of goods and saw them as a major investment opportunity. These industries led to the heavy pollution of the local environment (Gerard and Slater, 1996). Thus this diverse industrial past has left a legacy in the sediments. The Midlands have about 1000 km of canals, which is almost half of the national total of 2300 km (Environment Agency, 2003).

### **2.1.2 SITE SELECTION**

Forty-six urban canal sites were selected (Table 2.1) from the approximately 1000 km of classified canals within the West Midlands and were based on routine monitoring sites of the EA. The sites are predominately urban in nature (Fig. 2.1) and were chosen in order to represent as wide a range of water and sediment chemical characteristics as possible, with special reference to the range of metals (Tables 2.2 & 2.3). The sample sites take into account habitat structure (emergent and floating macrophyte stands or no macrophytes present) and known water quality, for example where metal problems are known to exist. It can be seen that different metals have distinct distribution among sites (Table 2.2). For example, at most sites the levels of cadmium are quite low ( $<1.0\mu\text{g/l}$ ) except for a small number of sites. One site in particular (CX-WG) stands out in having very high levels of dissolved cadmium in the water ( $5.14\mu\text{g/l}$ ). It was noticeable that this site did not have high levels of other metals. At SW-CB there were high levels of chromium, BL-BB high levels of lead and iron, (together with BL-BL and WS-MM), WB-LF, ST-SR, BW-KE and GU-NC high levels of zinc, and at BF-SB high levels of nickel.

For nutrients (Table 2.2), there are similar patterns. Levels of phosphorous ranged from  $0.05\text{--}118.72\mu\text{g/l}$  and nitrogen from  $0.58\text{--}22.23\mu\text{g/l}$ . An interesting pattern was observed on the Staffordshire/Worcester Canal where levels of phosphorous gradually fell from SW-OX ( $5.1\mu\text{m/l}$ ) close to a sewage outfall to  $2.55\mu\text{m/l}$  four sites later at SW-KD. A similar pattern was also seen with nitrogen, TON, nitrate, nitrite and O-P. An opposite pattern was seen with chlorophyll where levels gradually increased from the outfall to Kidderminster (SW-KD), a distance of about twenty miles. The sites are

also current Environment Agency GQA (General Quality Assessment) monitoring stations to take advantage of the chemical data taken at these sites.

#### *2.1.2.1 General Quality Assessment (GQA)*

##### *(i) Chemical GQA*

GQA is used to assess the status of water bodies and to show how the quality has changed over time. The Environment Agency currently uses chemical, biological and nutrient monitoring data to classify rivers and canals. The chemistry GQA scheme is based on dissolved oxygen (DO), biochemical oxygen demand (BOD) and ammonia (NH<sub>3</sub>), which act as indicators of organic pollution.

##### *(ii) Biological GQA*

The biological GQA scheme is based on measurements of macroinvertebrate communities. These can reflect the presence of pollutants that occur infrequently or in low concentrations that may not be reflected by chemical analysis alone. The Biological Monitoring Working Party (BMWP) system assigns a numerical value to about 80 different taxa according to their sensitivity to organic pollution (Wright et al. 1984). The average of the values for each taxon in a sample, known as ASPT (Average Score Per Taxon) is a reliable index of organic pollution (Wright et al. 1984). Values lower than expected indicate organic pollution.

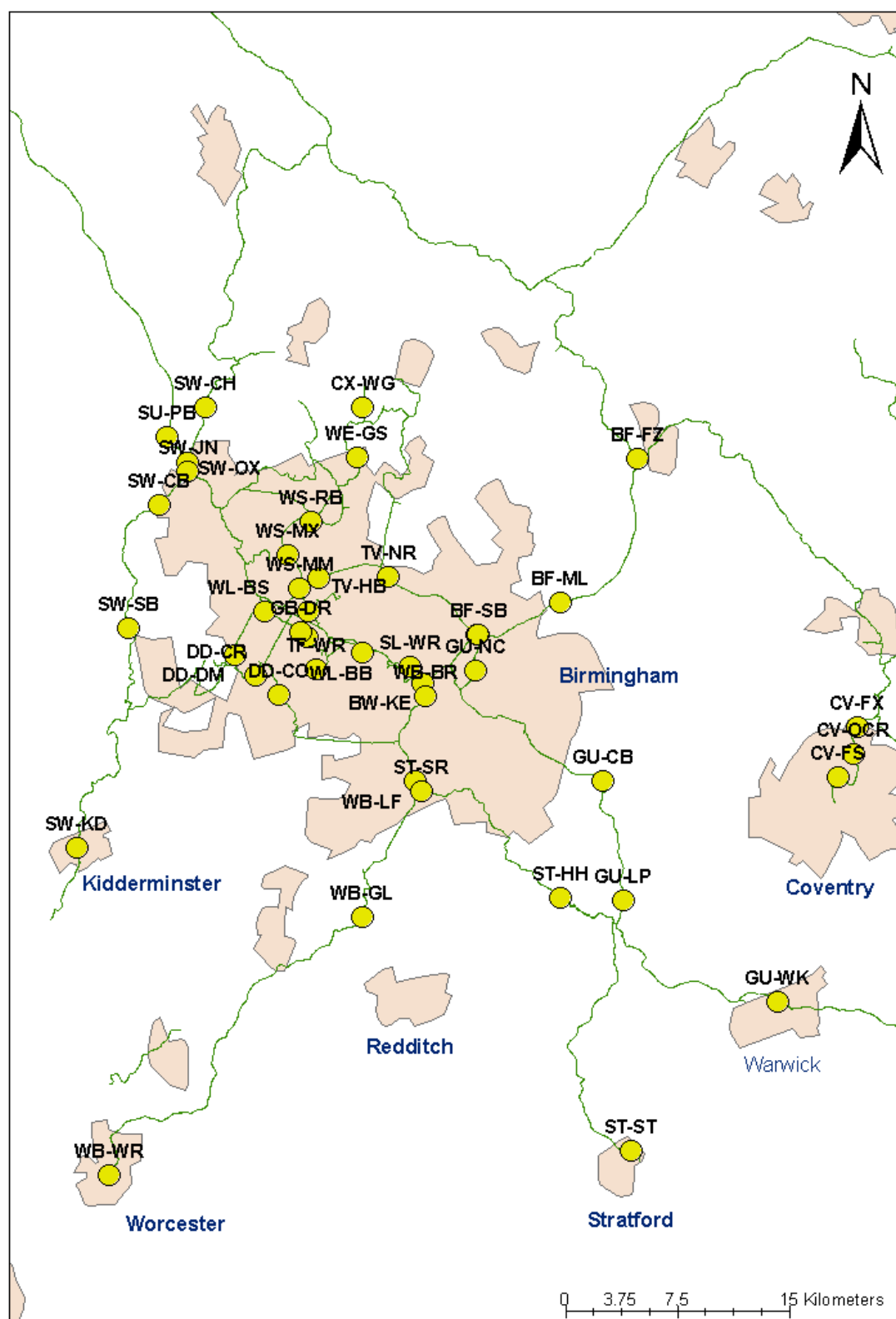
##### *(iii) Nutrient GQA*

Elevated nutrient levels in rivers and canals can lead to excessive plant growth and an associated shortage of dissolved oxygen especially at night (known as eutrophication), which can result in poor ecological water quality. The EA routinely analyse water samples to establish nitrate and phosphate concentrations. Using data collected from a given year plus the two preceding years, each stretch of river is given a grade for nitrate from 1 (very low) to 6 (very high) and for phosphate from 1 (very low) to 6 (excessively high). Nutrient concentrations cannot be categorised as 'good' or 'bad' in rivers in the same way that chemical and biological quality is assessed. As rivers naturally have different concentrations of nutrients, 'low' concentrations are not

necessarily good or bad but are merely ‘low’ relative to other rivers, so care must be taken when utilising data of this type.

**Table 2.1** Sample sites

Code	Canal	Site	Grid Ref	Urban, U or Rural,R
TV-HB	Tame Valley	Holloway Bank	SO 990 939	U
TV-NR		Newton Rd	SP 036 940	U
TV-SB		Salford Br	SP 096 901	U
CV-FX	Coventry	Foxford	SP 351 839	U
CV-OCR		Old Church Rd	SP 348 821	U
CV-FS		Foleshill Rd	SP 338 806	U
WE-GS	Wyrley & Essington	Goscot	SK 016 020	U
CX-WG	Cannock Extn	Wyrley Grove Br	SK 019 054	R
WS-RG	Walsall	Ryders Green Rd	SO 983 917	U
WS-MX		Moxley	SO 969 955	U
WS-MM		Moors Mill Lane	SO 977 932	U
WS-RB		Rayboulds Br	SO 985 977	U
SW-CH	Staffs & Worc	Coven Heath	SJ 914 054	R
SW-OX		Oxley	SJ 902 017	U
SW-JN		Junction	SJ 902 011	U
SW-CB		Compton Br	SO 883 988	R
SW-SB		Swindon Br	SO 862 906	R
SW-KD		Kidderminster	SO 828 758	U
SU-PB	Shropshire Union	Pendeford Br	SJ 888 034	R
WB-LF	Worc & B'ham	Pershore Rd, Lifford	SP 054 803	U
WB-GL		Grange Lane	SP 019 712	R
WB-WR		Diglis Basin, Worcester	SO 849 539	U
WB-BR		Bath Row Br	SP 061 860	U
ST-SR	Stratford	Stirchley	SP 059 796	U
ST-HH		Hockley Heath	SP 152 725	R
ST-ST		Stratford	SP 199 555	U
BL-BL	B'ham Level	Bromford Lane	SP 995 903	U
BL-PL		Park Lane East	SP 966 919	U
BW-KE	B'ham & W'ton	King Edwards Rd	SP 060 869	U
BL-BB	B'ham Level	Brasshouse Br	SP 019 889	U
WL-BB	W'ton Level	Brasshouse Br	SP 019 889	U
WL-BS		Baker St, Tipton	SO 954 917	U
WL-BR		Brades Rd	SO 982 900	U
GU-WK	Grand Union	Emscote Rd, Warwick	SP 297 655	U
GU-LP		Lapworth	SP 194 723	R
GU-CB		Catherine-de-Barne	SP 180 803	U
GU-NC		Nechells	SP 095 877	U
BF-SB	B'ham & Fazeley	Salford Br	SP 097 901	U
BF-ML		Minworth Lock	SP 152 923	U
BF-FZ		Fazeley	SK 203 019	U
DD-DM	Dudley	Dudley Mixed	SO 934 888	U
DD-CR		Cradley Rd	SO 948 874	U
DD-CO		Cherry Orchard	SO 963 861	U
TF-WR	Titford	W'ton Rd	SO 988 878	U
GB-DR	Gower Branch	Dudley Rd, Tipton	SO 978 903	U
SL-WR	Soho Loop	Western Rd Br	SP 051 880	U



**Figure 2.1** Map of the sample sites surveyed in the West Midlands

**Table 2.2 Mean annual chemical measurements (water)**

	Zn	Cr	Ni	Pb	Cu	Fe	Cd	NH3	P	N	TON	Chl A	NO3	NO2	O-P	DO	BOD	Cl	Ca	Alk	pH	Cond	Hdss
TV-HB	86.04	1.77	24.7	15.21	3.34	568	0.248	0.15	0.58	2.69	1.5	103.7	1.45	0.04	0.26	130	9.21	79	69.5	132	8.1	658	233
TV-NR	23.2	1.16	9.11	4.15	3.92	357	0.19	0.08	0.12	1.41	1.02	51.3	1.1	0.02	0.06	105	3.06	41.1	65.7	146	7.9	501	211
TV-SB	104.6	2.09	12.5	10	5.51	269	0.16	0.124	0.05	6.12	5.79	30.1	5.76	0.03	0.03	102	3.82	52.9	78.9	133	8	583	244
CV-FX	33.1	1.54	7.9	5	3	676	0.04	0.09	0.20	4.80	3.3	20.9	3.54	0.05	0.18	79	1.69	42	88.1	164	7.8	605	303
CV-OCR	41.2	2.4	7.8	14	4.1	610	0.17	0.08	0.13	5.07	5.26	29.7	4.66	0.04	0.13	87	1.9	47.5	91.5	182	7.9	708	332
CV-FS	27.6	1.39	6.18	7.7	2.03	285	0.15	0.33	0.31	3.80	1.6	35.9	3	0.02	0.03	77	2.82	39.4	74.8	151	7.9	602	264
WS-RG	122.53	3.86	15.44	6.76	5.93	1052	0.45	2.94	0.55	1.81	0.38	98.21	0.68	0.02	0.60	66	31.24	86.95	77.48	171	7.66	707	244
WS-MM	66.84	3.47	13.25	12	1.96	1583	0.515	0.25	0.62	1.86	0.332	120.21	0.049	0.03	0.47	57	31.86	107.33	74.14	150	7.6	616	243
WS-MX	32.49	1.06	7.05	4.36	3	481	0.17	0.21	0.16	1.63	1.27	123.16	1.43	0.04	0.09	95	7.47	59.71	83.54	223	7.7	601	296
WS-RB	64.49	2.54	15.43	7.48	3.46	448	0.367	0.107	0.12	0.85	0.66	52.26	0.74	0.01	0.06	61	3.9	58.28	90.67	249	7.72	644	380
WE-GS	34.16	0.72	16.3	3.82	3.88	150.5	0.09	0.11	0.07	0.74	1.01	18.2	0.97	0.01	0.06	74	4.04	55.64	82.35	227	7.65	788	354
CX-WG	36.29	0.683	13.59	3.7	5.28	221	5.14	0.06	0.07	1.73	2.19	27.32	3.08	0.02	0.06	71	2.83	41.83	62.55	138	7.71	572	235
SW-KD	75.54	4.73	20.4	16.76	3.93	281.78	0.21	0.077	2.55	12.43	9.66	25.4	9.63	0.03	2.61	90	2.31	93.93	73.11	177	7.88	794	269
SW-SB	99.7	8.33	14.97	7.93	4.84	224.9	0.29	0.11	4.28	14.38	12.44	17.62	12.44	0.08	4.07	108	2.23	104.57	76.66	177	8.03	838	261
SW-CB	105.76	120.47	10.19	5.34	4.36	206.18	0.3	0.177	4.52	18.60	15.35	9.66	15.2	0.16	4.31	81	2.16	106.18	92.2	181	7.45	874	279
SW-JN	92.46	44.89	8.94	3.39	4.67	149.9	0.199	0.27	4.80	17.51	14.97	8.94	14.82	0.15	4.62	72	3.4	102.43	76.04	169	7.48	823	251
SW-OX	104.44	6.17	10.15	1.73	4.96	82.46	0.15	0.13	5.10	17.94	15.07	5.36	14.94	0.13	4.90	86	2.67	106.79	74.38	166	7.43	814	244
SW-CH	42.92	3.23	7.14	2.14	6.59	178.9	0.186	0.98	3.42	13.36	10.69	32.18	10.59	0.12	3.89	83	2.94	115.65	73.05	166	7.84	894	254
SU-PB	126.95	38.27	11.39	4.06	5.08	193.1	0.31	0.36	5.36	22.23	18.7	48.46	18.68	0.08	4.57	87	2.17	106.28	75.51	156	7.61	809	250
WB-BR	237	33.83	40.78	21.82	12.3	355	0.46	0.08	0.16	1.99	1.65	80.57	1.57	0.02	0.10	97	4.4	68.52	63.3	124	8	700	205
WB-GL	27.18	1.84	6.84	5.58	4.48	527	0.12	0.12	0.24	1.79	1.34	45.1	1.16	0.02	0.09	76	2.88	31.97	50.08	150	7.75	460	201
WB-DG	27.62	1.12	4.99	8.51	2.78	322	0.09	0.12	0.22	3.83	3.23	22.4	2.33	0.04	0.08	110	2.61	51.23	83.7	192	8.07	642	359
WB-LF	317.83	75.33	54.37	42.96	12.87	761	1.2	0.04	0.14	1.38	0.84	116.23	1.12	0.01	0.10	103	4.63	73.13	61.22	120	8.02	610	199
ST-SR	284	69.91	42.4	38.38	8.21	799	1.06	0.3	0.22	1.98	1.56	81.48	2.05	0.03	0.15	74	3.31	61.15	61.73	153	7.67	635	221
ST-HH	103.35	2.94	15.73	6.63	4.93	422	0.09	0.19	0.17	1.65	1.29	94.62	1.09	0.02	0.09	68	4.35	47.77	53.48	130	7.62	538	195
ST-ST	34.33	1.1	6.81	3.85	2.86	980	0.09	0.1	0.13	2.04	1.24	47.22	1.33	0.03	0.24	82	3.57	42.32	61.58	146	7.6	615	223
BL-BL	155.05	11.01	11.4	40.2	4.29	1739	1.8	0.17	0.27	2.74	1.97	171.63	1.96	0.05	0.12	89	5.63	71	65.48	126	7.58	616	217
BL-PL	18.8	1.57	6.98	6.06	2.54	138	0.29	0.08	0.39	0.58	0.66	45.55	0.5	0.01	0.17	96	4.55	82.03	116.97	356	7.83	1410	514
BL-BB	83.9	18.75	12	52.89	3.68	1857	2.12	0.46	0.39	3.38	2.54	129.45	2.62	0.06	0.11	93	5.61	90.42	77.95	159	7.28	732	264
WL-BR	87.77	1.36	6.33	3.93	3.64	283	0.17	0.32	0.66	1.61	1.97	45.47	1.54	0.02	0.74	86	3	106.75	107.77	286	7.8	1258	428
WL-BS	157.88	0.73	6.15	2.63	3.21	224	0.12	0.22	0.44	1.23	1.65	17.91	1.28	0.01	0.45	77	1.63	94.22	124.08	365	7.57	1353	502
WL-BB	90.62	3.4	8.65	13.66	2.89	607	0.24	0.21	0.33	1.74	0.63	101.7	0.83	0.04	0.19	75	4.07	88.97	74.93	173	7.7	618	286
BW-KE	282.67	39.82	42.05	23.96	12.17	376	0.5	0.25	0.17	2.09	1.61	87.1	1.62	0.02	0.10	99	5.26	66.88	66.13	133	7.95	696	218
BF-SB	141.82	77.8	94.3	61.02	8.4	839	1.52	0.07	0.31	4.25	3.63	52.83	6.4	0.01	0.05	98	2.59	59.15	90.2	152	7.78	947	290
BF-MN	167.9	5.61	28.58	10.8	6.77	227	0.27	0.05	118.72	5.31	5.26	75.93	4.88	0.04	0.05	94	3.57	58.35	82.4	147	7.78	640	270
BF-FZ	147.73	5.16	51.68	23.73	3.91	512	0.24	0.11	0.09	2.79	2.34	54.24	2.26	0.02	0.05	70	2.01	56.7	94.25	147	7.47	646	307
GU-NC	256.17	7.64	43.08	17.13	15.77	292	0.39	0.06	0.15	3.51	3.81	89.38	2.97	0.04	0.14	98	3.37	73.12	83.6	151	7.83	857	298
GU-CB	16.87	0.68	9.78	5.15	1.93	916	0.1	0.16	0.31	1.25	0.92	79.77	1.03	0.02	0.12	46	3	87.62	50.75	125	6.88	600	192
GU-WK	17.73	1.97	5.76	8.65	2.38	770	0.1	0.07	0.14	11.47	4.32	61.1	4.12	0.03	0.12	91	2.46	60.78	65	192	7.88	730	296
GU-LP	29.88	0.92	7.65	4.02	2.21	419	0.1	0.06	0.20	1.86	2.69	66.77	1.35	0.04	0.17	77	3.59	58.98	52.45	127	7.47	430	211
SL-WR	110.42	44.13	25	21.85	8.02	497	0.38	0.24	0.17	2.31	1.98	92.4	1.68	0.04	0.05	63	2.08	40.4	50.4	147	7.7	428	170
TF-WT	40.67	1.11	6	6.3	3.83	436	0.43	0.44	0.34	3.37	2.62	99.53	2.56	0.08	0.31	76	4.11	79.13	52.7	119	7.67	533	161
DD-CO	46.3	1.25	5.28	2.66	1.62	283	0.1	0.08	0.12	1.98	1.68	93.33	1.23	0.02	0.05	103	6	120.5	87	234	8.15	1168	370
DD-CR	58.37	1.47	9.3	7.07	2.08	285	0.28	0.05	0.42	0.70	0.62	106	0.37	0.01	0.14	116	5.59	104.03	121	333	8.22	1358	516
DD-DM	36.73	1.22	8.15	10.23	1.9	405	0.26	0.05	0.45	1.17	0.63	111.22	0.3	0.02	0.15	101	4.61	103.82	117.5	320	8.17	1303	493
GB-DR	89.94	1.26	6.31	2.62	2.46	229	0.17	1.37	0.41	1.45	0.81	42.92	0.94	0.02	0.78	79	3.11	123.48	122	359	7.76	1612	534
min	16.87	0.68	4.99	1.73	1.62	82.46	0.04	0.04	0.05	0.58	0.332	5.36	0.049	0.01	0.03	46	1.63	31.97	50.08	119	6.88	428	161
max	317.83	120.47	94.3	61.02	15.77	1857	5.14	2.94	118.72	22.23	18.7	171.63	18.68	0.16	4.90	130	31.86	123.48	124.08	365	8.22	1612	534
mean	95.41	14.38	17.36	12.78	4.85	511.25	0.48	0.26	3.45	4.75	3.80	64.57	3.78	0.04	0.78	85.82	4.85	75.00	78.65	183.13	7.75	773.17	288.85

**Table 2.3** Mean annual chemical measurements (sediment)

	Cu-s (mg/kg)	Zn-s(mg/kg)	Cd-s(mg/kg)	Pb-s(mg/kg)	Cr-s(mg/kg)	Fe-s(mg/kg)	Ni-s(mg/kg)	N-s(mg/kg)	P-s(mg/kg)	volatiles (%)	Fines (%)
TV-HB	1200	535	24.8	697	136	25700	153	0.591	0.997	29.8	39.65
TV-NR	605	3770	12.1	278	60.9	18100	115	1.38	0.329	33.5	37.31
TV-SB	149	2620	7.7	243	43	19400	123	0.924	0.21	33.5	44.2
CV-FX	124	377	0.886	100	45	20400	37.5	0.417	0.158	14.6	76.66
CV-OCR	1110	1450	4.8	513	63.5	21500	60.6	0.515	0.161	22.1	58.31
CV-FS	377	945	3.47	293	40.7	17800	38.8	0.42	0.098	14.7	39.57
WS-RG	2490	2530	7.22	523	87.8	21800	334	0.878	0.087	21.2	34.05
WS-MM	332	1540	7.32	150	29.3	19100	121	0.812	0.091	30.4	44.54
WS-MX	4800	9910	60.5	782	291	41200	387	1.62	1.22	30.5	36.06
WS-RB	369	3280	8.09	292	66.3	25200	103	0.703	0.095	22.3	46.46
WE-GS	2000	7120	35.4	687	224	39000	158	0.983	0.613	28.2	72.12
CX-WG	1100	7650	48.4	550	268	29100	307	0.808	0.149	21.7	58.19
SW-KD	166	704	10.1	76.7	92.8	17600	53	1.65	0.533	22.5	17.67
SW-SB	74.2	1100	2.48	50.8	77.4	18400	47.3	0.747	0.42	12	17.36
SW-CB	140	1570	4.43	115	102	17100	63.6	0.855	0.379	17.9	12.35
SW-JN	173	2240	6.99	169	155	16100	104	0.612	0.666	35.4	23.46
SW-OX	84.9	1040	4.2	118	77.4	31500	86.3	0.285	0.281	9.03	11.29
SW-CH	195	1030	3.83	294	107	22100	113	0.639	0.367	22.7	73.57
SU-PB	45.4	351	3	23.3	42.2	7220	26.8	0.113	0.095	4.7	14.03
WB-BR	5330	6320	28.3	409	1010	39000	405	0.548	0.867	21.6	44.07
WB-GL	116	259	0.43	65.3	48.9	33300	57.7	0.282	0.129	9.56	42.11
WB-DG	262	724	3.12	431	72.2	39600	37.8	0.526	0.281	16.5	81.46
WB-LF	6270	5520	24.6	450	1430	37300	397	0.693	0.618	19.5	86.85
ST-SR	130	583	72.8	72.8	46.9	27300	51.5	0.299	0.129	9.52	62.14
ST-HH	44.4	87.2	0.09	59.1	38.1	34600	33.3	0.184	0.087	5.1	32.48
ST-ST	525	2130	37.4	265	176	73000	65.1	0.155	2.4	7.13	38.62
BL-BL	169	1550	18.1	122	79.6	57100	47.2	0.141	0.817	8.52	26.37
BL-PL	268	3830	5.58	130	49.6	34800	59.1	0.438	5.58	16.4	29.04
BL-BB	324	4220	6.87	318	67.1	55600	77	0.359	0.916	11.3	38.09
WL-BR	8210	7950	30	754	2170	44600	795	0.587	0.754	27.6	62.12
WL-BS	544	1950	12.5	462	139	36000	78.8	0.279	0.631	21.9	28.37
WL-BB	817	7470	27	737	309	57900	135	0.598	1.42	25.3	57.24
BW-KE	2190	3420	11.2	356	609	18600	276	0.222	0.385	8.55	36.72
BF-SB	88.2	331	0.95	100	41.1	26900	37.6	0.607	0.147	16.8	94.58
BF-MN	86	554	1.06	57.9	31.8	23100	45.9	0.749	0.265	11.2	93.12
BF-FZ	114	518	2.74	140	30.8	19800	39.6	0.507	0.135	7.37	91.6
GU-NC	5380	5990	13.7	1280	620	67200	390	0.371	0.471	13.7	89.36
GU-CB	4090	5240	22.9	702	1010	49400	902	0.436	0.477	21.8	98.09
GU-WK	1360	5740	6.72	358	145	37500	291	0.851	0.32	6.16	87.96
GU-LP	1080	2480	6.54	231	247	32100	244	0.443	0.102	7.05	90.66
SL-WR	139	760	0.622	174	73.7	44000	35.7	0.352	0.306	16.6	99.45
TF-WT	1390	2590	11.9	475	97.4	60200	120	0.664	0.495	21.4	99.39
DD-CO	169	4120	3.21	235	36.2	45300	54.4	0.485	0.108	23.9	99.94
DD-CR	115	415	1.13	94.6	26.2	39200	23.3	0.212	0.11	7.56	84.33
DD-DM	480	8330	15.9	69.2	152	51000	69.2	0.485	3.24	20.9	91.09
GB-DR	3060	2740	8.22	383	1080	24300	196	0.426	0.346	12.5	79.99
min	44.4	87.2	0.09	23.3	26.2	7220	23.3	0.113	0.087	4.7	11.29
max	8210.00	9910.00	72.80	1280.00	2170.00	73000.00	902.00	1.65	5.58	35.40	99.94
mean	44.40	87.20	0.09	23.30	26.20	7220.00	23.30	0.11	0.09	4.70	11.29

### **2.1.2 PUPAL EXUVIAE**

Pupal exuviae are skins shed by emerging adults after which they generally float for only two or three days. As a result, a sample of these skins will represent recently emerged adults within the canal (Wilson and Bright, 1973; Ruse, 2008). They accumulate at the edge of the water, amongst vegetation and flotsam or behind obstacles such as lock gates. Water and wind currents aid this accumulation, although on very still days on canals with low boat traffic, skins can accumulate across the entire width of the canal. This makes sampling easy and a collection made will represent individuals from all habitats over the emergence period. The larvae will have been subjected to the prevalent conditions over their entire development and so the pupae will therefore reflect the environmental conditions of the water and sediments during previous months (Wilson and Wilson, 1983, 1984).

#### *(i) Collection*

Debris that had accumulated on the water surface was collected with a long-handled pond net, with a 250  $\mu\text{m}$  mesh. The residue was placed in a plastic pot and returned to the laboratory to be preserved in Industrial Methylated Spirit (IMS). The collection was examined on site to determine if enough exuviae have been collected as most assays are based on a count of at least 200 exuviae (Wilson & Ruse, 2005). The samples were sieved through a coarse and fine (250  $\mu\text{m}$ ) mesh to remove most of the debris.

Three samples were collected during April to September, which cover as much of the different emerging periods of the adults as possible (Ruse and Wilson, 1984; Ruse, 1995; Wilson and Ruse, 2005). Different species also emerge at different times of the day (Wilson and Bright, 1973). Collecting accumulated exuviae from the edge of a water body will overcome any diurnal bias in the sample, providing a 2- or 3-day rolling average of the exuvial emergence.

#### *(ii) Laboratory Procedure*

The material was placed in a large white tray with water and subsamples taken and placed in small, shallow trays. In the laboratory, the sample residue was resuspended in water, stirred and small aliquots removed. The subsample was placed in a small,

Petri dish with a grid. All chironomid pupal skins were removed under a low-powered (x60-300) microscope, working along the grid. This method was followed until approximately 200 skins were removed. This eliminated bias when picking large and small exuviae.

With the aid of daylight illuminated magnifier all exuviae were taken and placed in glass vials until at least 200 were collected. This ensures that a representative subsample of the whole collection is made (Ruse, 1993; Wilson and Ruse, 2005). Careful examination is required as some of the exuviae are small and transparent (<2mm). Skins were examined under both low powered and high-powered binocular microscopes and identified using the keys of Langton (1991), Wilson (1996), Langton and Visser (2003) and Wilson and Ruse (2005). Exuviae were also examined where necessary under high power on temporary mounts on slides. Body length measurements of the specimens were also taken during identification.

### **2.1.3 ENVIRONMENTAL DATA**

Chemical data for this study were collected from two sources: i) Environment Agency routine monitoring data, and ii) supplementary water samples collected in the field. Chemical analysis of the water and sediment samples was carried out by the Environment Agency laboratory in Nottingham. The analytical methods used are detailed in Table 2.4. A range of water chemistry determinands was taken to reflect the nature of urban canals (Table 2.2). Samples were collected six times over a one-year period up to the final month of exuviae sampling. Due to time constraints, a four year rolling programme of sample collection was carried out.

The chemical composition and physical properties of the sediment has obvious implications as to the chironomid community it can support. Random sediment samples were collected by 2-3 trawls of a dredge or long-handled net and subsampled. Sediments were characterised by the % weights of their particle size distribution and organic matter. In order to determine possible food source that might affect the distribution of the detritivore species. Chemical analyses of the sediment were carried

out to determine the levels of the major metals and nutrients (Table 2.3). As with the water there were wide variations in values. For example, zinc ranged from 87.2 mg kg<sup>-1</sup> to 9910 mg kg<sup>-1</sup>, with maximum value being observed at WS-MX. For copper and chromium, the maximum (8210 mg kg<sup>-1</sup> and 2170mg kg<sup>-1</sup>) were found at WL-BR; cadmium (72.8mg kg<sup>-1</sup>) at ST-SR; lead (1280 mg kg<sup>-1</sup>) at GU-NC; iron (73000 mg kg<sup>-1</sup>) at ST-ST and nickel (902 mg kg<sup>-1</sup>) at GU-CB. With respect to the main limiting nutrients, nitrogen ranged from 0.113 to 1.65 mg kg<sup>-1</sup> and phosphorous from 0.087 to 5.58 mg kg<sup>-1</sup>. For nitrogen, only two sites had values above 1.0 mg kg<sup>-1</sup> (SW-KD and TV-NR) and five sites for phosphorus were above 1.0 mg kg<sup>-1</sup> (WS-MX, ST-ST, BL-PL, WL-BB and DD-DM).

**Table 2.4** Chemical analysis methods

	Determinand	Method
<b>water</b>		
	Chlorophyll	Acetone extraction/spectrophotometry
	TON	Colourimetric discrete analyser
	Total N	On-line digestion/continuous-flow absorption spectrophotometry
	Total P	Digestion/ continuous flow absorption spectrophotometry
	Cd, Cr, Pb, Ni	ICPOES/ ICPMS
	Fe	ICPOES
<b>sediment</b>		
	Grain Size	Particle size analyser
	Dry Weight metals	Aqua Regia extraction / ICPMS
	Dry Weight Kjeldahl N	Digestion / Colourimetry
	Dry Weight P	Digestion / Colourimetry
	Dry Weight Fe	Aqua Regia extraction / ICPOES

#### 2.1.4 BOAT TRAFFIC DATA

Typical annual boat densities were obtained from British Waterways for all the canals to be surveyed (Table 2.5). Murphy and Eaton (1983) found that there was a critical level above which the macrophyte community was affected. They observed differences in the emergent macrophytes present in canals and linked these to recreational boating densities. Areas of highest boating activities (above 4000 movements per year) frequently featured no emergent plants, while in areas of low

boating activity (less than 2000 movements) an extensive macrophyte cover prevailed. Between these two levels (2000-4000 movements) was the critical level.

**Table 2.5** Typical annual boat densities

Waterway	Sites	Boat Movements per Year
Tame Valley	All	500-1000
Coventry	All	3000-4000
Shropshire Union	Pendeford Br	11000-12000
Staffs & Worcs	Coven Heath	8000-10000
	Oxley/Junct B'ham Canal	10000-12000
	Swindon Br/Worcs Rd	5000-7000
Wyrley & Essington	All	1000-2000
Cannock Extn	All	c. 2000
Walsall	All	0-500
Birmingham New Main Line	All	4000-5000
Birmingham Old Main Line	All	500-1000
Worcs & Birmingham	All	3000-4000
Stratford	All	5000-6000
Dudley	All	2000-3000
Titford	All	500-1000
Gower Branch	All	500-1000
Soho Loop	All	u/k
Grand Union	Warwick/Lapworth	4000-6000
	Catherine de Barnes/Nechells	2000-3000
Birmingham & Fazeley	All	3000-4000

## 2.2 ANALYTICAL TECHNIQUES

A range of univariate and multivariate statistical techniques were used to analyse the results. The analyses are necessarily complex and the techniques selected differ across the chapters and the output is used in the next chapter (Fig. 2.2). In Chapter 3 *Pearson product moment correlation coefficients* were first constructed to establish relationships between concentrations of chemical variables. This was followed by *Factor Analysis* (Meglen, 1992; Qu and Kelderman, 2001) to identify factors that may be controlling these correlations. *Hierarchical cluster analysis* (Sneath and Sokal, 1973) was then carried out to identify groups of sites with similarities of underlying numerical structure within the metals data. To investigate how chironomid taxa varied with metal concentration, a measure of total metal concentration and toxicity was used: the *cumulative criterion unit* (CCU) (Clements et al., 2000).

The first three techniques were also used in Chapter 4. In addition, *discriminant analysis* was used to assess the percentage of sites that had been correctly highlighted by cluster analysis. *ERM* (effects range median) values, the median metal concentration associated with toxic effects, were used to calculate mean ERM quotients (*MERM-Q*) (MacDonald et al., 1996). This is a sediment quality guideline for assessing the toxicity potential of complex mixtures in contaminated sediment. For each metal, the observed concentration was compared to the ERM. A toxic unit index (*TUI*) (Ingersoll et al., 1996), was constructed by adding the ratios for each metal. *Contamination factors (CF)* were calculated as the ratio between observed metal content and a reference site. From this a *Metal Pollution Index (MPI)* was calculated, by taking the linear sum of the concentration factors together with a weighted value that takes into account the differences in the toxicity of the various metals. Linear regression analysis was also carried out to show the sensitivity of each metal to variations in MPI.

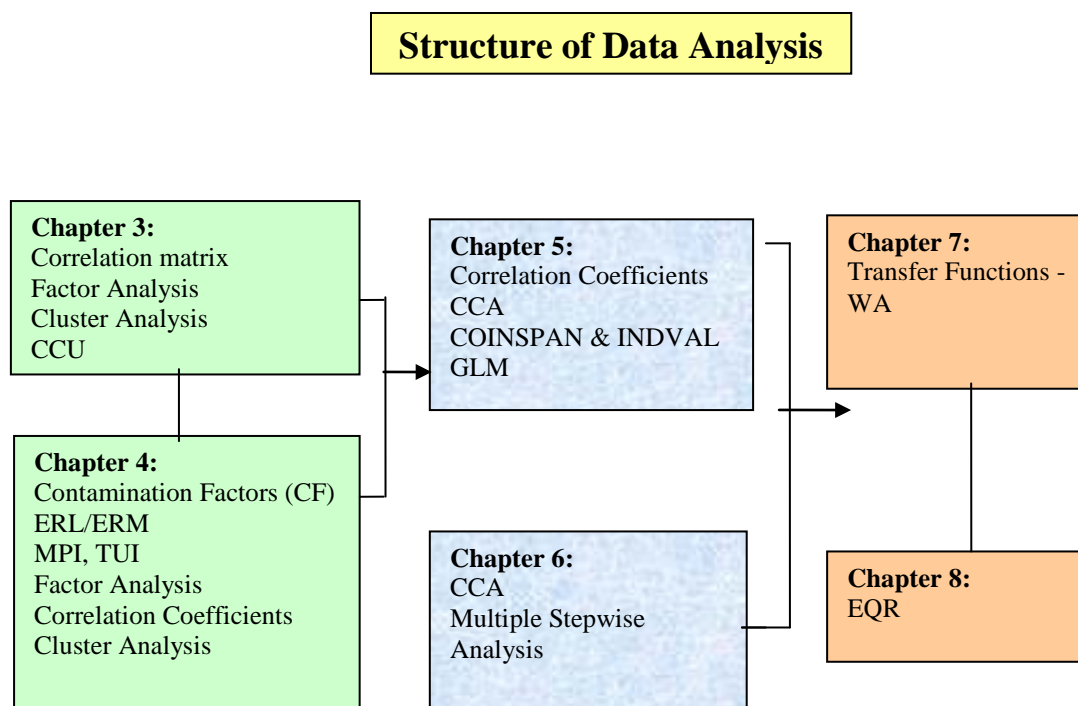
The focus of Chapter 5 is the response of species to the environmental variables. Relationships between environmental and multivariate space for selected species were also interrogated using *Generalized Linear Modelling (GLM)* (McCullagh and Nelder, 1983) . The relationships between the environmental variables and exuviae assemblage data were revealed by *Canonical Correspondence Analysis (CCA)* (ter Braak, 1986, 1987). A classification of sites constrained to significant environmental variables was carried out using *COINSPAN* The hierarchical classifications produced by COINSPAN (Carleton, 1996) were used with the original species data in a further analysis, using *INDVAL* (Dufrêne and Legendre, 1997). This was used to produce indicator assemblages that characterised groups of sites produced by COINSPAN. Regression analysis was performed in order to determine if chironomid species respond to metal pollution and if the CCU approach discussed in Chapter 3 provides a useful method for evaluating metal toxicity in canals.

Chapter 6, dealing with functional ecology, also uses *CCA* and *multiple stepwise regressions*. The relationship between functional feeding groups and metals were investigated using Pearson's correlation coefficients and regression analysis. Pupal exuviae were ordinated against chemical variables and key taxa used to establish

whether there were significant differences in body length within species at different sites. Ordination of size classes against environmental variables were used to test whether metals were significant in determining distribution of body size.

In Chapter 7, transfer functions using *Weighted Average (WA) calibrations* (Birks et al., 1990) were used to produce inference models and model testing. To test the ability of selected environmental variables to infer water chemistry from new sites, a selection of weighted averaging tests (transfer functions), were performed on the forty-six sample sites. Weighted averaging, weighted averaging partial least squares and partial least squares regression were performed on the dataset. Both unimodal weighted averaging (WA) regression and calibration models were tested and their predictive capabilities compared.

Chapter 8 focuses the application of the WA models and their use to create a robust monitoring tool. *Ecological Quality Ratios (EQR)* using weighted-averaging formulae were used to calculate biotic scores for each significant variable highlighted in the CCA (WFD, 2000; Ruse, 2002, 2007a,b).



**Figure 2.2** Flow diagram outlining structure of the data analysis through the project

## **CHAPTER 3**

# **CHARACTERISING HEAVY METAL CONCENTRATIONS IN THE WATER OF URBAN CANALS IN THE WEST MIDLANDS**

### **APPROACH**

Concentrations of heavy metals at 46 canal sites in the West Midlands were characterised by applying a Critical Criterion Unit (CCU). Patterns of metal contamination were determined by cluster analysis on the basis of CCU. The results show that the CCU can be used as a proxy for individual metal concentrations and that the combined measure of toxicity can be used successively to classify canals.

### **3.1 INTRODUCTION**

Water bodies within terrestrial environments (rivers, reservoirs, canals) serve a wide range of purposes, including domestic, leisure and wildlife. However, particularly in urban areas, water quality is generally poor as a direct result of past and present input of polluted waste. Within these polluted water systems problems of major concern are the development of water column anoxia, gas generation (the production of CH<sub>4</sub> and H<sub>2</sub>S gases) and the mobilisation of toxic chemicals (metals and organics) into the water column (Boult and Rebbeck, 1999). These may arise from the presence of contaminated sediment beneath the water column. Many polluted sediment-water systems have accumulated sediment that is both highly reactive, due to enhanced levels of sewage, and highly contaminated by industrial wastes (Boyd et al., 1999). Therefore, in these systems sediment-pore-water interactions will be critical in

determining the composition and quality of the water column. Controlling the release of toxic species are organic matter oxidation reactions occurring within the sediment. These reactions may act as both a sink and a source of solutes (Canfield et al., 1993). These reactions also indirectly release species associated with redox sensitive compounds such as metals present within organic compounds, nutrients and metals adsorbed onto iron oxides (Boyd et al., 1999).

One of the main operational issues in all canal systems is maximising the potential for the sustainable use of canal water in terms of local amenity, ecological value, fishing and industrial use (WFD, 2000). In order to meet these objectives, therefore, water quality in the canal must be maintained to as high a standard as possible. Water quality problems are often complex within canals, especially those within an urban context, as they incorporate metals, nutrients, as well as other organic compounds. The large sediment component that is also present acts as a chemical sink, which can itself be a pollution source when released by, for example, re-suspension due to boat traffic (Murphy et al., 1995).

Contaminants in urban catchments are a major cause of water quality impairment (Perdikaki and Mason, 1999; Sriyaraj and Shutes, 2001; Wagner and Geiger, 1996) and are derived from indirect sources such as surface runoff as well as some direct discharges from industry (Paul and Meyer, 2001; Gurnell et al., 2007; Egodawatta et al., 2009). Routine chemical monitoring only detects a limited range of determinands. To understand more fully the physical-ecological links a wider suite of chemical variables needs to be measured.

The Environment Agency (EA) uses General Quality Assessment (GQA) to assess the state of rivers and canals and to show how the quality has changed over time. The chemistry GQA scheme is based on dissolved oxygen (DO), biochemical oxygen demand (BOD) and ammonia, which act as indicators of organic pollution (Environment Agency, 2003). Additional variables are sometimes added to this basic suite and they can be grouped into the following broad categories:

- Basic variables (temperature, pH, conductivity, dissolved oxygen) that are used for a general characterisation of water quality;
- Suspended particulate matter (suspended solids, turbidity, organic matter (Total Organic Carbon (TOC), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD));
- Eutrophication indicators (nutrients (nitrogen and phosphorous), and various biological effect variables (chlorophyll a, Secchi disc transparency);
- Indicators of acidification (pH, alkalinity, conductivity);
- Metals at a small number of certain sites;
- Organic micropollutants such as pesticides.

From this it can be seen that there is a gap in our monitoring of metal distribution in water bodies especially canals. Urban canals can contain metals at elevated concentrations. A number of studies have been carried out on water bodies that receive elevated levels of metal and their effects on macroinvertebrates (Clements, 1991; Kifney, 1996; Clements et al., 2000; Hirst et al., 2002). A further complicating factor is that there is likely to be a variety of metals impacting at a site, so a cumulative measure of total metal concentration may be required.

### **3.1.1 CHARACTERISING HEAVY METAL CONCENTRATIONS**

The realisation that there can be additive chronic toxicity of metal mixtures in aquatic systems (Enserink et al. 1991) led to the development of the cumulative criterion unit (CCU), defined as a measure of total metal concentration and toxicity (Clements et al. 2000). This is calculated for individual sites and is based on the U.S. Environmental Protection Agency criterion continuous concentration (CCC) values for toxicity (EPA 1986) that derive from US EPA guidelines on critical concentrations, which when exceeded may harm aquatic life. Its usefulness lies in expressing the additive effects of each metal relative to postulated toxic thresholds as a single variable. So far this CCU has not been tested on UK canals, although Hirst et al. (2002) applied this in UK

rivers and noted that diatom and macroinvertebrate communities reacted in a similar manner to metal pollution and compared the responses to US rivers. Similarly, a more recent study (Robson et al., 2006) incorporated the CCU measure into his study of urban rivers to assess whether it could indicate metal toxicity in relation to macroinvertebrate assemblages.

This technique has yet to be applied to canals, and there is a pressing need because of their complex chemical nature, especially in urban areas. Therefore, the main aims of this chapter are to:

- (i) Characterise heavy metal concentrations;
- (ii) Use these values to classify the toxicity gradient.

## **3.2 MATERIALS AND METHODS**

### **3.2.1 SAMPLING**

Chemical data for this study were collected from two sources: i) Environment Agency routine monitoring data and (ii) supplementary water samples collected in the field at all sites for different determinands. Each routine monitoring site will have different requirements as to what is collected. A list was therefore made of likely determinands needed for the study and supplementary samples taken of those determinands not collected by the EA. A range of metal determinands was taken to reflect the nature of urban canals. Samples were collected six times over the course of a sample year. Table 2.4 shows the analytical methods used to obtain the chemical constituents of the water samples. The basic chemical grades given by the Environment Agency to rivers and canals are defined by standards for the concentrations of Biochemical Oxygen Demand (BOD), ammonia and dissolved oxygen (DO).

### **3.2.2 NUMERICAL CLASSIFICATION AND ANALYSIS OF WATER SAMPLES**

A pearson product moment correlation matrix was constructed to show the relationship between concentrations of chemical determinands within the water column. To identify factors that may possibly control these correlations, the best linear association of the associations among the variables were determined by Factor Analysis (FA). In FA varimax rotation, maximizes variance of squared loadings within factors (i.e. simplifies the columns of the loading matrix). This method therefore attempts to make the loadings either large or small to ease visual interpretation (Minitab Inc., 2003). Rotation places the factors into positions where only variables distinctly related to a factor will be associated. Previous work (Meglen, 1992; Qu and Kelderman, 2001) indicated that factors that either correspond to a cumulative percentage of 85-95% and variables with loadings greater than 0.7 should be chosen.

Cluster analysis was carried out to identify groups of sites with similarities of underlying numerical structure within the metals data, by means of CCU. A quantitative Euclidian distance measure with Ward's complete linkage method was utilized to identify similarities and cluster the data.

### **3.2.3 CHARACTERISATION OF HEAVY METAL CONCENTRATIONS**

The cumulative criterion unit (CCU), which is a measure of total metal concentration and toxicity, was used to investigate toxicity gradients within the canals of the West Midlands. From the metal concentrations obtained, the CCU was calculated for each site, as follows:

$$CCU = \sum m_i / c_i$$

Where  $m_i$  is the total recoverable metal concentration and  $c_i$  is the criterion value for the  $i$ th metal. The criterion value is the critical concentration at which when exceeded

becomes harmful to life. For Fe, a criterion level of 1000  $\mu\text{gL}^{-1}$  was adopted, following the USEPA (1986) chronic criterion values. Water hardness affects the toxicity and bioavailability of certain metals, so criterion levels for Cd, Cu, Pb, Zn, Cr and Ni vary and were modified as follows:

$$C_i = \exp\{m_A[\ln(\text{hardness})] + b_A\} (\text{CF})$$

where CF is a freshwater conversion factor (Table 3.1).

The criteria levels were based on maximum concentrations (MC), which is an estimate of the highest concentrations of a material in surface waters to which an aquatic community can be exposed briefly without resulting in any detrimental effects (Robson et al., 2006).

The relative percentage contribution of each metal to the CCU values was also calculated to evaluate which metals might be contributing most to toxicity. This was calculated for all sites combined, as well as for each site individually.

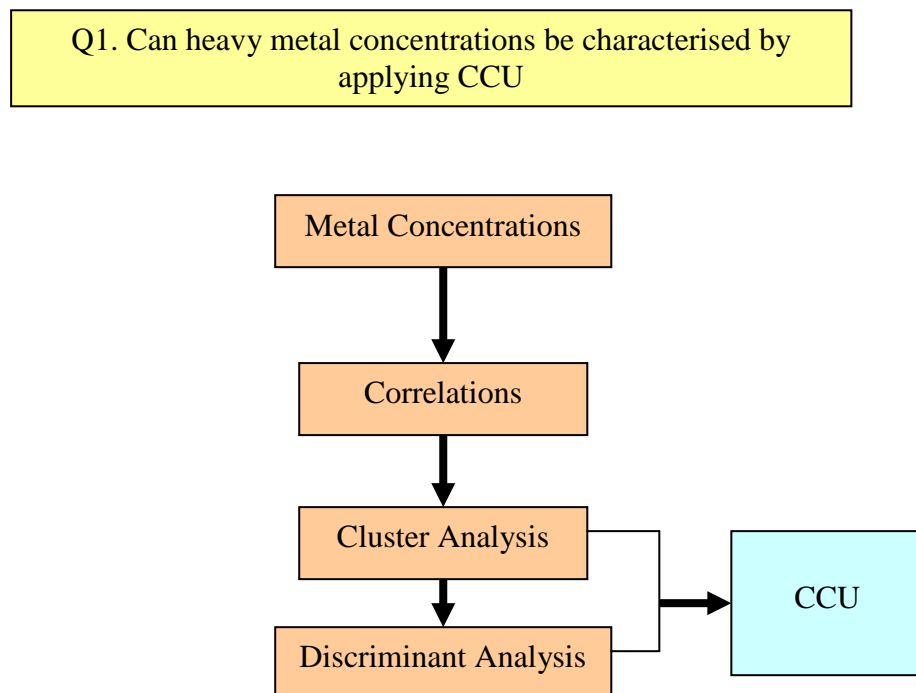
**Table 3.1** Parameters for calculating freshwater dissolved metals criteria that are hardness-dependent

Chemical	$m_A$	$b_A$	Conversion Factor (CF)
Cadmium	1.0166	-3.924	$1.136672 - [(\ln \text{hardness})(0.041838)]$
Chromium	0.819	3.7256	0.316
Copper	0.9422	-1.7	0.96
Lead	1.273	-1.46	$1.46203 - [(\ln \text{hardness})(0.145712)]$
nickel	0.846	2.255	0.998
zinc	0.8473	0.884	0.978

$$\text{Criteria} = \exp\{m_A[\ln(\text{hardness})] + b_A\}(\text{CF})$$

Unpolluted sites, where no effects on aquatic organisms would be expected, have been defined as having CCU values less than 1.0 (Clements et al., 2000). The CCU level of 1.0 would represent the point, if metals are additive, where an adverse effect on the biota should be evident. A low-metal category has arbitrarily been defined as

between 1.0 and 2.0; a medium category defined as having CCU levels between 2.0 and 10.0. This was selected because benthic communities are likely to be severely affected by metal levels 2-10 times the criterion level. Highly polluted sites would have CCU levels above 10.0 (Clements et al., 2000).

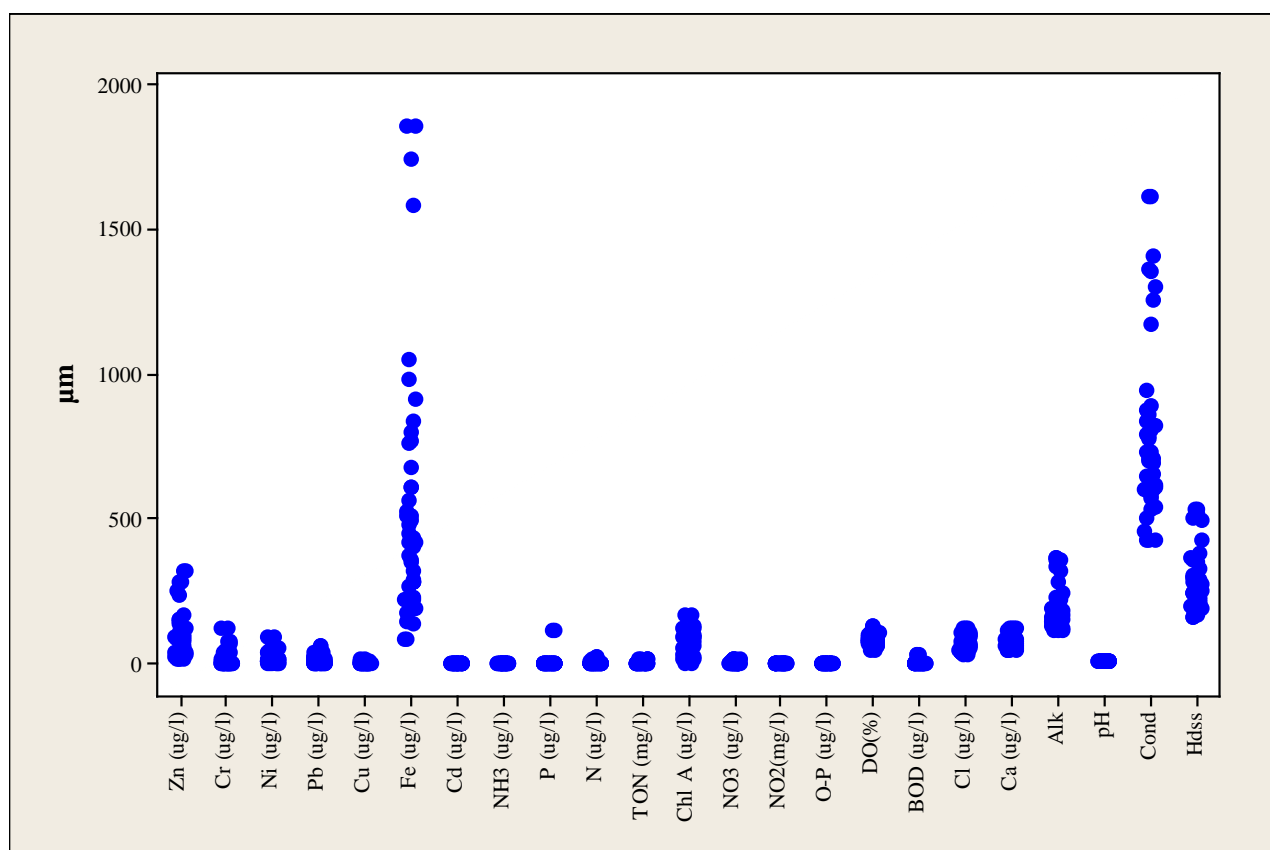


**Figure 3.1** Analysis route for the analysis of water chemistry

### 3.3 RESULTS

#### 3.3.1 PHYSICOCHEMICAL CHARACTERISTICS

Mean annual chemical measurements for the 46 canal sites are provided in Table 2.2, illustrating the variation among the sites. For example, iron ranged from 86( $\mu\text{m}$ ) to 1857( $\mu\text{m}$ ); total P from 0.05 to 118.72; Zn from 16.87 to 317.83; BOD from 1.63 to 31.86 (Fig. 3.2).



**Figure 3.2** Plot to show variation in chemical measurements

### 3.3.2 CORRELATIONS BETWEEN VARIABLES

**Table 3.2** Correlation coefficients

	Zn	Cr	Ni	Pb	Cu	Fe	Cd	NH3	P	N	TON	Chl A	NO3	NO2
Cr	-0.73													
Ni	-0.69	0.62												
Pb	0.51	-0.60	-0.64											
Cu	0.72	-0.66	-0.70	0.44										
Fe				0.60										
Cd	-0.53	0.53	0.55	-0.58	-0.48									
NH3														
P		0.33												
N		0.47							0.39					
TON		-0.38				-0.44			-0.37	-0.86				
Chl A				0.52		0.66	-0.40			-0.36	-0.58			
NO3		-0.38			0.36	-0.41			-0.29	-0.82	0.94	-0.57		
NO2		-0.37						-0.29	-0.49	-0.80	0.75		0.65	
O-P				0.33										
DO														
BOD						-0.30	0.34							
Cl														
Ca						-0.32								
Alk		-0.34	-0.45	0.39	0.46	0.37								
PH														
Cond						0.38								
Hdss			-0.33		0.41	0.32								

Table 3.2 shows the significant correlations between variables ( $p < 0.05$ ). It can be seen that the metals are generally intercorrelated. The exception was iron, which was only positively correlated with lead. Chromium was positively correlated with phosphorous and nitrogen as well as negatively correlated with both nitrate and nitrite. Nutrient variables were also generally intercorrelated, although orphosphosphate was only positively correlated with lead. Alkalinity was correlated with the metals (Cr and Ni, (-ve); Pb, Cu and Fe (+ve)) apart from zinc and cadmium.

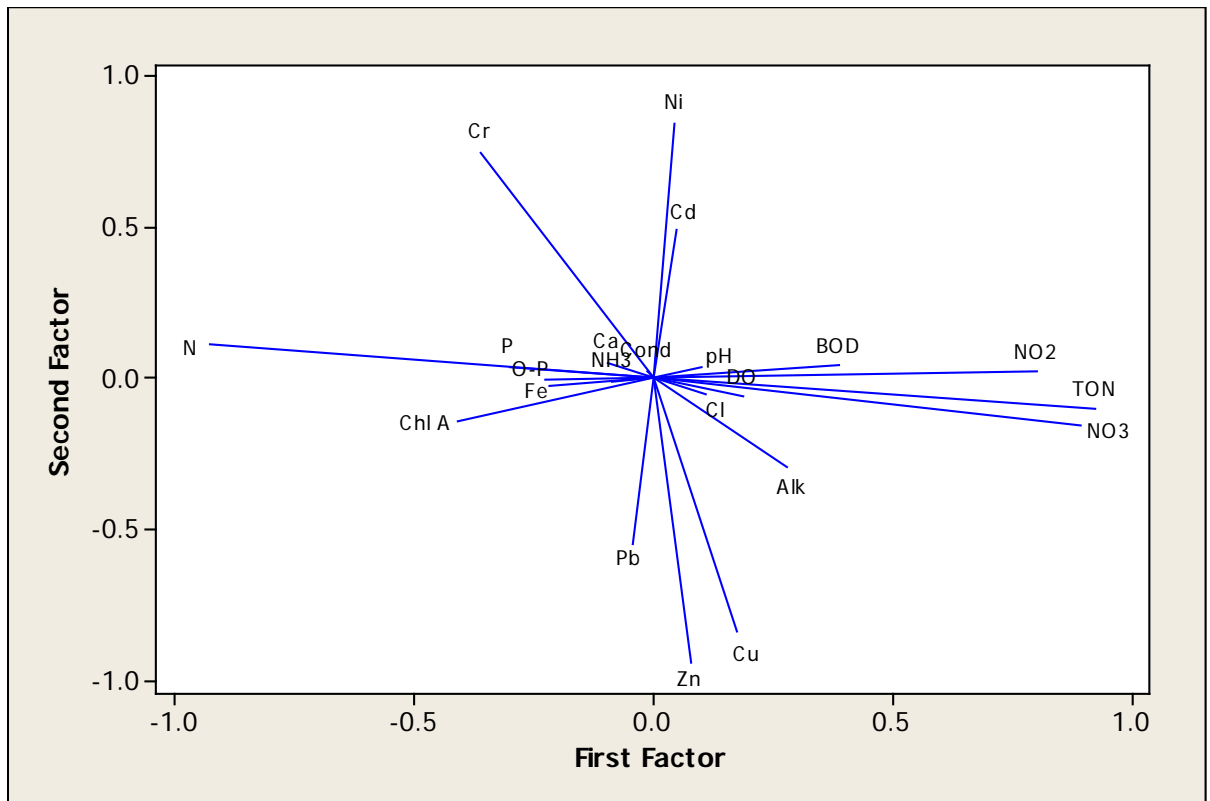
### 3.3.3 FACTOR ANALYSIS

Factor Analysis identified 8 significant factors with eigenvalues greater than 1.0, explaining about 83% of the total variance in the data. Factor 1 is clearly related to nutrients. Sites associated with the positive loadings of  $\text{NO}_2$ ,  $\text{NO}_3$  and TON included SW-OX, SW-JN, SW-CB and SU-PB (Table 2.1); Sites associated with high negative loadings of N included BL-PL. Factor 2 is related to inorganic elements and Factor 3 to metals (Table 3.3; Figs. 3.3 & 3.4). A second analysis of only metals found 5 significant factors with eigenvalues greater than 1.0, explaining 78% of the total variance in the data. Factors 1-3 (explaining 49% of the variance) show high negative loadings for iron, cadmium and chromium respectively (Table 3.4; Fig. 3.5 & 3.6).

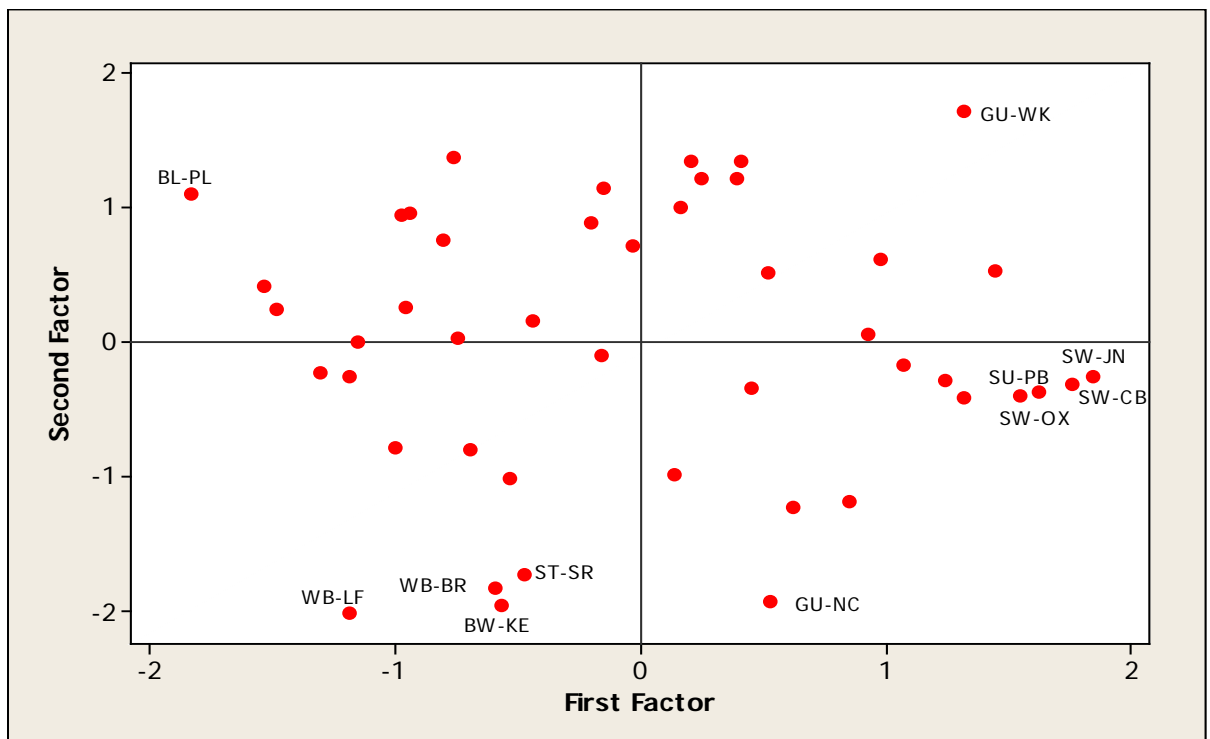
Factor 1 was related to iron and sites associated with low values included WB-LF, ST-SR, WS-RG and WS-MM. Factor 2 was associated with cadmium and sites with low values included CV-FX and ST-HH.

**Table 3.3** Eigenvalues, cumulative % of variance and variables with absolute loading values >0.7(+/-) in the factor analysis of 46 canal water samples

2	3.5601	34.3	Zn, Cr, Ni, Cu
3	3.0371	48.1	Ca, Alk, Cond
4	1.8068	56.3	Fe
5	1.7338	64.2	DO, pH
6	1.6852	71.9	O-P
7	1.2015	77.4	BOD
8	1.1182	82.5	NH <sub>3</sub>
9	0.8977	86.6	Cd
10	0.7835	90.2	P



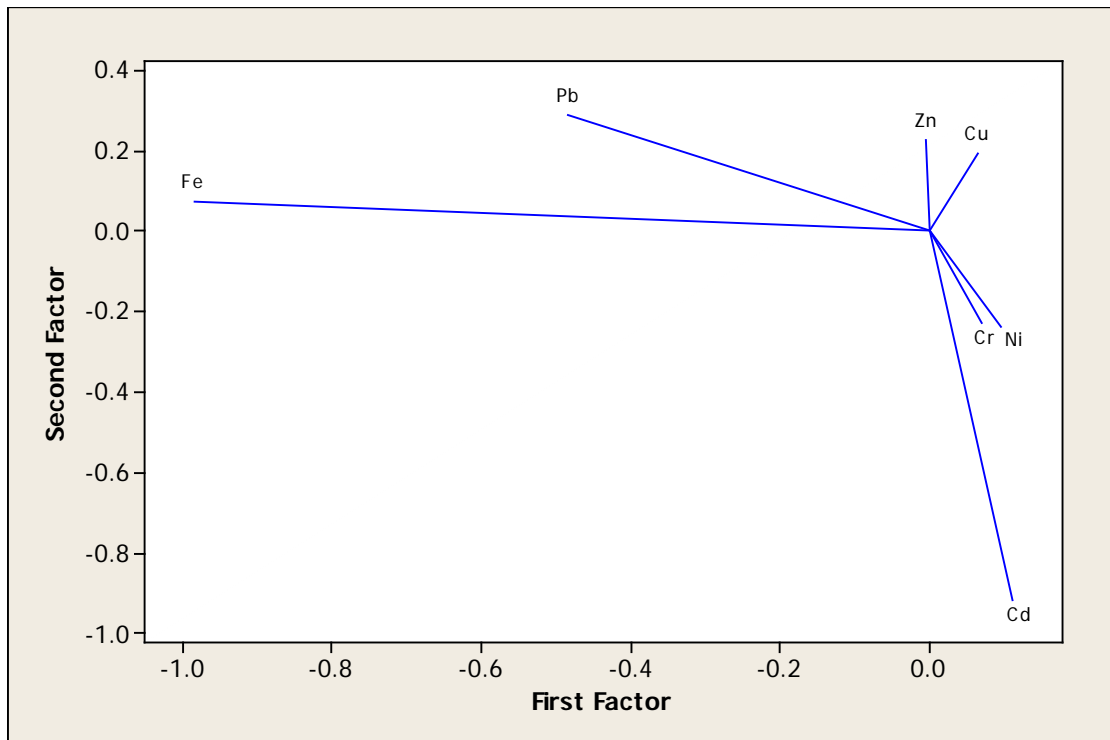
**Figure 3.3** Loading plots (all variables)



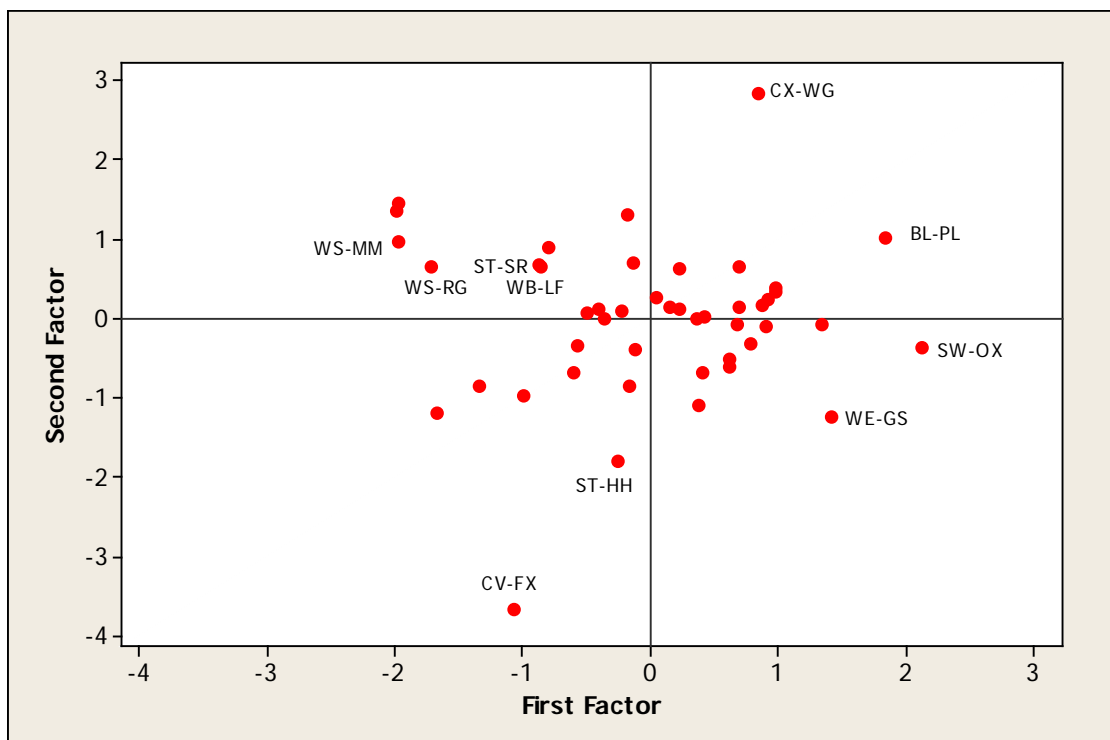
**Figure 3.4** Score plot (all variables)

**Table 3.4** Eigenvalues, cumulative % of variance and metalvariables with absolute loading values >0.7(+/-) in the factor analysis of 46 canal water samples

Factor	Eigenvalue	Cumulative % of variance	Variables with loading >0.7(+/-)
1	1.2363	17.7	Fe
2	1.1293	33.8	Cd
3	1.0483	48.8	Cr
4	1.0398	63.7	Cu
5	1.0119	78.2	Ni
6	0.9332	91.5	Zn



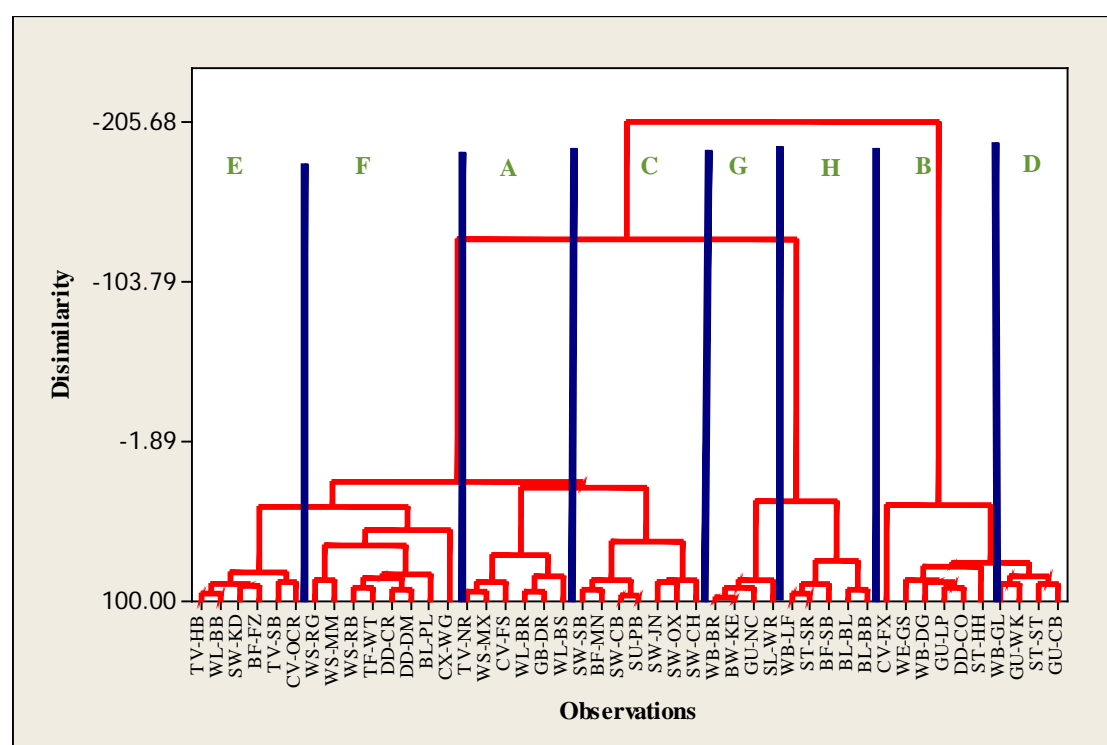
**Figure 3.5** Loading plot (metals)



**Figure 3.6** Score plot (metals)

### 3.3.4 CLUSTER ANALYSIS

A cluster analysis was carried out on the combined effects of metals and groups were assigned on the basis of CCU measurements to allow for the combined measure of toxicity (Fig. 3.7). Table 3.5 summarises mean values of individual metal concentrations and CCU.



**Figure 3.7** Dendrogram showing distribution of metals within the water

**Table 3.5** Mean values of metal concentrations and CCU

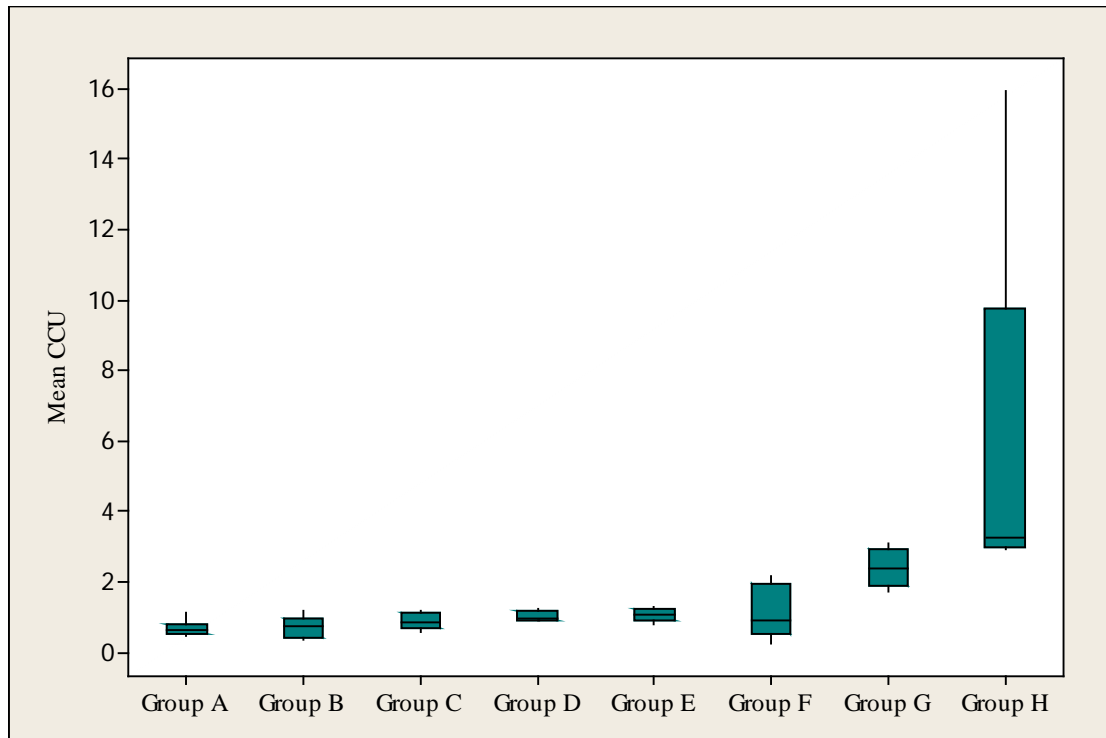
	Cu		Zn		Cd		Pb		Cr		Ni		Fe		CCU	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Group A	2.66	0.65	61.05	47.78	0.16	0.06	4.81	2.35	1.22	0.25	6.16	0.72	280.63	98.64	0.69	0.23
Group B	3.43	2.12	51.46	45.76	0.09	0.01	5.20	1.41	1.45	1.29	13.94	3.61	496.17	388.1	0.74	0.29
Group C	5.08	0.78	95.37	28.16	0.24	0.07	4.10	2.29	36.89	44.60	10.46	2.63	172.57	50.92	0.89	0.21
Group D	3.14	0.89	27.57	6.29	0.11	0.05	5.21	1.81	1.42	0.43	7.35	1.15	621.5	233.86	1.03	0.16
Group E	4.04	1.51	93.80	46.20	0.22	0.04	14.30	4.53	3.30	1.68	20.31	14.99	434.97	159.92	1.07	0.18
Group F	3.76	1.62	64.87	30.90	1.20	1.93	7.22	2.70	2.19	1.30	12.17	3.76	670.83	535.42	1.12	0.60
Group G	12.07	3.17	221.57	76.43	0.43	0.06	21.19	2.89	31.36	16.36	37.73	8.54	380	85.78	1.75	0.54
Group H	7.49	3.71	196.52	99.70	1.54	0.43	47.09	9.59	50.56	32.81	42.89	34.35	1199	549.09	5.74	5.70

**Table 3.6** Indices and group membership

	<b>CCU</b>	<b>Group</b>
TV-HB	1.23	E
TV-NR	0.67	A
TV-SB	0.97	E
CV-FX	0.91	B
CV-OCR	0.94	E
CV-FS	0.53	A
WS-RG	2.16	F
WS-MM	2.1	F
WS-MX	0.73	A
WS-RB	0.79	F
WE-GS	0.38	B
CX-WG	1.52	F
SW-KD	0.83	E
SW-SB	0.88	C
SW-CB	0.9	C
SW-JN	0.77	C
SW-OX	0.72	C
SW-CH	0.61	C
SU-PB	1.14	C
WB-BR	2.3	G
WB-GL	0.91	D
WB-DG	0.73	B
WB-LF	3.55	H
ST-SR	2.93	H
ST-HH	1.19	B
ST-ST	1.24	D
BL-BL	3.29	H
BL-PL	0.27	F
BL-BB	3.01	H
WL-BR	0.6	A
WL-BS	1.12	A
WL-BB	1.15	E
BW-KE	2.44	G
BF-SB	15.93	H
BF-ML	1.19	C
BF-FZ	1.29	E
GU-NC	3.08	G
GU-CB	1.08	D
GU-WK	0.89	D
GU-LP	0.73	B
SL-WR	1.76	G
TF-WR	1.06	F
DD-CO	0.47	B
DD-CR	0.5	F
DD-DM	0.59	F
GB-DR	0.49	A

### 3.3.5 CHARACTERISATION OF HEAVY METAL CONCENTRATIONS

Heavy-metal concentrations as expressed as cumulative criterion units (CCU) differed greatly among the 46 sites and ranged from 0.28 to 15.93 (Table 3.6). The CCU was less than 2.0 at the majority of sites (78%) and only one site exceeded 10.0. Mean CCU values between groups (Fig. 3.8 and Table 3.6) are shown in Fig. 3.9.



**Figure 3.8** Mean CCU values found between groups

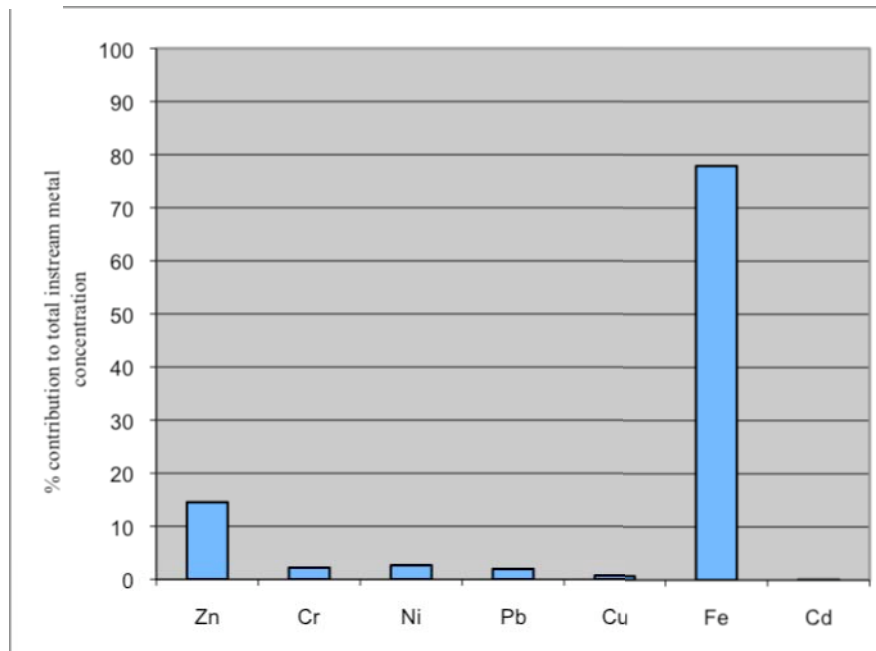
As can be seen, there is little difference between groups A-E with low mean CCU levels, but mean CCU increased in groups F-H, illustrating the presence of a gradient of pollution within the conurbation.

Mean annual metal measurements for the 46 canal sites are provided in Table 2.2. Iron had the highest absolute concentration in the canals, but its contribution to the CCU was considerably lessened once criterion values and the effects of water hardness on bioavailability were taken into account (Fig. 3.9a & b). Zinc and chromium together had significant contribution to CCU alongside iron, water hardness effects and criterion values were taken into account.

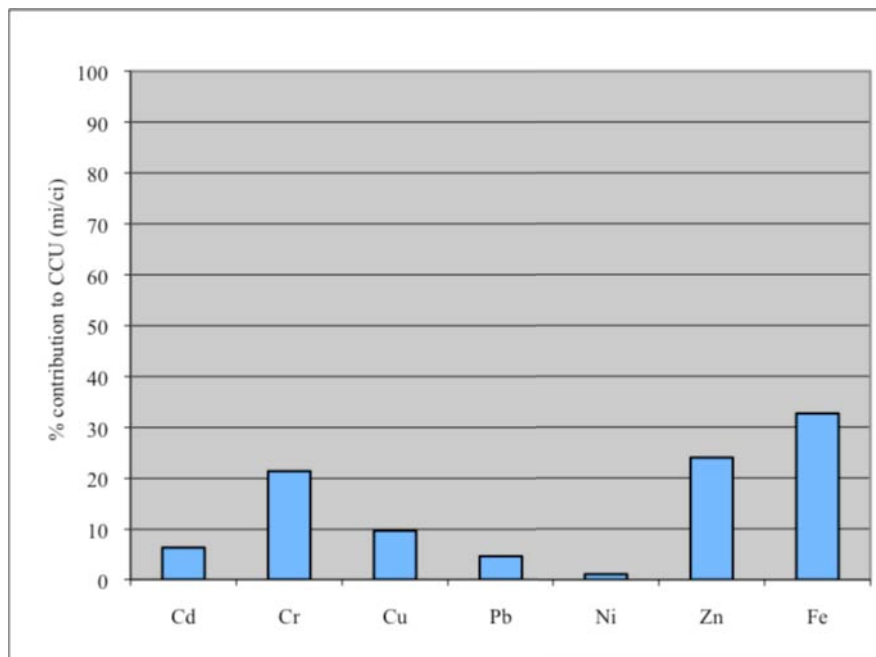
Figures 3.10 and 3.11 illustrate sites with high and low CCU values. For the high CCU value sites, some differences are shown at different sites. For example, at site BF-SB, chromium accounts for about 85% of the CCU, but at sites WB-LF and BL-B, chromium accounts for less than 5% at each site. At WB-LF, zinc accounts for the greatest contribution to CCU (42%), followed by iron (21%) and copper (14%). However, at all these sites, iron makes up the greatest contribution to total instream metal concentrations: WB-SB (68%), WB-LF (60%), and B-BL (88%). Zinc, the next significant source of instream metal concentration, accounts for 11%, 25% and 8%.

For the low CCU sites, at all three sites selected zinc and iron accounted for 86%, 86%, and 65% of CCU at GB-DR, DD-CO and BL-PL. These two metals also contributed 96%, 96% and 91% of total metal concentrations.

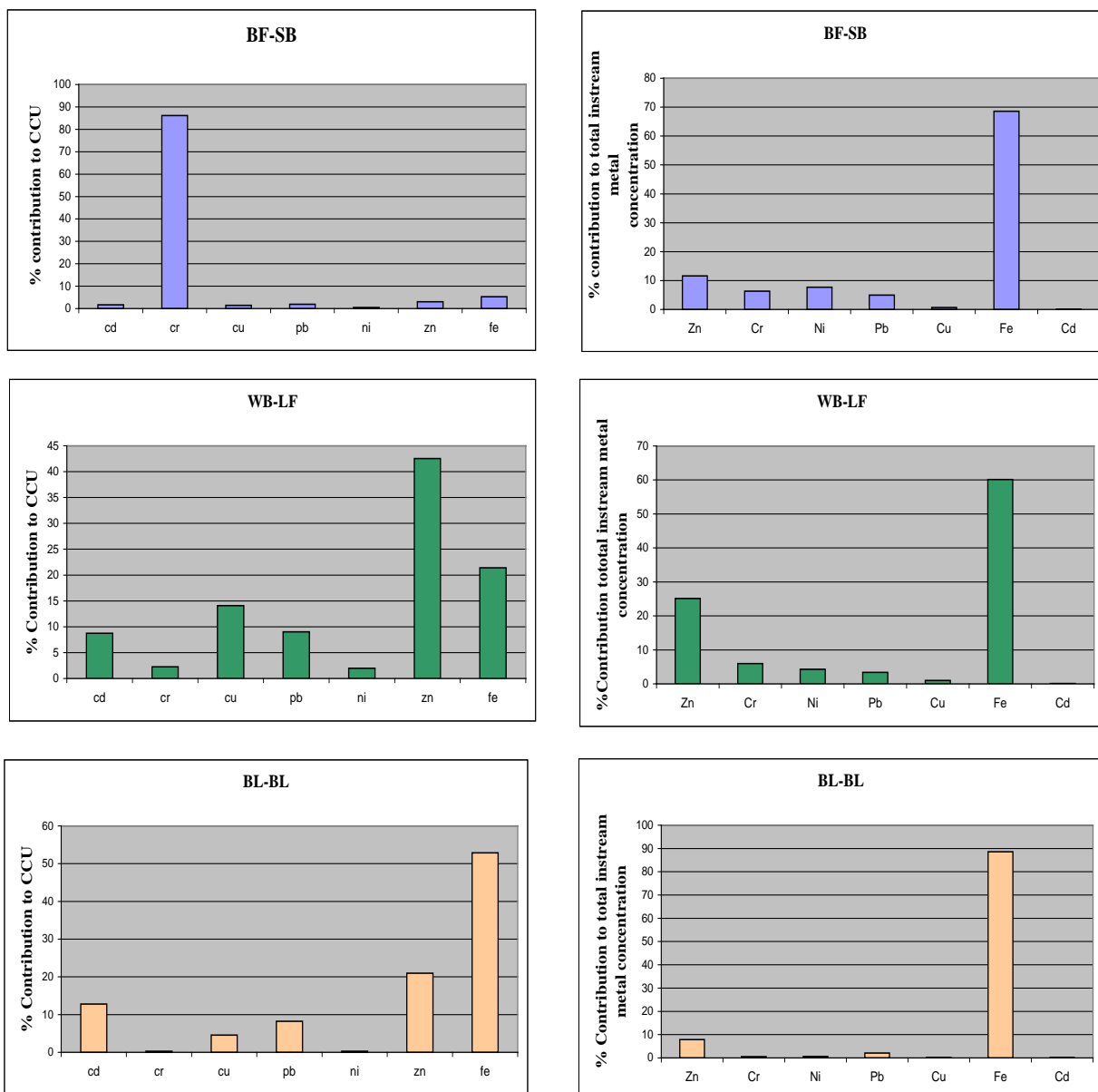
(a)



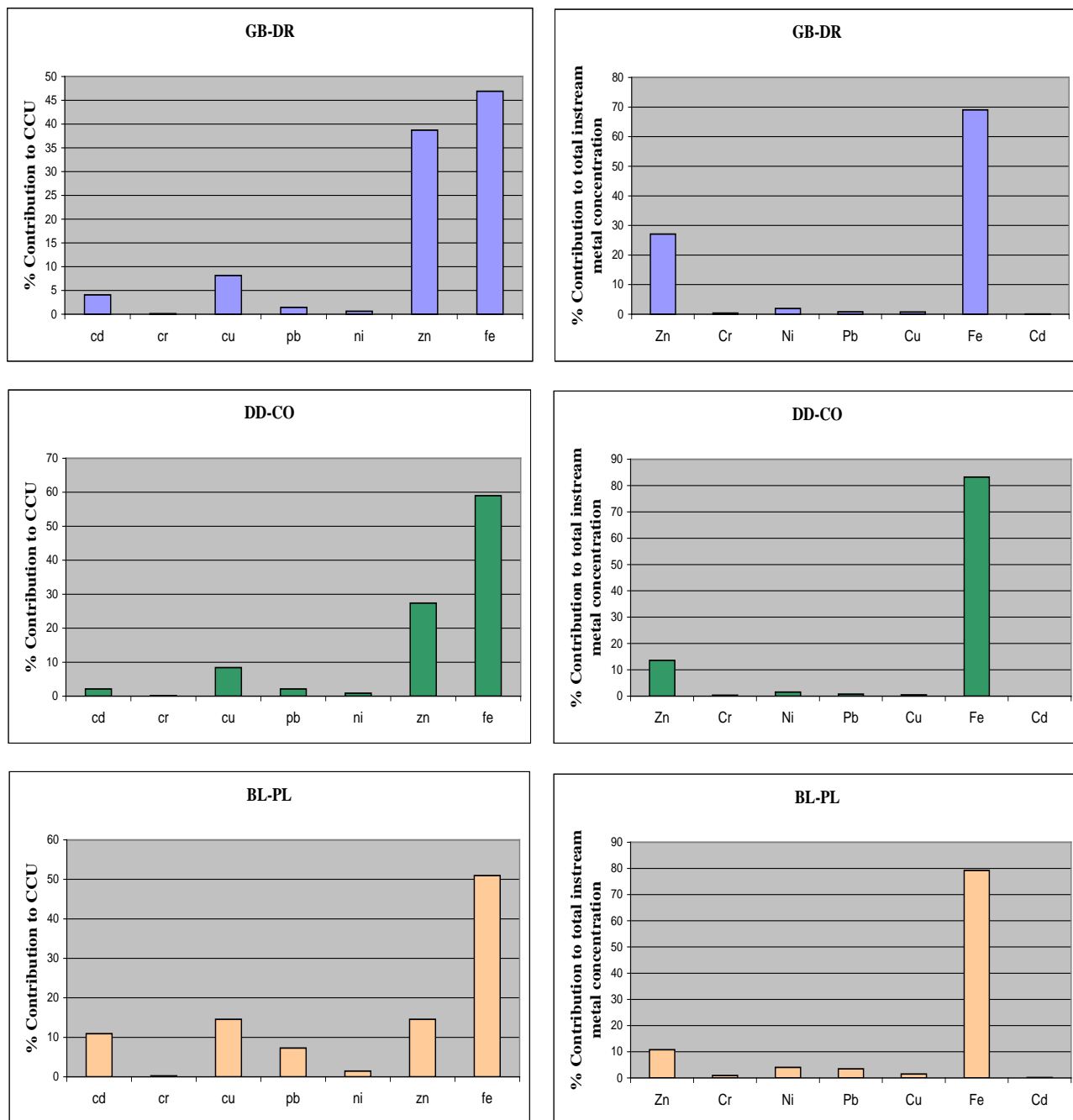
(b)



**Figure 3.9** The relative contribution of each metal to the total metal loadings of canal sites (a) and as the ratio of metal concentration to criterion value, mi/ci (b)



**Figure 3.10** The relative contribution of metals to total metal concentrations and to CCU in sites with high CCU values



**Figure 3.11** The relative contribution of metals to total metal concentrations and to CCU in sites with low CCU values

### 3.4 DISCUSSION

The concentrations of dissolved Cd, Cr, Cu, Ni, Pb, Fe and Zn, exhibit distributions that reflect their input and show wide variation in values. For example Pb ranged from  $1.73 \mu\text{g l}^{-1}$  to  $61.02 \mu\text{g l}^{-1}$ ; Cr from  $0.68 \mu\text{g l}^{-1}$  to  $120.47 \mu\text{g l}^{-1}$ ; Zn from  $16.83 \mu\text{g l}^{-1}$  to  $317.83 \mu\text{g l}^{-1}$ ; Cd from  $0.04 \mu\text{g l}^{-1}$  to  $5.14 \mu\text{g l}^{-1}$ ; Fe from  $82.46 \mu\text{g l}^{-1}$  to  $1857 \mu\text{g l}^{-1}$ ; Cu from  $1.62 \mu\text{g l}^{-1}$  to  $15.77 \mu\text{g l}^{-1}$ . Industrial inputs are the likely causes of high dissolved metals, either present or historic. Automobile exhausts may likely be another source of lead as well as urban storm run-off. Several of the sites were located close to major road routes (e.g. M6 motorway). Cadmium is commonly used in electroplating and battery manufacture. Zinc is primarily used for galvanizing iron and steel products and in Zn-based alloys for die-casting. All these industries are present in the Birmingham area. The West Midlands has a long history of motor vehicle assembly together with linked industries (Paul and Meyer, 2001; Bernhardt and Palmer, 2007; Hutchinson and Rothwell, 2008). For example, in West Bromwich, workshops produced laminated and coil-springs and in Wolverhampton, there were metal-working workshops that specialised in weldless steel tubes. Manufacturers that produced wheels were mainly located in Dudley and Bilston (Gerard and Slater, 1996).

#### 3.4.1 CLASSIFYING A TOXICITY GRADIENT

The use of CCU to cluster sites was successful, in that sites clustered according to their pollution levels showing a strong spatial gradient across the conurbation. For example, CV-OCR was in Group E. This site has had historic problems with metals and has elevated levels of iron and lead. Most of the higher value CCU groups could be found within the central Birmingham area (Fig. 3.12). It can also be seen that similar CCU groups are concentrated together. Group H sites are mainly located around the centre, together with Group G in southern parts of Birmingham; Groups A and F are located towards the west of the area; Group C sites are generally arranged along the Staffordshire/Worcester Canal from Wolverhampton and Groups B and D are mainly located away from the main urban centres in rural locations.

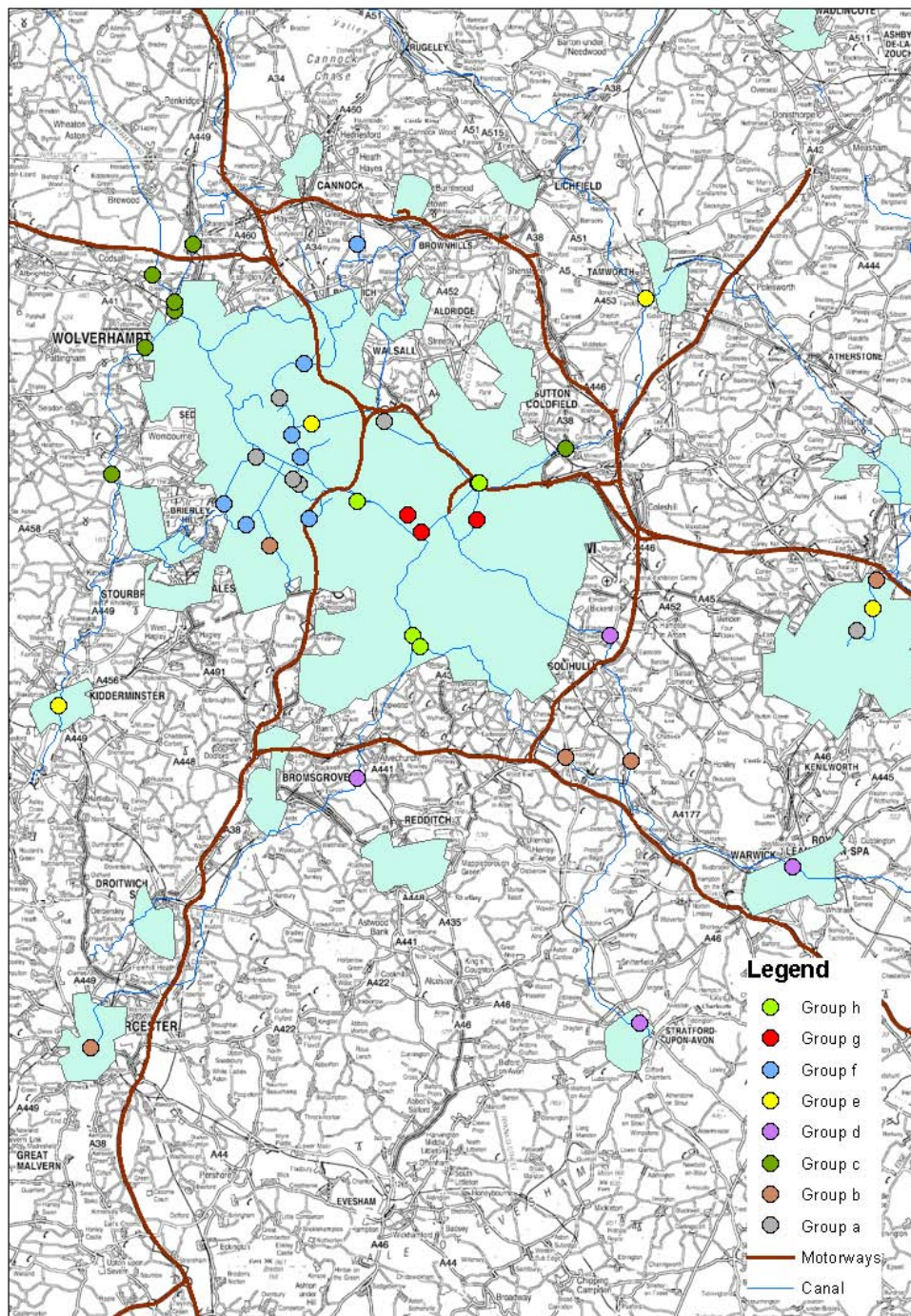
### **3.4.2 CHARACTERISATION OF HEAVY METAL CONCENTRATIONS**

At 22 of the 46 sites, CCU scores were greater than 1. This is the level at which, if exceeded, represents a conservative estimate of the total metal concentration likely to cause harm to aquatic organisms (Clements et al, 2000). Interpretation of the background water quality samples indicates that iron and zinc comprise about 90% of the total loading in the canals, but only contributed about 55% to CCU. Copper and chromium and to a lesser extent, cadmium and lead added very little to the total concentration in the water but had a major contribution to potential toxicity. Iron and zinc contributed most to total instream metal concentration at the three low CCU value sites, with little variation shown between the sites. Iron and zinc also provided the greatest contribution to CCU, again with low variation between sites. Copper made significant contribution at each site, together with lead. Despite having CCU values below 1, there will still be some urban runoff contributing low levels of metal to the water.

For comparison, in a study by Robson et al. (2005) of a small urbanised stream catchment in Sheffield, they found that the threshold of likely harm to aquatic life was exceeded at their study sites. They highlighted cadmium, copper and lead as the major sources of potential chronic instream toxicity.

At sites with high CCU values, greater variation was observed in the individual metals contribution to CCU. This may be a possible consequence of different urban land-use in the surrounding area. Heavy metal pollution is a problem associated with areas of intensive industry and this is certainly the case in much of the West Midlands. However, roads and vehicles are considered one of the largest sources of metals (Akbar et al. 2006). At BF-SB, although chromium contributed less than 10% of the total metal concentration, it accounted for about 85% to CCU. Chromium is used in metal alloys such as stainless steel, protective coatings on metal and pigments for paints. Many of these sites are located in areas of heavy urban runoff from roadways, such as the M6 motorway, which runs directly over the Tame Valley and Birmingham & Fazeley Canals (TV-SB, BF-SB). The presence of heavy traffic most likely

accounts for a significant amount of metals, together with the heavy industry (Bryson et al. 1996).



**Figure 3.12** Distribution of CCU within the West Midlands

Previous studies have shown that CCU scores were effective at assessing the effects of metal pollution on benthic communities (Clements et al., 2000), who postulated that the integration of different metal concentrations into a single variable was a significant advantage of the index. Moreover, they went on to speculate that a single variable could represent additive effects and might express toxicity relative to thresholds. Hirst et al. (2002) found that CCU was selected over individual metals as a predictor of diatom assemblages and it was also significantly correlated with macroinvertebrate richness.

The application of CCU as a predictor of chironomid assemblages is explored in Chapter 6.

### **3.5 CHAPTER SUMMARY**

This chapter has illustrated that CCU scores can be used effectively as a proxy for individual metal concentrations and it has demonstrated that the combined measure of toxicity (CCU) can be used successively to classify the canals. This was especially apparent when using cluster analysis.

## **CHAPTER 4**

# **VARIABILITY IN SEDIMENT CHEMISTRY OF URBAN CANALS IN THE WEST MIDLANDS**

### **APPROACH**

Spatial analysis trends for sediment metals at 46 sites in the West Midlands canal network were determined using the metal pollution index (MPI). The MPI was based on the concentrations of Cu, Zn, Cd, Pb, Cr and Ni, normalized to reference sites. The MPI was designed to vary between 0 for pristine sites and 100 for extremely impacted sites. A general classification of the pollution levels, in relation to metals, was achieved and the most heavily impacted sites identified.

### **4.1 INTRODUCTION**

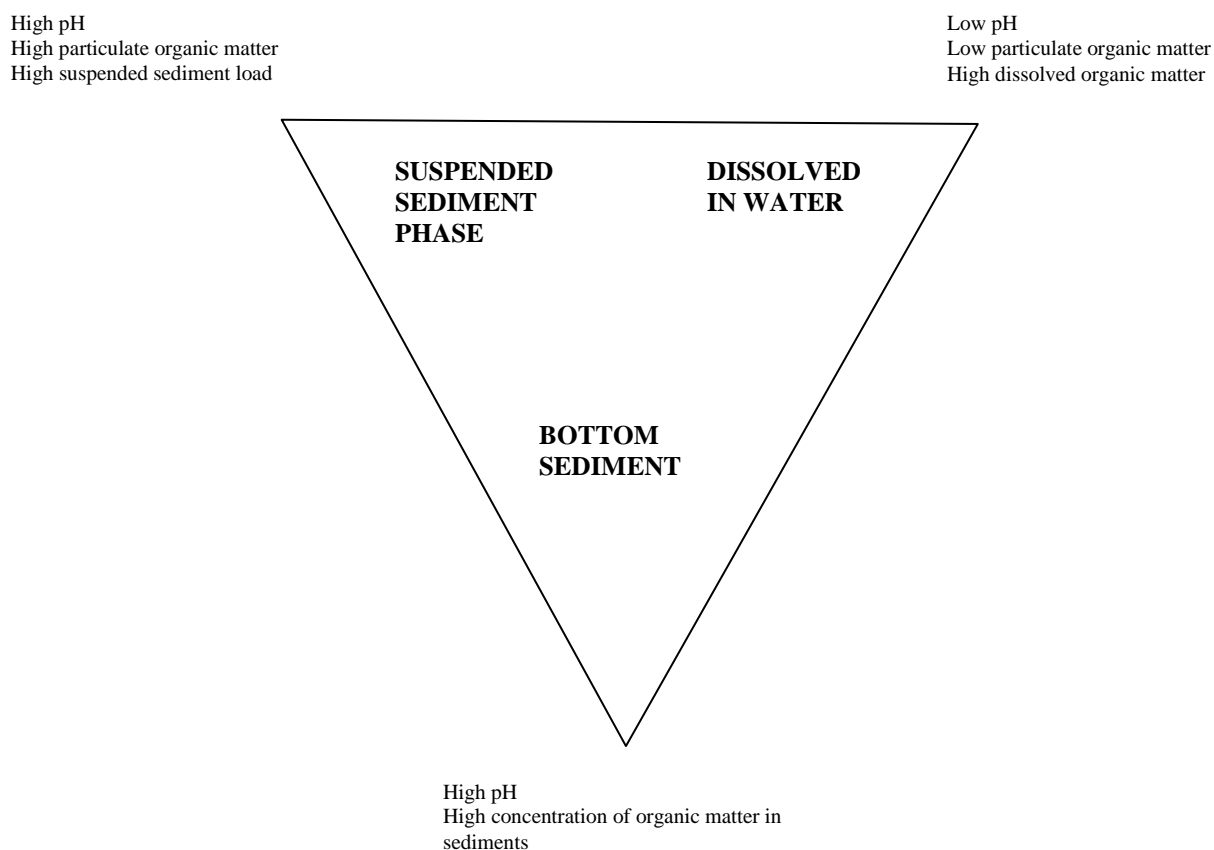
Sediments have a high capacity to accumulate pollutants especially trace elements such as metals, either from direct discharges or runoff inputs or via the water column by means of changes in pH or redox potential and adsorption for example. As a result, sediments have been recognised as environmental indicators and have been used to assess and monitor chemical contaminants in many different environments, including rivers, mangroves, estuaries, lakes and oceans (Soares et al., 1999; Smol, 2002; Liu et al., 2003). Pollutants transported on suspended particles within the water body are frequently deposited onto the surfaces of freshwater plants, which is an important process in the contamination of aquatic biota (Sansone et al., 1998). Urban canal sediment is predominantly derived from anthropogenic inputs and, as a result, it has a different composition to that of most natural sediments, being characterised by high concentrations of organic matter, Fe, P and heavy metals (Bromhead and Beckwith 1994; Dodd et al., 2003; NRA, 1996 Bijlsma et al., 1996; Boyd et al., 1999).

Figure 4.1 illustrates how metals are partitioned in aquatic systems among three major phases (solution, suspension and sedimentation) and highlights the importance of sediments in aquatic ecosystems. Metals are partitioned amongst the soluble phases, suspended and bottom sediments, as well as the biota. The major pathways of partitioning include adsorption, complexation, precipitation and biological uptake (Elder, 1988). Metals tend to accumulate in bottom sediments by the process of adsorption, as they have strong affinities for iron and manganese oxyhydroxides, particulate organic matter and clay minerals. In natural media, metal contaminants undergo reactions with ligands in water and with surface sites on the solid materials with which the water is in contact. Reactions in which the metal is bound to the solid matrix are referred to as sorption reactions and metal that is bound to the solid is said to be sorbed (EPA, 1995). During transport of metals in soils and surface water systems, metal sorption to the solid matrix results in a reduction in the dissolved concentration of metal and this affects the overall rate of metal transport. There are many chemical and physical changes (diagenesis) that occur within the sediment. Diagenesis plays an important role in the binding of contaminants. An important factor in early diagenesis is the biological oxidation of organic carbon. This together with the resulting anoxia produces large changes to the form of iron, manganese and sulphur which play key roles in the binding of trace elements in sediments and releasing them to the overlying water (Williamson et al., 1995).

Suspended solids have been shown to be important in reducing zinc toxicity. For example in certain species of *Daphnia*, sorption of zinc to suspended solids and/or changes in water chemistry due to the addition of suspended solids appear to have been factors causing reductions in zinc toxicity (Scott Hall et al, 1986). Where suspended solids were above  $483\text{--}734\text{ mg}^{-1}$  that increased total alkalinity, total hardness and total dissolved carbon reduced the toxicity of  $20\text{mg}^{-1}$  zinc to *P. promelas*. The toxic form of zinc to these organisms appeared to reside in the aqueous phase.

Reducing conditions of sediments encourage the formation of insoluble metal sulphides that decrease the bioavailability and thus toxicity of these metals to aquatic life. Also, oxidation of sediments containing metal sulphides will encourage the

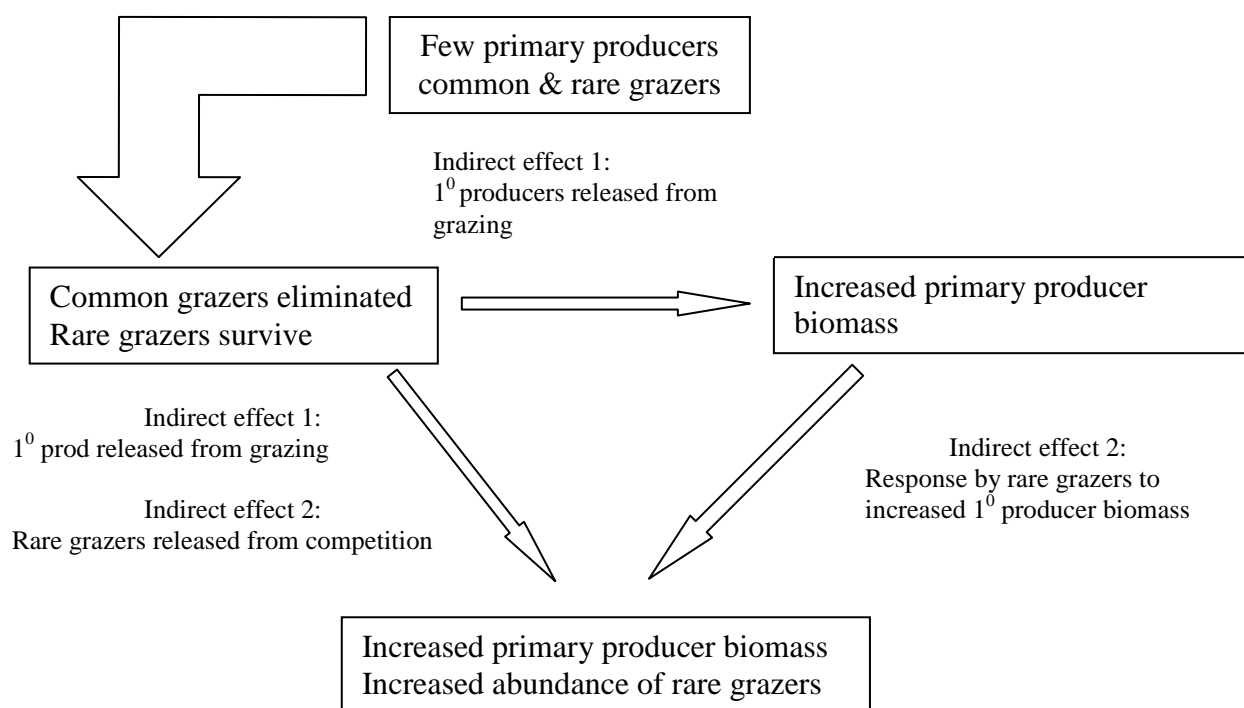
release of these metals into solution, at least temporarily, which may affect aquatic life.



**Figure 4.1** Metal partitioning in aquatic systems among 3 major phases (inside triangle) and some environmental conditions that favour each phase (outside triangle) (adapted from Elder 1988)

Due to their toxicity and accumulative behaviour, great ecological significance has been placed on the presence of heavy metals within the environment (Pardo et al., 1990; Meybeck et al., 2004). They enter aquatic organisms through body and respiratory surfaces and by ingestion of particulate matter and water. Metals may cause direct toxic effects and sensitive species may be impaired by sublethal effects or decimated by lethal effects. Direct effects vary with the intensity duration of exposure

to the toxicant. This has been studied in part because species responses have been used to predict criteria and establish permissible levels of contamination (Long et al., 1995). Trophic cascades (Pace et al., 1999) can lead to indirect effects where tolerant species can be affected, and where the effects can be mediated through consumer-resource interactions (Fig. 4.2). Ecosystem function can be impaired, for example, if there are changes in the nutrient or oxygen dynamics of the system under consideration. Direct effects of toxicants generally reduce species richness in the absence of competition, whereas, indirect effects may lead to an increase or decrease in abundance (e.g. from reduced availability of preferred food) (Fleeger et al., 2003).



**Figure 4.2** The indirect and direct effect of contamination in aquatic systems (adapted from Fleeger et al. 2003)

Sediments can also be important secondary sources of pollution in their own right, as they can influence the nature of the overlying and interstitial water, via physical, biological and chemical processes (pH or Eh changes). Changes in the pH and redox potential (Eh) can affect physicochemical equilibria, which in turn controls the complexation (solutes adsorbing from the aqueous solution to minerals) and solubility of trace metals. For example, as water becomes more alkaline, iron and manganese precipitate out of solution as oxides and hydroxides. Manganese may return into

solution if the redox potential of the sediments promotes the reduction of manganese (Philips and Rainbow, 1993). The presence of phytoplankton or macrophytes may also change the pH and redox potential on a local scale. This also affects the availability of other trace metals. The interstitial water between sediment grains is also important in the availability of trace metals. Anoxic sediments which are organically enriched contain many metal binding sites. This can favour reduction in certain metals that can release other metals previously adsorbed onto hydrous oxides (Luoma, 1983). Boat traffic may also create stresses through remobilization of contaminants adsorbed onto particulate organic matter (Murphy et al., 1995). It has also been found that re-suspension by boat traffic has a major influence on pore water chemistry (Dodd et al., 2003).

Although some heavy metals such as copper and zinc are important trace elements for aquatic life, they can be toxic to some aquatic biota at higher concentrations. They also exhibit additive effects where two or more metals and their individual toxicities are combined. Synergistic effects occur when the combined effect is greater than the sum of the individual effects (Wah Chu & Chow, 2002). In a study on a burrowing crustacean Jackson et al. (2005) found that increasing concentrations of lead and zinc caused significant increases in the mortality of the larvae. Synergistic effects were also observed, leading to higher mortalities than the metals on their own.

Water quality problems are often complex within canals, as they incorporate metals, nutrients as well as other organic compounds. The large sediment component that is also present acts as a chemical sink which can itself be a pollution source. Disturbance by boat traffic can create stress through increased nutrient cycling of phosphate for example (Yousef, 1980) or by remobilization of industrial contaminants such as heavy metals (Bordas and Bourg, 1998; Zuyin et al., 2007). The resuspended particles increase turbidity and reduce light available to submerged plants, causing restricted development and eventual loss of species (Murphy, 1995). Canals are extremely difficult to sample in a representative and replicable manner. Therefore, to satisfy water quality managers integrated indicators are required that can be used to address temporal and spatial variations of multiple constituents.

#### **4.1.1 IDENTIFYING METALS WITH BIOLOGICALLY ADVERSE EFFECTS**

Traditionally (especially in the US and Australia), sediment quality has been based on numerical Sediment Quality Guidelines (SDGs) derived on the basis of pre-existing ecotoxicological studies that assessed possible adverse biological effects of various sediment contaminants on a variety of species (Long and Macdonald, 1998; MacDonald et al., 1996; Birch and Taylor, 2002).

Two questions have been used to investigate which metals are most likely to be present in toxic amounts:

- i) Have the metals found at the levels present in this study been observed to have had toxic effects elsewhere?
- ii) How much higher are the levels found in the most polluted sites than those found at reference sites?

The first question characterizes the approach taken in the United States of America, by the National Status and Trends Program (Long and Morgan, 1990), where sediment quality guidelines were developed to determine when levels of metal concentration became toxic to biota using extensive laboratory experiments. Three ranges of metal concentration were defined: No-effects, possible effects and probable effects. Once quantified it is possible to define the effects range low (ERL) as the lower 10<sup>th</sup> percentile concentration in sediments associated with toxic effects. ERL reflects toxicity and concentration of metals. The effects range median (ERM) is the median metal concentration associated with toxic effects, and where concentration levels fall below the ERL, no toxic effects are expected. Concentration levels above the ERM are likely to lead to probable toxic effects and Table 4.1 shows ERM and ERL values for the metals. When calculating ERM, it is the toxicity of the metal as well as the concentration that is taken into account.

**Table 4.1** ERL/ERM values (from Long et al., 1995)

	ERL	ERM
Cd	1.2	3.9
Cr	81	270
Cu	34	190
Ni	20.9	45
Pb	46.7	99
Zn	150	550

These can be used as sediment quality guidelines (SDQ) for assessing the toxicity potential of complex mixtures in contaminated sediment by relating a sediment concentration to possible/probable toxic outcomes (Birch and Taylor, 2002) and they have been used effectively in this manner in several studies, especially in marine and estuarine environments (e.g. MacDonald et al., 1996). The values of each metal are normalised to its ERM value, the results summed and then divided by the total number of metals analysed. In this study, the mean ERM quotient (MERMQ) will be determined for six metals (Cu, Pb, Cd, Cr and Ni – MERMQ6m). From this an overall measure of metal concentration can be developed. For each metal, if the observed concentration/ERM>1 then the likelihood of sediment toxicity is high. By adding the ratios for each metal together, a toxic unit index (TUI) can be constructed. As toxic effects are not necessarily additive or maybe synergistic or antagonistic (Ingersoll et al., 1996), the index is an approximation. It is reasonable, however, to use the TUI as a measure of metal contamination for metals that exceed toxic thresholds. The success of such an approach has been demonstrated by research on overall metal concentration in stream-bottom sediments and vegetative habitats in urban streams (Rogers et al., 2002).

#### **4.1.2 CONTAMINATION (ENRICHMENT) FACTORS (CF) AND METAL POLLUTION INDEX (MPI)**

To enable sensitive assessments, indices have been developed that give different weights to an assortment of trace elements that are based on their assumed toxicity (Goncalves et al., 1992; Soares et al., 1999; Meybeck et al., 2004). One such index is the Metal Pollution Index (MPI) which can be viewed as a multiple normalization technique.

The use of such indices has not been carried out in the UK before, despite periodic sediment monitoring in British canals by British Waterways and rivers by the Environment Agency. The WFD stresses the importance of an adequate knowledge of sediment chemistry, highlighting its likely importance in the future. Sediments are considered in the Directive in two distinct aspects, chemical quality and physical and physico-chemical aspects conditioning the biological communities. In Article 16(7): “the Commission shall submit proposals for quality standards applicable to the concentrations of the priority substances in surface water, sediments or biota”. In Annex V: 1.2.6. “...standards may be set for water, sediments or biota”. Article 2 also states “Member states shall ensure, on the basis of monitoring, that concentrations of substances in Annex 1 do not increase in sediments and biota”.

In terms of conditioning the biological communities, Annex V mentions “Presence of taxa indicative of pollution” in the definition of “moderate status” for benthos and there is mention of “physico-chemical quality of sediments” in the definition of “good and moderate ecological status”. In Annex II (Identification of pressures) “dredging” is cited as a pressure to be considered for evaluating “the risk of failing the objectives”.

To overcome the limitations of the numerical approach described above the WFD introduced the concepts of ‘good ecological status (or potential)’ and ‘reference sites’. Sediment quality thus gains a flexible dimension by which appropriate and specific evaluation of the overall ecological quality can be made. This is also important if

biological indicators are to be used to assess the quality of the sediment. It is important that sediment quality is taken into account for the WFD, and that new ecologically oriented tools are developed in order that this can be achieved. However, there is still the need to classify objectively where the boundaries are between ecological status classes and so this where the production of pollution indices fit in. The assessment of sediment in this study is important in that it will investigate local scale differences in urban canals, which is an under-researched issue in the UK.

The main aims of this chapter are to:

- (i) Differentiate anthropogenic from more 'natural' levels of trace metal contamination;
- (ii) Assess the distribution of metal contamination by means of pattern recognition techniques;
- (iii) Compute a sediment-based index of metal pollution;
- (iv) Investigate inter-site variability.

## **4.2 MATERIALS AND METHODS**

### **4.2.1 SAMPLING**

At each site, a small dredge or long-handled pond net was used to obtain three samples of surface sediment. These were combined and mixed together to provide as representative a sample as possible and placed in a 250 ml glass beaker. The methods used to obtain the chemical assays are described in Chapter 2 (Table 2.4).

### **4.2.2 NUMERICAL CLASSIFICATION AND ANALYSIS OF SEDIMENT**

This study has to account for several variables in parallel and therefore multivariate analyses will be used to examine the associations between samples and measured variables. The data were analyzed for covariance and relationships among the categorical variables examined (Fig. 4.3).

Pearson product moment coefficient of correlations was calculated for pairs of variables to look for covariation in the data. Factor analysis (FA) was then used to reduce data complexity and understand the relationship of the variables to ordination axes or factors. Varimax rotation, a popular rotation method (Abdi, 2003), was used to ease interpretation as it minimizes the association of variables with factors. The Kaiser criterion, which selects factors with an eigenvalue greater than one, was used to determine how many factors were used in the analyses (Camusso et al., 2002).

Cluster analysis was carried out to identify clusters of sites with similarities of underlying numerical structure within the sediment chemistry data. A quantitative Euclidian distance measure with Ward's complete linkage method was utilized to cluster sites with similar sediment metal chemistry (Sneath and Sokal, 1973). Discriminant analysis was used to indicate: (i) the percentage of correctly classified

sites in the groups obtained by cluster analysis, and (ii) misclassified samples. Cross-validation is one technique that is used to compensate for an optimistic apparent error rate. The apparent error rate is the percent of misclassified observations. This number tends to be optimistic because the data being classified are the same data used to build the classification function (McLachlan, 1976). The subgroups that resulted from the analysis were selected for further analysis based on the relative difference between the groups. Means and variance (derived from the subgroups) were calculated for each chemical parameter and these were plotted as box-and-whisker plots. Mann-Whitney U tests were used to determine the statistical significance between the various groups. Each succeeding pair of subgroups along the contamination gradient was also tested for significance to determine how much overlap there was between each subgroup. All the above analyses were carried out using MINITAB v14 and CANOCO 4.5 (ter Braak, 2003).

#### **4.2.3 CONTAMINATION FACTORS AND METAL POLLUTION INDEX**

Contamination factors (CF) were calculated as the ratio between the sediment metal content and that found at a reference site. The reference site used (SU-PB) had the lowest mean sediment metal levels out of the 46-site data set. Enrichment factors (EFs) above a certain threshold were considered to be indicative of contamination. The enrichment classes utilised were those adopted by the University of Sydney (<http://www.ozestuaries.org/indicators.html>), and are as follows: EF<1 indicates no enrichment; EF<3 is minor; EF 3-5 is moderate; EF 5-10 is moderately severe; EF 10-25 is severe; EF 25-50 is very severe and EF >50 is extremely severe.

The linear sum of the concentration factors together with a weighted value taking into account the differences in the toxicity of the various metals was used to calculate a metal pollution index (MPI) (Soares et al., 1999). This can be used to give an evaluation of overall metal pollution at a particular site and was defined as:

$$MPI = \sum_i (w_i/w_t) \times CF_i$$

where  $CF_i$  is the contamination factor for metal  $i$ ;  $w_i$  is the weight for metal  $i$ ; and  $w_t = \sum_i w_i$ .

The weights (Zn=1; Cu=5; Cr, Pb and Ni=100; Cd=500) were established by (Goncalves et al., 1992) and assume an inverse proportion to the maximum permissible level in surface waters for domestic supply (EPA 1976).

#### **4.2.4 LINEAR REGRESSION**

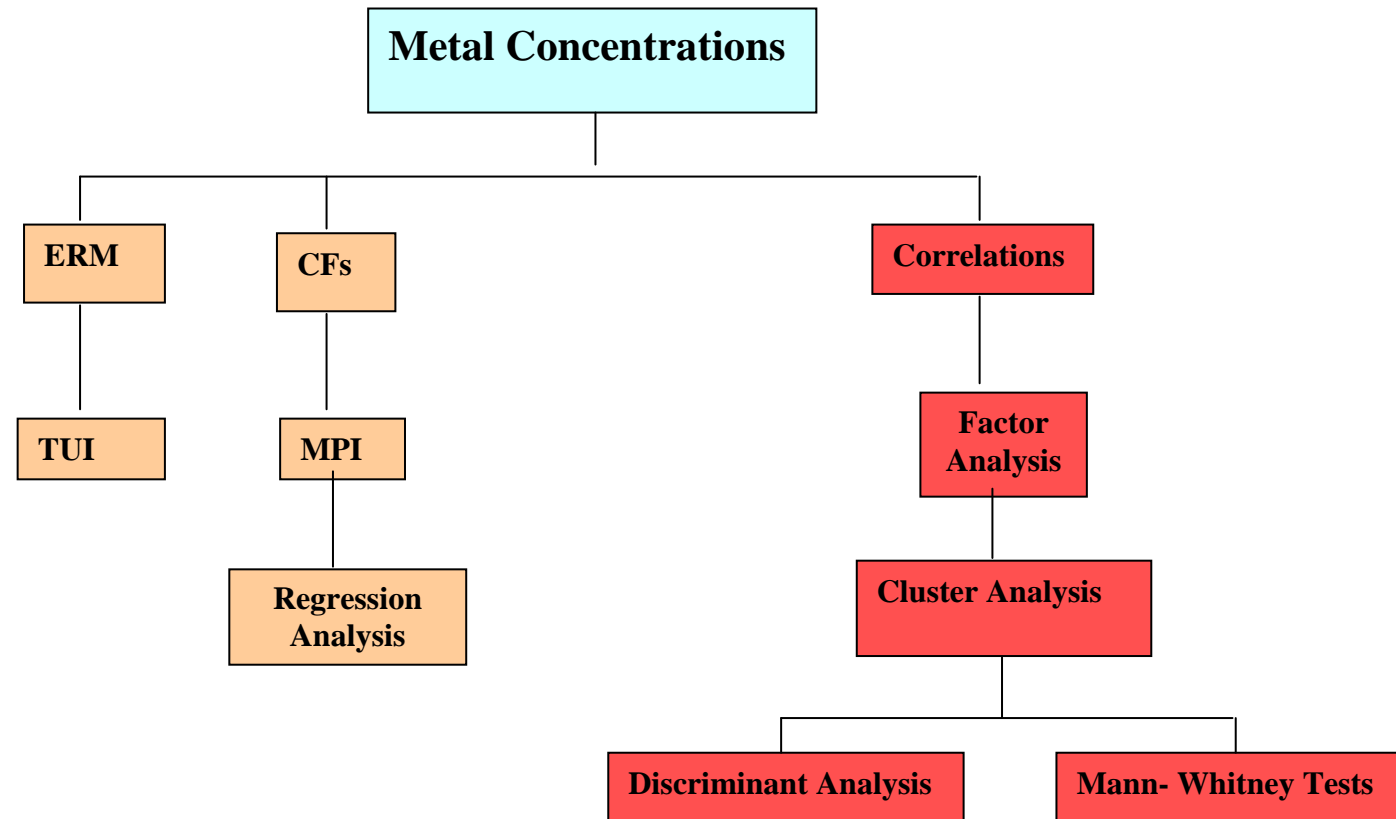
Following calculation of MPIs, linear regression (McCullagh and Nelder, 1983) was also carried out to show the sensitivity of each metal to variations in MPI. This was used to investigate and model the relationship between response variables and one or more predictors.

**Figure 4.3** Analysis route for sediment chemistry analysis

Q1. Can anthropogenic be differentiated from more 'natural' levels of trace metal pollution?  
Q2. Can pattern recognition techniques be used to assess distribution of metal contamination?  
Q3. Can a sediment-based metal pollution index be computed?

*i) Metal Pollution Indices*

*ii) Sediment classification*



## **4.3 RESULTS**

### **4.3.1 METAL CONCENTRATIONS**

Data for metal concentrations within the sediment are presented in Table 4.2, together with mean, minimum and maximum ranges. The data suggest that certain sites have higher concentrations of a wider range of contaminants. An example can be seen at BW-KE where metal concentrations range from 7.62 times ERM levels for lead and 43 times ERM levels for copper. This compares with sites such as ST-ST where sediment metal concentrations are all below published ERM values.

### **4.3.2 CORRELATIONS BETWEEN VARIABLES**

Correlation between the analysed parameters (Table 4.3) shows that the heavy metals were seen to be all intercorrelated. The fines (<5.8 $\mu$ ) were only correlated with iron and volatile matter only with lead.

**Table 4.2** Variable concentrations (mg kg<sup>-1</sup>)

	Cu	Zn	Cd	Pb	Cr	Fe	Ni	volatiles	Fines
TV-HB	1200	535	24.8	697	136	25700	153	29.8	39.65
TV-NR	605	3770	12.1	278	60.9	18100	115	33.5	37.31
TV-SB	149	2620	7.7	243	43	19400	123	33.5	44.2
CV-FX	124	377	0.886	100	45	20400	37.5	14.6	76.66
CV-OCR	1110	1450	4.8	513	63.5	21500	60.6	22.1	58.31
CV-FS	377	945	3.47	293	40.7	17800	38.8	14.7	39.57
WE-GS	2490	2530	7.22	523	87.8	21800	334	21.2	34.05
CX-WG	332	1540	7.32	150	29.3	19100	121	30.4	44.54
WS-RG	4800	9910	60.5	782	291	41200	387	30.5	36.06
WS-MX	369	3280	8.09	292	66.3	25200	103	22.3	46.46
WS-MM	2000	7120	35.4	687	224	39000	158	28.2	72.12
WS-RB	1100	7650	48.4	550	268	29100	307	21.7	58.19
SW-CH	166	704	10.1	76.7	92.8	17600	53	22.5	17.67
SW-OX	74.2	1100	2.48	50.8	77.4	18400	47.3	12	17.36
SW-JN	140	1570	4.43	115	102	17100	63.6	17.9	12.35
SW-CB	173	2240	6.99	169	155	16100	104	35.4	23.46
SW-SB	84.9	1040	4.2	118	77.4	31500	86.3	9.03	11.29
SW-KD	195	1030	3.83	294	107	22100	113	22.7	73.57
SU-PB	45.4	351	3	23.3	42.2	7220	26.8	4.7	14.03
WB-LF	5330	6320	28.3	409	1010	39000	405	21.6	44.07
WB-GL	116	259	0.43	65.3	48.9	33300	57.7	9.56	42.11
WB-WR	262	724	3.12	431	72.2	39600	37.8	16.5	81.46
ST-SR	6270	5520	24.6	450	1430	37300	397	19.5	86.85
ST-HH	130	583	72.8	72.8	46.9	27300	51.5	9.52	62.14
ST-ST	44.4	87.2	0.09	59.1	38.1	34600	33.3	5.1	32.48
BL-BL	525	2130	37.4	265	176	73000	65.1	7.13	38.62
BL-PL	169	1550	18.1	122	79.6	57100	47.2	8.52	26.37
WL-BR	268	3830	5.58	130	49.6	34800	59.1	16.4	29.04
WL-BS	324	4220	6.87	318	67.1	55600	77	11.3	38.09
BW-KE	8210	7950	30	754	2170	44600	795	27.6	62.12
BL-BB	544	1950	12.5	462	139	36000	78.8	21.9	28.37
WL-BB	817	7470	27	737	309	57900	135	25.3	57.24
WB-BR	2190	3420	11.2	356	609	18600	276	8.55	36.72
GU-WK	88.2	331	0.95	100	41.1	26900	37.6	16.8	94.58
GU-LP	86	554	1.06	57.9	31.8	23100	45.9	11.2	93.12
GU-CB	114	518	2.74	140	30.8	19800	39.6	7.37	91.6
GU-NC	5380	5990	13.7	1280	620	67200	390	13.7	89.36
BF-SB	4090	5240	22.9	702	1010	49400	902	21.8	98.09
BF-ML	1360	5740	6.72	358	145	37500	291	6.16	87.96
BF-FZ	1080	2480	6.54	231	247	32100	244	7.05	90.66
DD-DM	139	760	0.622	174	73.7	44000	35.7	16.6	99.45
DD-CR	1390	2590	11.9	475	97.4	60200	120	21.4	99.39
DD-CO	169	4120	3.21	235	36.2	45300	54.4	23.9	99.94
TF-WR	115	415	1.13	94.6	26.2	39200	23.3	7.56	84.33
GB-DR	480	8330	15.9	69.2	152	51000	69.2	20.9	91.09
SL-WR	3060	2740	8.22	383	1080	24300	196	12.5	79.99
Mean	1267.07	2947.46	13.68	323.60	257.52	33413.48	160.78	17.87	57.00
Min	44.4	87.2	0.09	23.3	26.2	7220	23.3	4.7	11.29
Max	8210	9910	72.8	1280	2170	73000	902	35.4	99.94

**Table 4.3** Spearman correlation coefficients for the investigated metals, volatile matter and fines (Bold indicates the highly significant ( $p < 0.05$ ) positive correlations).

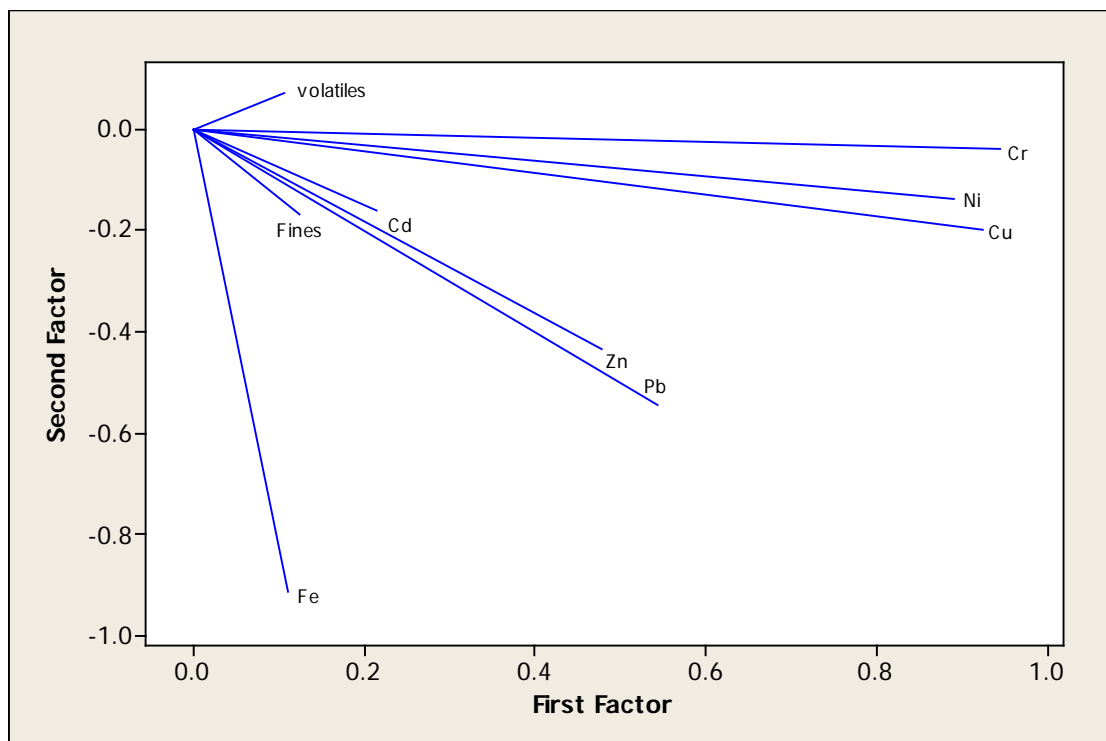
	Cu	Zn	Cd	Pb	Cr	Ni	Vol	Fines
Zn	<b>0.642</b> p<0.001							
Cd	<b>0.408</b> 0.005	<b>0.543</b> p<0.001						
Pb	<b>0.699</b> p<0.001	<b>0.623</b> p<0.001	<b>0.409</b> 0.005					
Cr	<b>0.897</b> p<0.001	<b>0.510</b> p<0.001	<b>0.295</b> 0.046	<b>0.485</b> 0.001				
Ni	<b>0.854</b> p<0.001	<b>0.613</b> p<0.001	<b>0.377</b> 0.010	<b>0.642</b> p<0.001	<b>0.807</b> p<0.001			
Vol	0.233 0.12	<b>0.396</b> 0.006	0.231 0.122	<b>0.379</b> 0.009	0.148 0.325	0.249 0.095		
Fines	0.195 0.199	0.163 0.278	-0.036 0.813	0.209 0.163	0.191 0.203	0.205 0.172	-0.069 0.649	
Fe	<b>0.300</b> 0.043	<b>0.447</b> 0.002	<b>0.294</b> 0.048	<b>0.438</b> 0.002	0.221 0.139	0.249 0.095	-0.105 0.489	<b>0.338</b> 0.022

### 4.3.3 FACTOR ANALYSIS

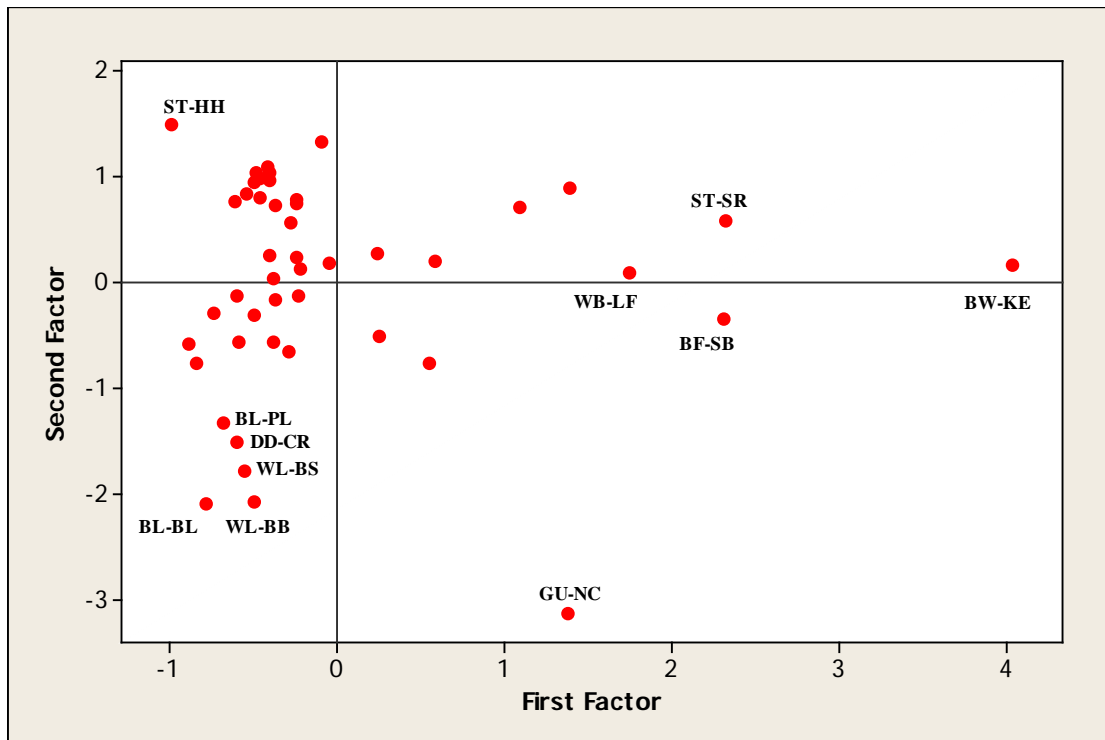
Table 4.4 shows the results obtained from the factor analysis. About 90% of the variation in the data can be explained by five factors. Factor 1, accounting for 34.9% of the variance reflects high chromium, copper and nickel; Factor 2 reflects high levels of iron, whereas Factor 3 reflects volatile organic matter, Factor 4 reflects high cadmium and Factor 5, by fines. The original eight variables have now been reduced to four new factors. These can be plotted to obtain a graphical interpretation of the overall picture. Fig. 4.7 illustrates the first two factors, which account for 51.0% of the total variance.

**Table 4.4** Eigenvalues, cumulative % of variance and variables with absolute loading values  $>0.7$ , in the factor analysis of sediment samples

Factor	Eigenvalue	Cumulative % of variance	Variables with loading value $>0.7$
1	3.1446	34.9	Cu, Cr, Ni
2	1.4402	50.9	Fe
3	1.3316	65.7	volatiles
4	1.1940	79.0	Cd
5	1.0163	90.3	Fines



**Figure 4.4** Factor analysis loading plot



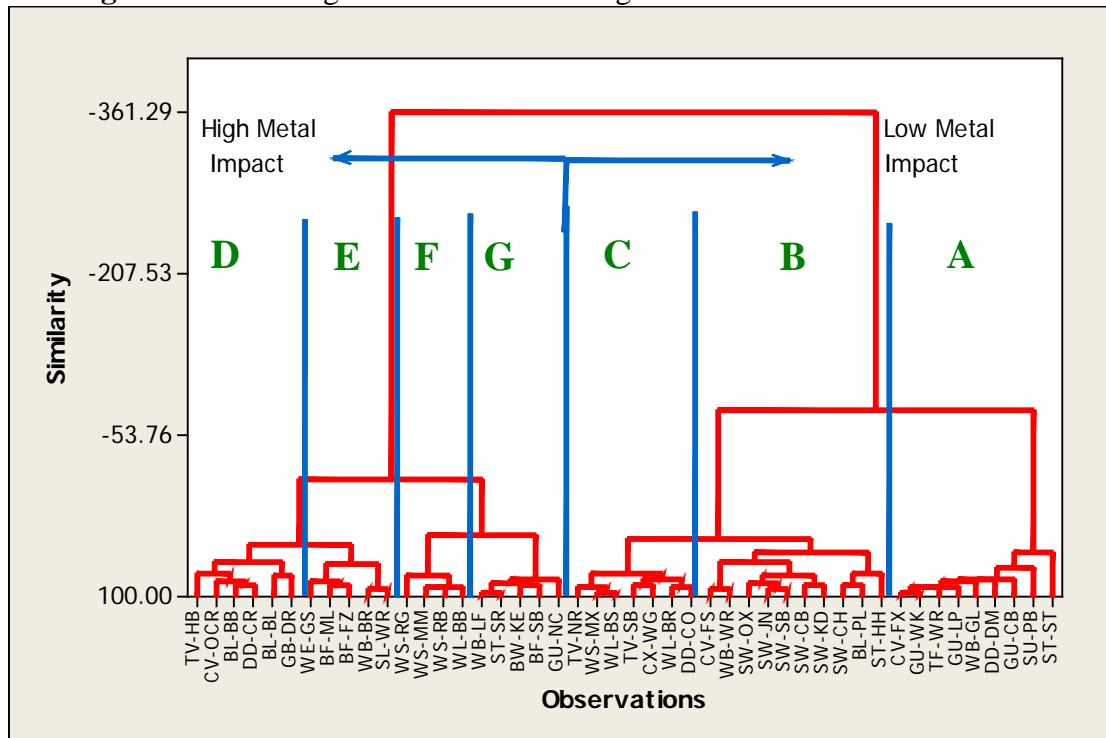
**Figure 4.5** Factor analysis score plot

The score plot (Fig. 4.5), shows a cluster of samples to the right of the plot (BW-KE, ST-SR, WB-LF, GU-NC and BF-SB) that represent sites associated with high loadings of chromium, nickel and copper (Fig. 4.4). Towards the bottom of the plot (GU-NC, WL-BB, BL-BL, WL-BS, DD-CR, BL-PL) are sites characterised by high levels of iron and top left low levels.

#### **4.3.4 HIERARCHICAL CLUSTER ANALYSIS**

The dendrogram (Fig. 4.6) shows a clear division between sites to the right with low metal impact and those sites to the left that are highly impacted by metal. Seven groups can be constructed, A-G, which delineate a gradient of metal impacted sites, with Group A having lower levels of metals up to sites with high levels of metal in the sediment, group G. Of the seven metal parameters, only iron was not significantly different between the groups making up the first two divisions of the cluster analysis.

**Figure 4.6** Dendrogram with Ward Linkage and euclidian distance measure

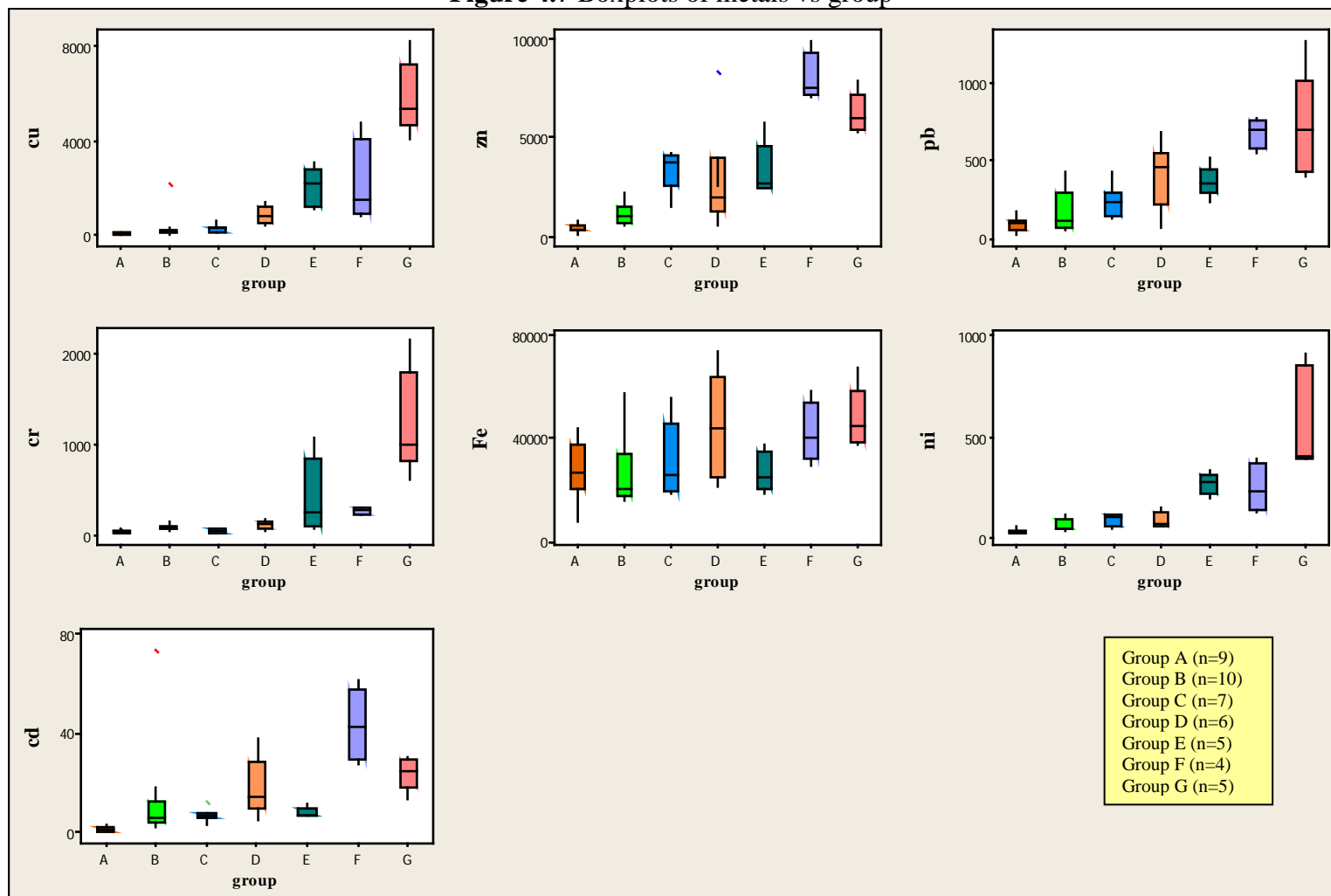


Means and variance for each variable are shown as a series of box-and-whisker plots (Fig. 4.7 & Table 4.5). Copper, lead and nickel present an observable gradient across subgroup means, before statistical significance was tested. Nickel proved to have the least amount of overlap between each subgroup. Four out of the six pairs of subgroups proved to be significantly different ( $\alpha < 0.05$ ). Copper showed that three of the pairs were significantly different with one pair borderline, whereas only one pair for lead proved to be significantly different (Table 4.6).

Discriminant analysis, correctly classified 89.1% of the sites (41 out of 46 sites). Of the five misclassified sites groups, four were reclassified as members of the next group, although these were borderline misclassifications according to probability values. Only one (SL-WR) site was placed into a group more than one from its true group. The misclassified sites were reassigned to their 'true' groups using these data. There are always going to be sites that are borderline between groups. Increasing the

number of sites or increasing the metal gradient might reduce the number of misclassifications, although there will still be some sites that will be close to the border between two groups.

**Figure 4.7** Boxplots of metals vs group



**Table 4.5** Mean values for metals in each group

	<b>Cu</b>		<b>Zn</b>		<b>Cd</b>		<b>Pb</b>		<b>Cr</b>		<b>Ni</b>		<b>Fe</b>	
	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>
<b>Group A</b>	491.00	419.51	3121.43	930.01	8.51	2.51	269.43	115.18	59.09	21.69	102.59	25.03	33200.00	17863.28
<b>Group B</b>	229.40	93.82	1301.80	609.75	4.37	1.54	260.40	123.28	95.38	42.60	71.44	35.52	22540.00	9807.80
<b>Group C</b>	480.44	1043.20	1073.25	775.87	15.21	23.87	123.33	111.95	190.89	359.91	68.46	54.22	25402.50	14729.14
<b>Group D</b>	104.60	954.70	954.70	1558.14	1.10	1.09	102.88	67.56	40.18	6.17	44.40	9.96	30600.00	9092.41
<b>Group E</b>	5680.00	6821.67	6821.67	1786.07	30.00	15.99	729.50	312.41	1088.50	656.37	546.00	236.83	46450.00	11040.61
<b>Group F</b>	605.50	1206.67	1206.67	740.50	13.54	14.79	367.60	228.51	102.40	56.62	69.42	45.68	39900.00	18266.47
<b>Group G</b>	1439.63	5592.50	5592.50	2431.74	19.80	15.62	438.90	227.77	255.23	160.40	226.78	94.69	35875.00	13538.07

**Table 4.6** Mann-Whitney significance tests between groups ( $p < 0.05$ )

<b>Group Pair</b>	<b>Cu</b>	<b>Zn</b>	<b>Cd</b>	<b>Pb</b>	<b>Cr</b>	<b>Fe</b>	<b>Ni</b>
A/B	0.02	0.001	0.0005	n.s	0.003	n.s	0.005
B/C	n.s	0.002	n.s	n.s	0.01	n.s	0.04
C/D	0.01	n.s	0.05	n.s	0.008	n.s	n.s
D/E	n.s	n.s	n.s	n.s	n.s	n.s	0.008
E/F	n.s	0.02	0.02	0.02	n.s	n.s	n.s
F/G	0.04	n.s	n.s	n.s	0.02	n.s	0.02

### 4.3.5 METAL POLLUTION INDICES

#### 4.3.5.1 *ERM/MERMQ*

Mean ERM quotients (MERMQ6m) (Table 4.7) indicate a wide range of contamination. Thirty-one of the 46 sites contain sediments with metal concentrations at levels that may have an adverse effect on the biota (MERMQ>1.5) (Birch & Taylor, 2002).

Sixty-seven per cent of the sites in this study had a mean quotient of >1.5 and therefore a 76% probability of sediment toxicity, whereas 28% of the study sites, contained sediment with a mean ERM quotient of between 0.5 and 1.5, so have a 49% probability of being toxic. None of the sites were considered to have low metal contamination (e.g. a mean ERM quotient of <0.11).

As mentioned previously, MERMQ values may give a better indication of sediment contamination. This is apparent when the mean values between groups are examined. These range from 2.61 for Group A to 5.54 for Group G and 12.25 for Group E (Table 4.8; Fig. 4.8).

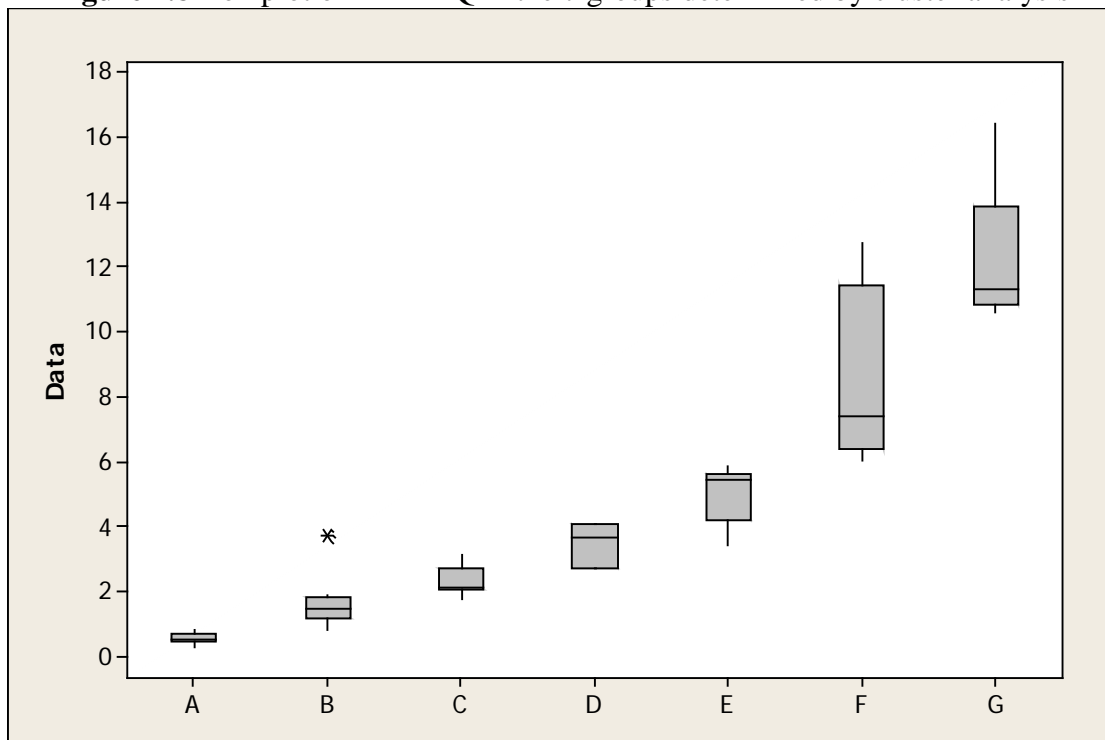
**Table 4.7** Metal pollution indices

	<b>MPI</b>	<b>TUI</b>	<b>MERMQ</b>
TV-HB	9.85	24.59	4.10
TV-NR	4.57	18.73	3.12
TV-SB	3.38	12.87	2.14
CV-FX	1.22	3.58	0.60
CV-OCR	4.44	16.47	2.75
CV-FS	2.76	8.56	1.43
WE-GS	5.26	32.59	5.43
CX-WG	2.78	10.74	1.79
WS-RG	18.93	76.37	12.73
WS-MX	3.92	15.46	2.58
WS-MM	12.61	43.83	7.30
WS-RB	14.40	45.48	7.58
SW-CH	3.10	7.04	1.17
SW-OX	1.34	4.88	0.81
SW-JN	2.15	7.68	1.28
SW-CB	3.11	11.37	1.89
SW-SB	2.28	6.81	1.14
SW-KD	3.08	9.76	1.63
SU-PB	1.00	2.63	0.44
WB-LF	12.42	63.67	10.61
WB-GL	1.17	3.31	0.55
WB-WR	3.87	8.96	1.49
ST-SR	13.20	68.01	11.33
ST-HH	16.07	22.46	3.74
ST-ST	1.05	1.89	0.32
BL-BL	11.00	21.00	3.50
BL-PL	5.64	10.92	1.82
WL-BR	2.64	12.62	2.10
WL-BS	4.33	16.31	2.72
BW-KE	18.51	98.68	16.45
BL-BB	6.15	16.55	2.76
WL-BB	11.55	36.39	6.07
WB-BR	6.63	32.60	5.43
GU-WK	1.33	3.31	0.55
GU-LP	1.03	3.45	0.58
GU-CB	1.76	4.65	0.78
GU-NC	13.38	66.61	11.10
BF-SB	12.87	67.80	11.30
BF-ML	4.57	29.94	4.99
BF-FZ	4.02	20.54	3.42
DD-DM	2.05	5.10	0.85
DD-CR	6.51	22.90	3.82
DD-CO	2.84	12.92	2.15
TF-WR	1.51	3.22	0.54
GB-DR	5.07	24.55	4.09
SL-WR	7.76	35.42	5.90

**Table 4.8** Group membership

	MPI		MERMQ		TUI	
	mean	SD	mean	SD	mean	SD
Group A	0.51	0.11	2.61	0.69	15.66	4.15
Group B	1.87	1.90	1.54	0.23	9.27	1.39
Group C	1.26	0.31	1.98	1.89	11.85	11.36
Group D	2.85	1.23	0.79	0.68	4.74	4.05
Group E	3.44	2.15	12.25	2.17	73.52	13.03
Group F	6.18	1.50	2.41	1.43	14.49	8.58
Group G	9.59	3.33	5.54	1.44	33.24	8.62

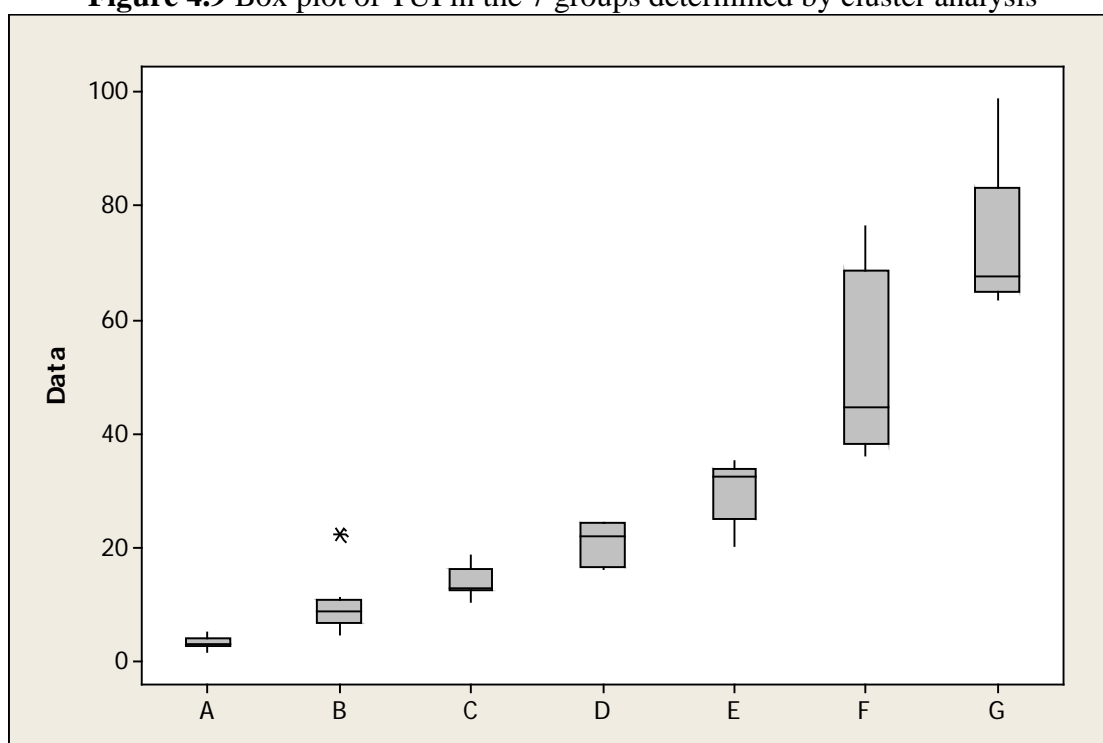
**Figure 4.8** Box plot of MERMQ in the 7 groups determined by cluster analysis



#### 4.3.5.2 Toxic unit index (TUI)

The results of the TUI are presented in Tables 4.7 and 4.8 and Fig. 4.9. Thirteen sites exceeded ERM values for cadmium but all the other metals fell below their respective values. Cadmium is therefore the only metal that could pose a toxic risk to the biota. The TUI also generally follow an increasing range from 10.81 (Group A) and 9.84 (Group B) to a mean TUI of 72.95 for Group G.

**Figure 4.9** Box plot of TUI in the 7 groups determined by cluster analysis



#### 4.3.5.3 Contamination factors (CF) and metal pollution index (MPI)

For the purposes of this study, site SU-PB was chosen as the reference site as this had the lowest concentrations of most of the metals present. These levels were lower for example than Dutch sediment standards (de Rooij, 2006). The highest contamination factors for copper (180.84) and chromium (51.42) were found at BW-KE; for zinc (28.23) at WS-RG; for cadmium (24.27) at ST-HH; for lead (54.94) at GU-NC; iron (10.11) at BL-BL and nickel (33.66) at BF-SB. Table 4.9 shows the contamination factors colour-coded to indicate to what degree each contaminant is causing the most contamination at a particular site. At a glance it can be seen which site has very or extreme enrichment of any particular metal. For example, it can be seen that site BW-KE has four metals (Cu, Pb, Cr and Ni) that are very or extremely enriched. In contrast there is insignificant enrichment at GU-LP.

Fig 4.10 illustrates the statistical distribution of CF for each of the individual metals within each of the groups determined by cluster analysis. Pb, Ni and Cu show a general increasing trend across the cluster groups. These are lacking for the other groups.

**Table 4.9** Contamination factors (CFs)

	Cu	Zn	Cd	Pb	Cr	Fe	Ni
TV-HB	26.43	1.52	8.27	29.91	3.22	3.56	5.71
TV-NR	13.33	10.74	4.03	11.93	1.44	2.51	4.29
TV-SB	3.28	7.46	2.57	10.43	1.02	2.69	4.59
CV-FX	2.73	1.07	0.30	4.29	1.07	2.83	1.40
CV-OCR	24.45	4.13	1.60	22.02	1.50	2.98	2.26
CV-FS	8.30	2.69	1.16	12.58	0.96	2.47	1.45
WE-GS	54.85	7.21	2.41	22.45	2.08	3.02	12.46
CX-WG	7.31	4.39	2.44	6.44	0.69	2.65	4.51
WS-RG	105.73	28.23	20.17	33.56	6.90	5.71	14.44
WS-MX	8.13	9.34	2.70	12.53	1.57	3.49	3.84
WS-MM	44.05	20.28	11.80	29.48	5.31	5.40	5.90
WS-RB	24.23	21.79	16.13	23.61	6.35	4.03	11.46
SW-CH	3.66	2.01	3.37	3.29	2.20	2.44	1.98
SW-OX	1.63	3.13	0.83	2.18	1.83	2.55	1.76
SW-JN	3.08	4.47	1.48	4.94	2.42	2.37	2.37
SW-CB	3.81	6.38	2.33	7.25	3.67	2.23	3.88
SW-SB	1.87	2.96	1.40	5.06	1.83	4.36	3.22
SW-KD	4.30	2.93	1.28	12.62	2.54	3.06	4.22
SU-PB	1.00	1.00	1.00	1.00	1.00	1.00	1.00
WB-LF	117.40	18.01	9.43	17.55	23.93	5.40	15.11
WB-GL	2.56	0.74	0.14	2.80	1.16	4.61	2.15
WB-WR	5.77	2.06	1.04	18.50	1.71	5.48	1.41
ST-SR	138.11	15.73	8.20	19.31	33.89	5.17	14.81
ST-HH	2.86	1.66	24.27	3.12	1.11	3.78	1.92
ST-ST	0.98	0.25	0.03	2.54	0.90	4.79	1.24
BL-BL	11.56	6.07	12.47	11.37	4.17	10.11	2.43
BL-PL	3.72	4.42	6.03	5.24	1.89	7.91	1.76
WL-BR	5.90	10.91	1.86	5.58	1.18	4.82	2.21
WL-BS	7.14	12.02	2.29	13.65	1.59	7.70	2.87
BW-KE	180.84	22.65	10.00	32.36	51.42	6.18	29.66
BL-BB	11.98	5.56	4.17	19.83	3.29	4.99	2.94
WL-BB	18.00	21.28	9.00	31.63	7.32	8.02	5.04
WB-BR	48.24	9.74	3.73	15.28	14.43	2.58	10.30
GU-WK	1.94	0.94	0.32	4.29	0.97	3.73	1.40
GU-LP	1.89	1.58	0.35	2.48	0.75	3.20	1.71
GU-CB	2.51	1.48	0.91	6.01	0.73	2.74	1.48
GU-NC	118.50	17.07	4.57	54.94	14.69	9.31	14.55
BF-SB	90.09	14.93	7.63	30.13	23.93	6.84	33.66
BF-ML	29.96	16.35	2.24	15.36	3.44	5.19	10.86
BF-FZ	23.79	7.07	2.18	9.91	5.85	4.45	9.10
DD-DM	3.06	2.17	0.21	7.47	1.75	6.09	1.33
DD-CR	30.62	7.38	3.97	20.39	2.31	8.34	4.48
DD-CO	3.72	11.74	1.07	10.09	0.86	6.27	2.03
TF-WR	2.53	1.18	0.38	4.06	0.62	5.43	0.87
GB-DR	10.57	23.73	5.30	2.97	3.60	7.06	2.58
SL-WR	67.40	7.81	2.74	16.44	25.59	3.37	7.31

<1	no enrichment	10-25	severe enrichment
<3	minor enrichment	25-50	very severe enrichment
3-5	moderate enrichment	>50	extremely severe enrichment
5-10	moderately severe enrichment		

**Figure 4.10** Statistical distribution of contamination factors (CF) within each group determined by cluster

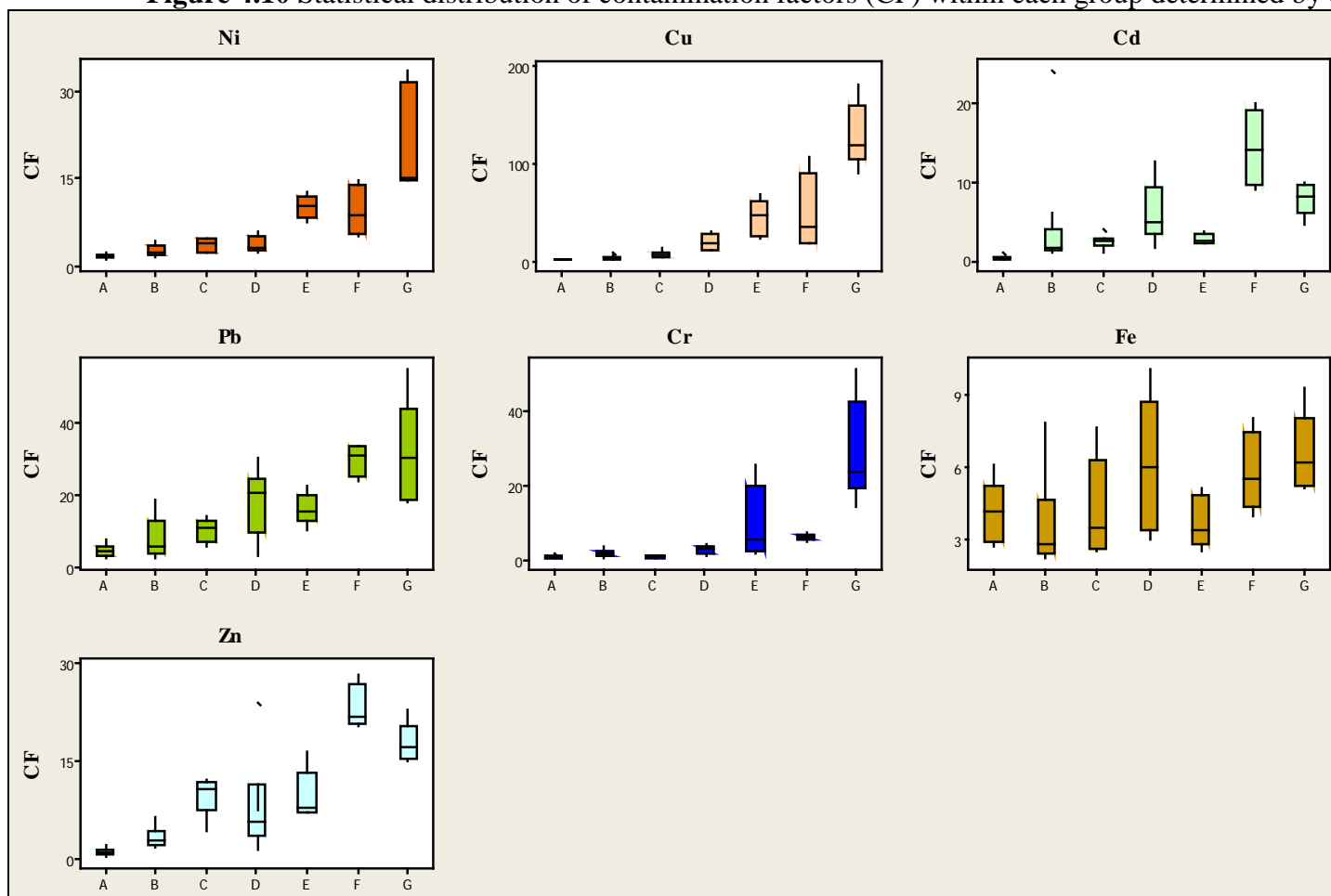
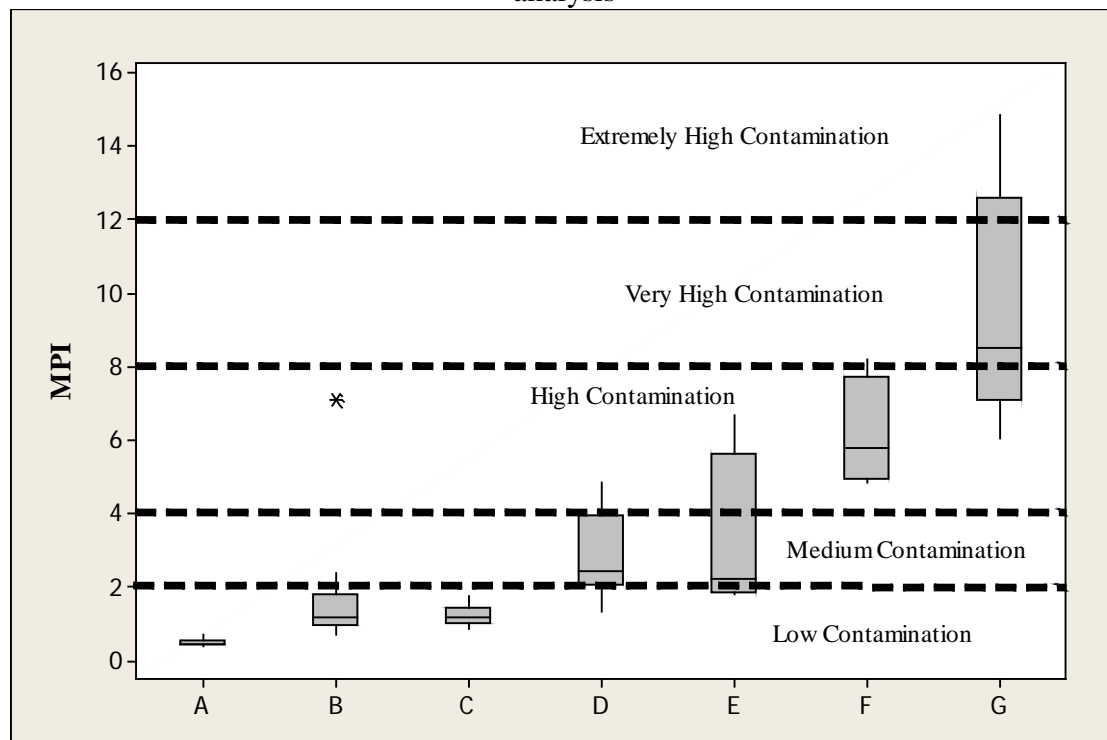


Table 4.7 illustrates the variation in the metal pollution index (MPI) at the 46 sites, while Table 4.8 and Fig. 4.11 give the mean MPI in each of the groups determined by cluster analysis. It can be seen that MPI values are generally increasing in successive groups. Contamination bands have also been added.

**Figure 4.11** Statistical distribution of MPI among the 7 groups determined by cluster analysis



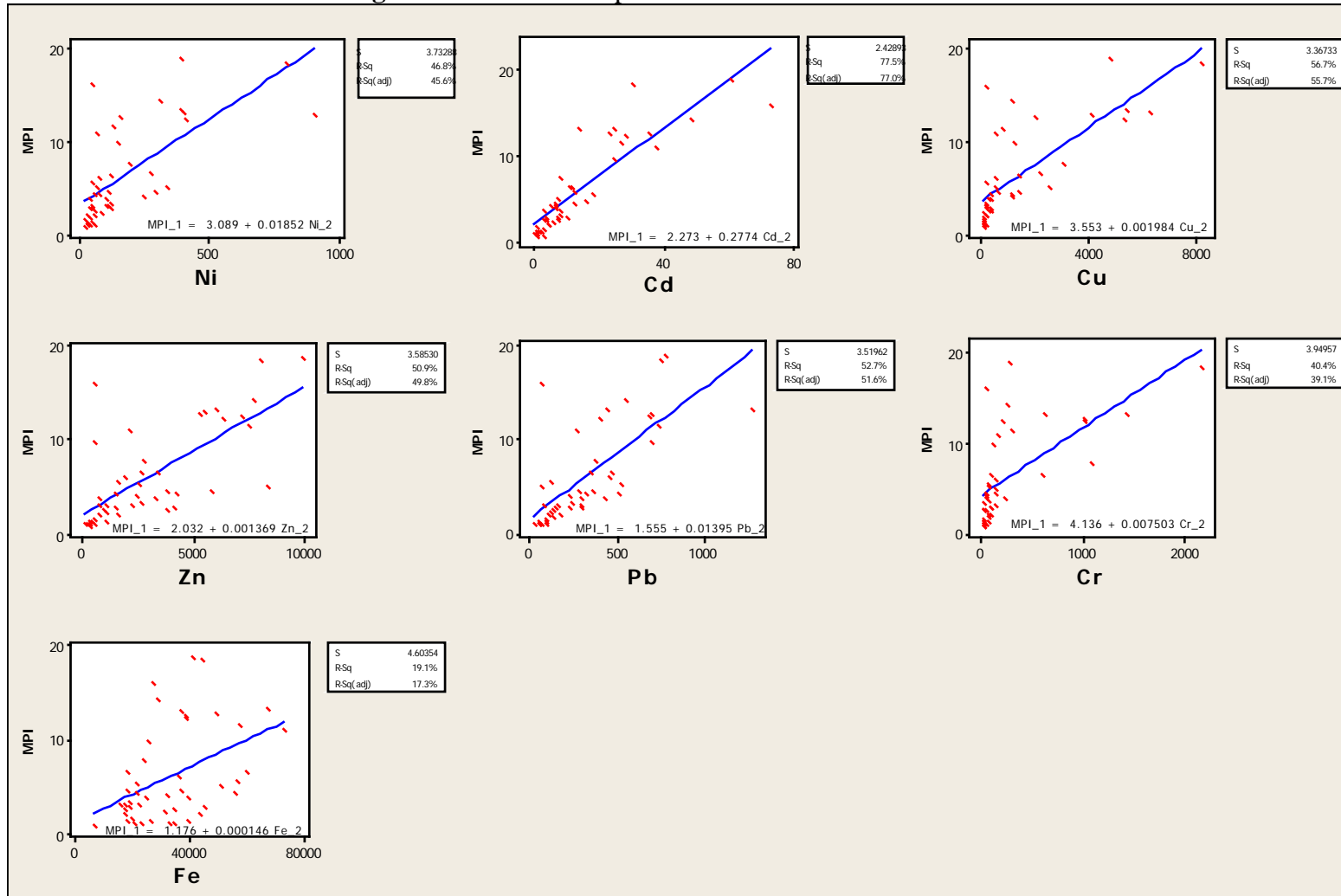
In Table 4.8 it can be seen that Group A have mean MPI values of about 0.5. Therefore, these sites can be classified as being unpolluted by the seven heavy metals measured in this study. This compares with mean MPI values of about 9.5 in group G.

#### 4.3.6 REGRESSION ANALYSIS

Fig. 4.12 shows the sensitivity to each metal to variations in MPI. The only metal to show a strong correlation was cadmium ( $r^2_{\text{adj}}$  77%). Reasonable correlation was shown by copper ( $r^2_{\text{adj}}$  55.7%) and lead ( $r^2_{\text{adj}}$  51.6%), while a low correlation was exhibited by iron ( $r^2_{\text{adj}}$  17.3%).

The sensitivity of individual metals to MPI variations (Fig. 4.12) illustrates that cadmium shows the strongest correlation with MPI and therefore suggests that cadmium will be the most sensitive pollution indicator. This perhaps shows that this element will also be better used as a general indicator of sediment pollution and other metals better to pinpoint specific problem areas. Cadmium and lead showed slightly less sensitivity, followed by nickel/copper. Chromium and iron showed low correlation to MPI. Fines and volatile organic matter were not correlated at all to MPI.

**Figure 4.12** Relationships between trace metal concentrations and MPI



## **4.4 DISCUSSION**

### **4.4.1 HOW THIS STUDY COMPARES WITH OTHER SYSTEMS**

Previous studies have shown that metals interact in solution with dissolved organic matter (by the process of chelation and complexation) and in turn are concentrated by adsorption onto fine particulates (Qu and Kelderman, 2001). Iron, was significantly correlated with fine particles in this study. Other geochemical support phases may also occur. For example, co-precipitation of lead with iron sulphide may occur in canals (Evans, 1989). Under anaerobic conditions that are often present in canals, especially in summer, iron sulphides are formed by interactions between iron and hydrogen sulphides. Iron oxides may also play a role in retaining lead (Izquierdo et al., 1997). In this study there was indeed a significant correlation between lead and iron. Fe/Mn hydroxides have been shown to be the main carrier for certain metals as Cd, Zn and Ni (Salomons et al., 1987). Carrier particles with similar sedimentological properties have been shown to occur in other canal systems (Qu and Kelderman, 2001). Transportation in suspended solids was seen in other canals, such as the Delft canals (Kelderman et al., 1998; Kelderman et al., 2000; Qu and Kelderman, 2001). The transport of trace metals is dependent on the partitioning of the metals between dissolved and particulate phases and other environmental conditions. Trace metals may be recycled via chemical and biological processes within the sediment and back to the water column. Grain size of sediment is one of the major controlling factors for the distribution of trace metals. Metals on coarse particles are in a crystalline solid state, usually in low concentrations and are environmentally immobile. On the other hand fine particles such as clay and colloidal materials are generally surface active and contain organic matter while Fe/Mn oxide surface coatings can play an important role in controlling deposition of trace metals to sediments (Ip et al., 2006). As well as physical transportation of metals on fine grains, other factors controlling spatial variations include the chemical condition of the sedimentary environment (via e.g. sorption-adsorption of trace metals, flocculation etc). Distribution of metals between solution and particulate materials is strongly affected by changes in the chemical property of particles and their surrounding area which is strongly related to the

distribution of trace metals in sediments. They may show geochemical behaviours or originate from anthropogenic sources.

Previous studies have used mean ERM quotients in relation to the probability of toxicity. Studies matching chemical and toxicity data from estuaries in the USA, showed that a mean ERM quotient of  $<0.1$  has a 9% probability of being toxic; a quotient of 0.11-0.5 has a 21% probability of toxicity; a mean quotient of 0.51-1.5 a 49% probability of being toxic and a mean ERM quotient of  $>1.5$  has a 76% chance of toxicity (Long & MacDonald 1998; Long et al., 2000). On these criteria, 31 of the sites in this study would be considered to have a high probability of toxicity, while only two of the sites (SU-PB and ST-ST) would be considered to have low toxicity according to the above.

The analytical procedure used here has proved to be a useful tool for the classification of sediment pollution levels in urban canal sites. A general classification of the pollution levels, in relation to metals, was achieved and the most critical sites identified. Highly contaminated sites were revealed and the exploratory analysis of the data allowed the identification of abnormal metal concentrations at various sites.

Sixty six percent of the sites in the current study had a mean quotient of  $>2.5$  and therefore a 76% probability of sediment toxicity. These therefore would be considered the highest priority areas for attention.

#### **4.4.2 RELATIVE EFFECTIVENESS OF MEASUREMENTS OF TOXICITY**

The various indices have different aims and can be divided into two groups: (i) contamination and background enrichment indices which measure the contamination or enrichment levels and (ii) ecological risk indices, which evaluate the potential for observing adverse biological effects.

##### **(i) Contamination and background enrichment indices**

MPI is only a contamination index and does not compare the contaminants with any value. There is no threshold classification from unpolluted to high pollution. It calculates a geometric average which has advantages when compared with other aggregate methods since it highlights concentration differences.

## (ii) Ecological risk indices

Ideally heavy metal assessment indices should not be used as the only evidence of sediment quality. The integration of contamination assessment with biota and toxicity evaluation should also be carried out to allow a weight of evidence for sediment quality assessment.

CCU in water measures total metal concentration and toxicity. The criterion values are based on toxicity tests on species from different trophic levels. The postulated advantages are in integrating different metal concentrations into a single variable that might represent additive effects and in expressing toxicity to thresholds. Therefore, the CCU concept represents a possible method of linking ecotoxicological data with ecological assessment.

## **4.5 SUMMARY**

It has been shown in this chapter that sediment quality guidelines can be effectively used as a screening tool when identifying, ranking and prioritising metals which are of most concern by comparing their concentrations to ERL and ERM values. However, because the mean quotient is used, the contribution of individual metals to the overall toxicity of the sample may be diminished. A metal with a very high concentration in a sample containing metals with low concentrations will see the overall toxic effect reduced, so may not reflect the true toxicity of the sediment.

## **CHAPTER 5**

# **RELATING ENVIRONMENT TO CHIRONOMID ASSEMBLAGES**

### **APPROACH**

Forty-six sites on canals in the English Midlands were sampled using the Chironomid Pupal Exuviae Technique (CPET). Species data were associated with water and sediment chemistry at each site. Separate CCA analyses were carried out on (i) all species and all chemical variables, Pb (water and sediment), TON and fines were the best variables discriminating between sites) (ii) epibenthic species and water chemistry Cr, Pb & Chlorophyll) (iii) inbenthic (sediment dwelling) species and sediment chemistry (Fines, Pb & N. Biological classifications constrained by each of the significant variables were used to calculate indicator species scores and reveal species assemblages. The use of chironomid exuviae was found to be an effective tool to determine water and sediment quality within urban canals.

## 5.1 INTRODUCTION

The recent implementation of the European Commission legislation and the water Framework Directive (WFD) (WFD, 2000) requires that all member states protect and enhance all surface and groundwaters. ‘Good ecological status’ has to be achieved by 2015. Canals, however, do not fall under this designation. The Framework Directive provides for separate designations, that of Artificial (AWB) and Heavily Modified Water Bodies (HMWB), with canals falling under the former designation. Under the directive an “Artificial Water Body” is surface water created by human activity where no water body existed before and which has not been created by direct physical alteration, movement or realignment of an existing water body. Unlike all other surface waters, which have to achieve good ecological status, AWBs will be required to reach an objective of “Good Ecological Potential” (GEP), which should allow for the water body to be used in a sustainable manner. Quite how this should be achieved is a matter for some debate and it is certainly a complex question.

In designing biomonitoring programs for aquatic systems, consideration is normally given to the type of waterbody being considered, the type of stress potentially affecting the ecosystem and the timeframe of the study. By incorporating knowledge of how different organism groups react to different stressors a more cost-effective monitoring programme can be designed. Four organism groups are being addressed by the WFD (fish, macroinvertebrates, macrophytes and diatoms). These are often correlated and it has also been suggested that it is not necessary to monitor all these groups simultaneously (Hering et al., 2006).

Current bioassessment tools are inadequate for canals. Applying the BMWP (Biological Monitoring Working Party (Armitage et al., 1983) system of water quality assessment would be problematic as this is based upon the sensitivity of macroinvertebrate taxa to organic pollution, and therefore it cannot be used to assess toxic, metal or inorganic forms of pollution. To address this problem a revised assessment method, PSYM (Predictive SYstem for Multimetrics), (Pond Conservation Trust, 2002) was developed to provide sensitive and robust quality assessments. This combines the predictive approach of RIVPACS (River Invertebrate

Prediction And Classification System (Wright et al., 2000) with the multimetric-based methods used for ecological quality assessment in the United States. In multimetric assessment, values from individual metrics are combined into a single measure. For canals specific invertebrate metrics can independently assess both water quality and habitat quality, although it is difficult to gain insight into the complex nature of urban situations using this method.

Bioindicators are generally identified by establishing a strong relationship with some environmental characteristic (Kitching et al., 2000; Davis, 2001; McGeoch et al., 2002; Ruse, 2002). To establish the robustness of the relationship, the same environment should be resampled at different times and in different habitats, elsewhere in the geographical area where the bioindicator will be used (Weaver, 1995). Indicator species are indicative of particular groups of sites. 'Good' indicator species should be found mostly in a single group of a classification and be present at most of the sites belonging to that group, or at least have the potential of being present at most sites.

It has been found that large abundance of individuals are generally associated with high fidelity (frequency of occurrence) of a species over sample sites (Brown, 1984; Gaston et al., 1997). These characteristics facilitate monitoring, which is an important criterion for a useful biomonitoring tool (Kremen et al., 1994). Species that have other combinations of specificity and fidelity can also be useful indicators, as detector species. These can be used to monitor environmental change when they span a range of conditions, therefore having low specificity, and could be more useful in indicating direction of change. Bioindication in aquatic systems has made use of such species, which have a range of preference for different environmental conditions (Williams et al., 1986; Weatherley and Ormrod, 1990).

A fundamental requirement of any bioassessment technique that is used for water quality monitoring is the ability to distinguish natural variability from human-induced changes (Resh, 1995). In developing ecological assessment tools a number of factors have to be considered: (i) number of species in the taxonomic group (ii) reliability of species (iii) survey methodology (iv) how represented the species is in all the

available habitats in the ecosystem to be assessed (Dodkins et al., 2005). To be of diagnostic value, the species or groups should be both sensitive and stressor specific (Hamalainen, 2000). Current approaches in the assessment of waterbodies for systems that use specific biotic indices or scoring systems (Armitage et al., 1983; De Pauw and Vanhooren, 1983) intend to measure either water (or habitat) quality or pollution. In these cases the quality assessed is more general (mainly organic) pollution and so indicator values of the taxa are more subjective and the resulting index values are also subjective. In the US, a metrics-based system is used. Metrics are measures (taxa richness, functional groups) that are used to develop multi-metric systems (Simon and Lyons, 1995). Using a metrics-based system can be problematic in that the most commonly used metrics are highly variable and can be insensitive to subtle changes in water quality (Resh et al., 1995).

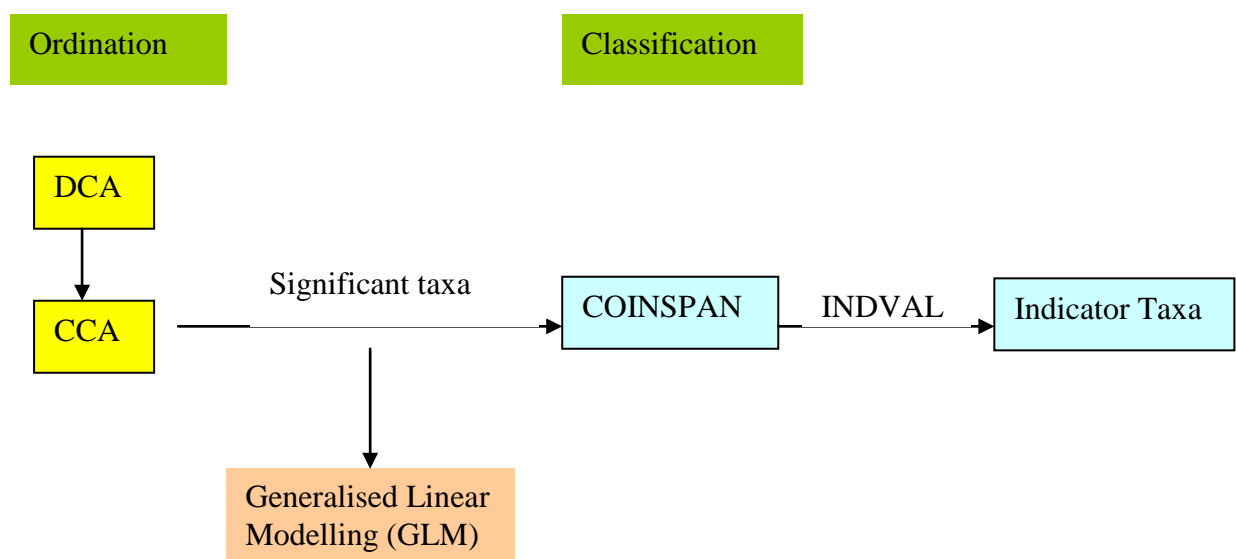
A further complication is the fact that different sampling strategies are required for biological assessment using these methods which varies in relation to the habitat being studied. The use of chironomid pupal exuviae is a sampling method that overcomes these sampling difficulties. This group have been extensively used to monitor lake status, being first successfully demonstrated by (Thienemann, 1910). Subsequent work has demonstrated that they can be used to classify lakes according to hypolimnetic oxygen concentration (Brinkhurst, 1974; Saether, 1979) and food availability (Saether, 1979).

To circumvent the above-mentioned problems this chapter uses weighted averaging (WA) regression to create a robust model of species responses. The weighted averaging approach is based upon ecological theory – species having unimodal responses segregated along environmental gradients (ter Braak, 1987). Percentage abundance of taxa is used, therefore, WA is an appropriate method. Indicator values for species are thus expressed quantitatively in terms of the environmental variable of interest. It is used routinely to reconstruct past environments (Birks, 1998; Bennion and Smith, 2000; Brodersen and Anderson, 2002), but only a few studies have used the technique to model present-day species-environment relationships (Brodersen et al., 1998; Brodersen and Lindegaard, 1999). All training sets model present day

species-environmental relationships but relatively few paleoecological studies investigate change in more recent decades.

In order to develop a tool for the monitoring of urban canals, the aims of this study are therefore to (Fig 5.1):

- (i) investigate which chemical parameters influence the variation in chironomid taxa composition;
- (ii) determine indicator chironomid taxa for significant chemical variables;
- (iii) determine if chironomid species respond to metal pollution and does the CCU approach (Chapter 3) provide a useful method for evaluating metal toxicity in canals.



**Figure 5.1** Diagram of the analysis steps taken to create indicator taxa

## 5.2 MATERIALS AND METHODS

### 5.2.1 NUMERICAL ANALYSIS

#### 5.2.1.1 ordination

Species data at each site were combined and abundance recorded as a percentage of the total number of exuviae collected. To improve the signal to noise ratio, the species data were square-root transformed, as recommended by (Prentice, 1980). All environmental data were tested for normality (MINITAB <sup>TM</sup> v14) and transformations performed where necessary. Reciprocal square roots were used for chromium (water and sediment), cadmium, ammonia, phosphorous, nitrogen, ortho-phosphate, pH and nickel (sediment). Nickel, BOD, conductivity and hardness were all reciprocal transformed. Reciprocal square transformations were used for alkalinity. All remaining variables were log-transformed (Table 6.1).

**Table 5.1** Statistical transformations

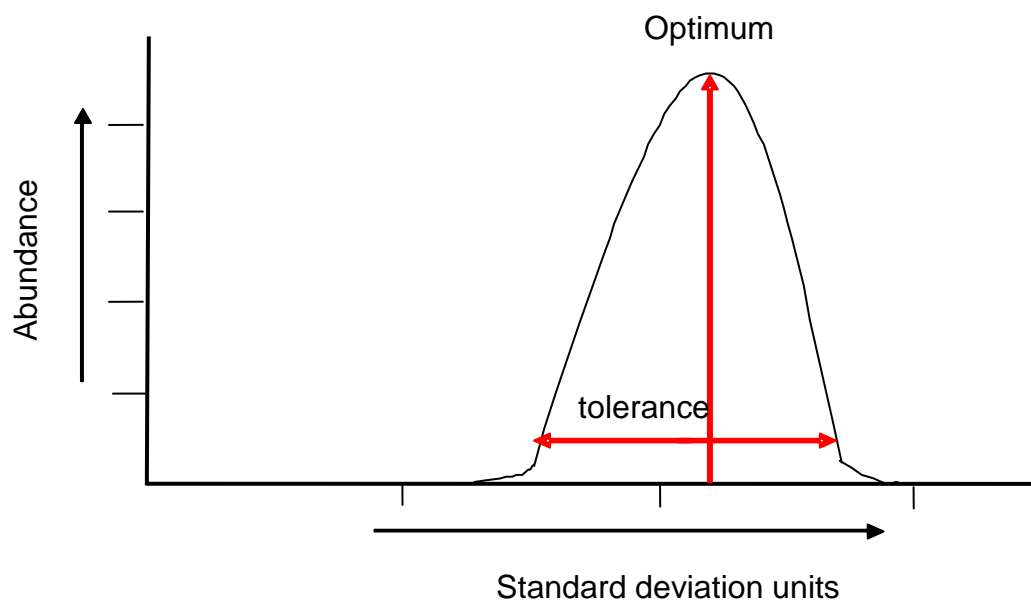
(a) water

Variable	Transformation
Zn	log10
Cr	reciprocal square root
Ni	reciprocal
Pb	log10
Cu	log10
Fe	log10
Cd	reciprocal square root
NH <sub>3</sub>	reciprocal square root
P	reciprocal square root
N	reciprocal square root
TON	log10
Chl A	sqrt
NO <sub>3</sub>	log10
NO <sub>2</sub>	log10
O-P	reciprocal square root
DO	no transformation
BOD	reciprocal
Cl	no transformation
Ca	log10
Alk	reciprocal square root
pH	reciprocal square root
Cond	reciprocal
Hdss	reciprocal

(b) sediment

Variable	Transformation
Cu	log10
Zn	log10
Cd	log10
Pb	log10
Cr	reciprocal square root
Fe	log10
Ni	reciprocal square root
N	log10
P	log10
Volatiles	square root
fines	square root

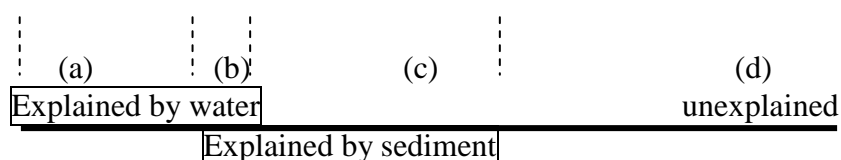
Detrended Correspondence Analysis (DCA) was carried out to determine whether there was a unimodal (bell-shaped) relationship apparent between the species abundance data and the primary ordination axis (Leps and Smilauer, 2003). This was employed to investigate gradient lengths and relationships between surveys based on species data unconstrained by environmental data. The first DCA axis had a gradient length of 2.32 standard deviation units, which is too long for linear ordination but suitable for unimodal analysis. As a result the relationships between environmental data and chironomid species were investigated using Canonical Correspondence Analysis (CCA). CCA is a direct gradient analysis technique in which the ordination axes are constrained as linear combinations of environmental variables (ter Braak, 1986; ter Braak, 1987). Biplot-scaling with emphasis on inter-species distances was used. A species maximum abundance, the highest point of the distribution curve, being its optimum value (species score) in relation to the ordination axis; while its niche breadth is measured by the width of the bell-shaped curve and known as its tolerance (Fig. 5.2).



**Figure 5.2** Species optimum and tolerance defined when unimodally related to ordination axis

Forward stepwise regression was used to select the minimal number of explanatory variables that could significantly ( $p < 0.05$ ) explain the largest amount of variation in the species data. The significance of the variables was tested by 999 unrestricted Monte Carlo permutations using a Bonferroni adjustment to avoid the inclusion of redundant variables (Manly, 1991). Probability levels of significance began at  $\alpha = 0.05$  for selection of the first variable and adjusted with  $P = \alpha/n$ , where  $n$  is the variable rank. Ordination analyses were performed using CANOCO 4.5 (ter Braak, 2003).

To test which water or sediment chemical variables were the most significant in explaining the distribution of chironomid species, a partial CCA was carried out. This is used to isolate effects of particular explanatory variables (Borcard, 1992; Anderson and Gribble, 1998). Partial CCA was used to investigate the fraction of the variation explained by [a] water chemical variables only, [b] sediment chemical variables only [c] auto correlated water and sediment variables and [d] unexplained variation (Fig, 5.3).



1. CCA of species data constrained by water variables [a+b]
2. CCA of species data constrained by sediment variables [b+c]
3. CCA of species data constrained by sediment + water [a+b+c]
4. Partial CCA of species data constrained by water with sediment as covariable [a]
5. Partial CCA of species data constrained by sediment with water as covariable [c]

**Figure 5.3** Method of partial canonical correspondance analysis (adapted from Legendre & Legendre, 1998). Horizontal line represents 100% of the variation in the species data

### 5.2.1.2 *Indicator taxa*

A series of steps were followed to build a two-way table from the hierarchical clusters indicator values. The first species group (centre of figure 5.4) contains species that are common in all habitats (i.e., having their indicator value maximum when all sites are pooled in one group). At the next step, two species groups are created: one with species dominating in all wet habitats, and the other one with species that are common in all dry habitats. The procedure is repeated for each site cluster.

A classification of sites constrained to significant environmental variables was carried out using COINSPAN (Carleton, 1996). COINSPAN is a segmentation of gradients produced by CCA, as opposed to segmentation of gradients produced by Correspondence Analysis (CA), as with TWINSPAN (Hill, 1979). The use of an individual taxon to indicate particular environmental preferences is a potentially useful tool. The resulting classifications produced by COINSPAN can be further analysed, together with the original species data, using INDVAL (Dufrêne and Legendre, 1997; McGeoch, 1998). This method examines the characteristic species from the predetermined group of sites according to the presence and abundance of each taxon in each group independently of the others. Each species has an associated indicator value (IV-value) and a p-value obtained by Monte Carlo permutations (999 runs). Significance was tested at the 0.05 level. INDVAL v2004 (Dufrêne and Legendre, 1997) was used to carry out this analysis. Species with higher than 5% appearance in all samples were allocated the highest indicator values.

The index value changes along a typology and decreases (increases) for generalist (specialist) species. This method combines measures of specificity and fidelity (McGeoch et al., 2002) producing an indicator value (INDVAL) for each species as a percentage, as follows:

Specificity measure:  $A_{ij} = N_{\text{individuals}_{ij}} / N_{\text{individuals}_i}$

Where  $N_{individuals_{ij}}$  is the mean number of species  $i$  across sites of group  $j$  and  $N_{individuals_i}$  is the sum of the mean numbers of individuals of species  $i$  over all groups;

Fidelity measure:  $B_{ij} = N_{sites_{ij}}/N_{sites_j}$

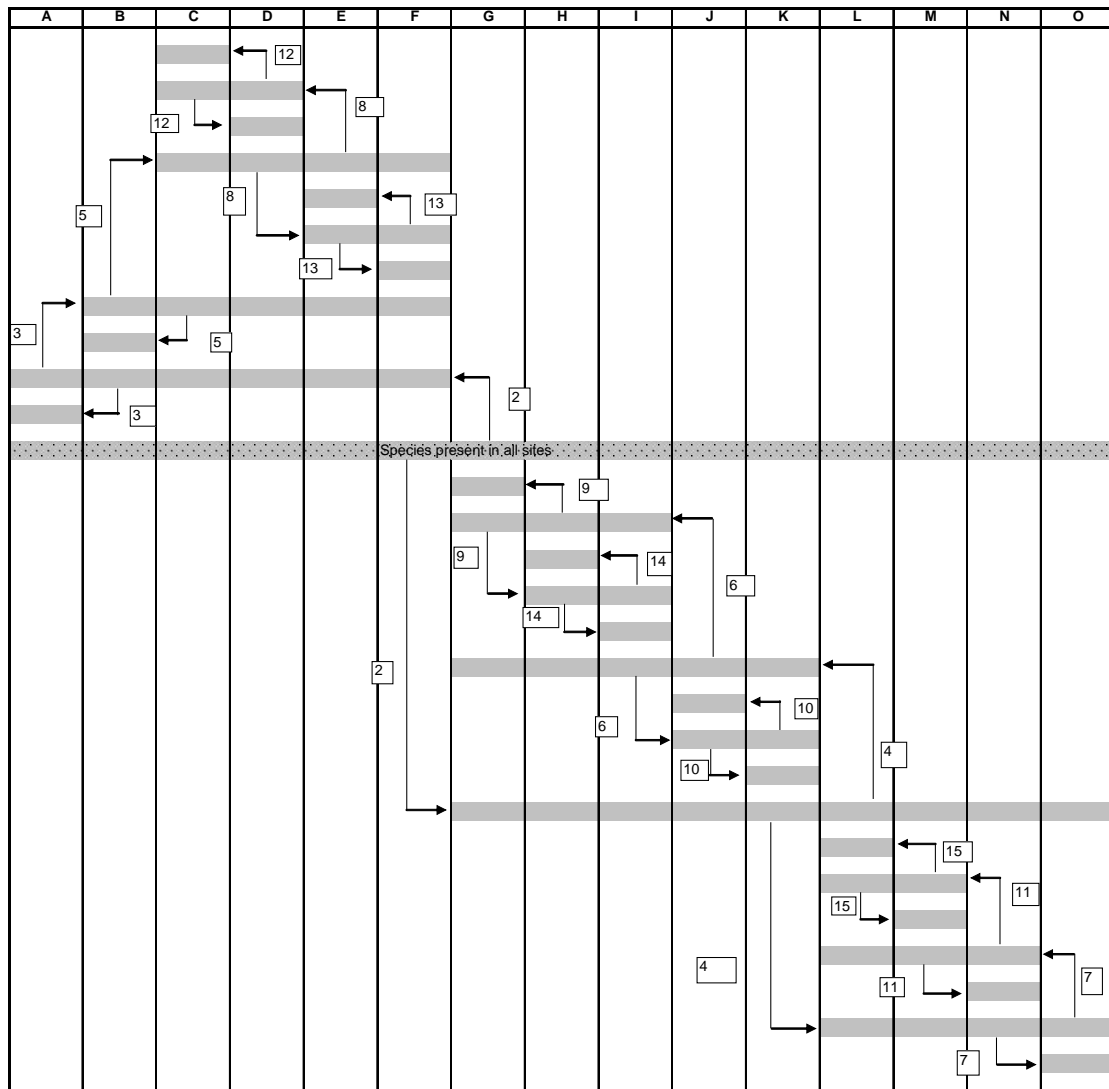
Where  $N_{sites_{ij}}$  is the number of sites in cluster  $j$  where species  $i$  is present and  $N_{sites_j}$  is the total number of sites in that cluster.

The percentage indicator value for species  $i$  in cluster  $j$  is therefore:

$$IndVal_{ij} = A_{ij} \times B_{ij} \times 100.$$

The INDVAL index is computed for all levels of a classification, fitting (eurytopic) species with larger niche breadths into the typology.

INDVAL scores were calculated for every species at each division and arranged in a two-way table. Fig 5.4 shows the steps that are followed to build up a two-way table for chromium from the hierarchical COINSPAN classification using INDVAL. The first species group in the centre of the figure contains those species common to all sites (their indicator values are maximum when all sites are pooled in one group). In the next step, two species groups are created, separating high from low levels of the variable under consideration. The procedure was repeated for each site cluster. A minimum threshold level of 25 was used for the INDVAL value (Dufrene & Legendre, 1997). This supposes that a characteristic taxon is present in at least 50% of one site group and that its relative abundances in that group reaches at least 50%. If one of the two values reaches 100%, the other is always greater than or equal to 25%.



**Figure 5.4** Steps that are followed to build a two-way table from the COINSPAN clusters indicator values (Chromium)

#### 5.2.1.3 Generalized Linear Modelling (GLM)

Relationships between environmental and multivariate space for selected species were also interrogated using Generalized Linear Modelling (GLM) (McCullagh, 1983) using Canodraw (ter Braak, 2003).

#### *5.2.1.4 Regression analysis*

Best subset regression was performed to find the best two- and three predictor models that were then evaluated using multiple regression analysis. Best subsets regression generates regression models using the maximum R criterion (MINITAB, 2004). All one-predictor regression models were examined first, followed by all the two models giving the largest R. The two models with the largest R are chosen and this process continues until the model contains all predictors.

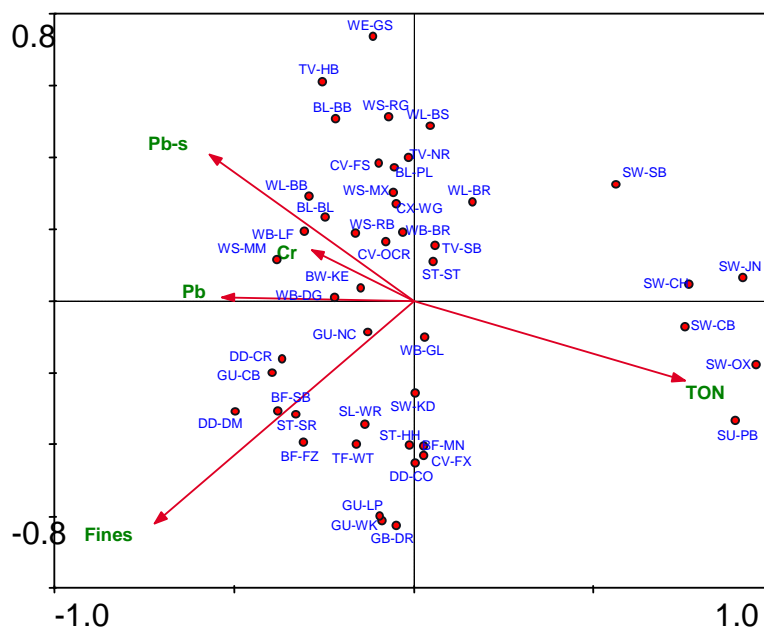
The methods to calculate CCU can be found in (Chapter 3). Following ordination by CCA, scores on the first two axes were related to metal concentrations and CCU using multiple stepwise regressions with forward selection. A second regression was carried out including all environmental variables. For chironomid species assemblages, a third regression was carried out using as predictors metal concentrations and CCU.

## 5.3 RESULTS

### 5.3.1 SPECIES-ENVIRONMENT RELATIONSHIPS

#### 5.3.1.1 Combined dataset

A total of 28,772 chironomid pupal exuviae were identified to 88 taxa from 46 sites. After forward selection with the original 34 chemical variables, 5 were selected (Table 5.2). These were sediment fines, total oxidised nitrogen (TON), lead (Pb) and chromium (Cr) (Fig. 5.5). The first two axes explained 17% of the variation seen in the species data and both axes were significant in a restricted permutation test ( $P < 0.001$ ). Table 5.2 shows the results of variation partitioning, of each significant variable when each was constrained in a CCA. TON accounted for most of the variability on the canonical axes and became less important for sediment fines, sediment lead, dissolved lead and finally chromium.



**Figure 5.5** CCA biplot of sites constrained by all chemical variables combined and both epi- and enbenthic taxa

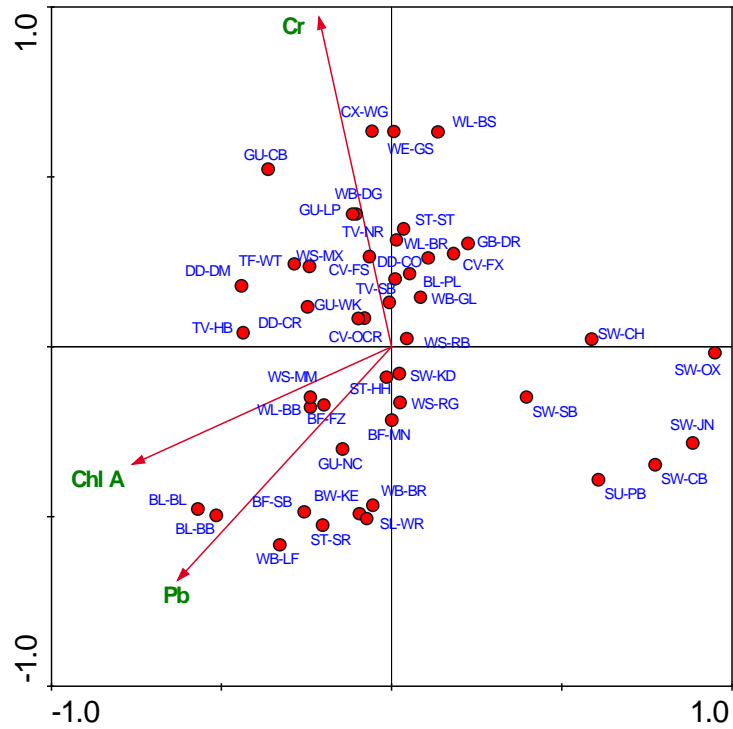
**Table 5.2** Strength of the relationship between species data and selected environmental variables constrained separately to the first canonical correspondence analysis (CCA) ordination axis. Strength is expressed as the eigenvalue ratio axis 1 vs. axis 2 ( $\lambda_1/\lambda_2$ )

Environmental variable	$\lambda_1$	$\lambda_2$	$\lambda_1/\lambda_2$
TON	0.113	0.152	0.74
fines	0.098	0.152	0.65
Pb-s	0.097	0.178	0.55
Pb	0.084	0.175	0.48
Cr	0.077	0.182	0.42

#### 5.3.1.2 Epibenthic taxa

52 epibenthic species (Wilson, 1993) remained in the dataset after the removal of the enbenthic species. The gradient length in a DCA was 2.319 suggesting that unimodal analyses were an appropriate direct gradient tool; thus CCA was the preferred analytical method.

After forward selection and Bonferroni-type adjustments, three variables were significantly related to the species, chlorophyll, lead and chromium (Fig. 5.6). The first two axes were significant in a restricted permutation test and captures 16.6% of the variance in species data and 82.5% of this could be explained by the three environmental variables.



**Figure 5.6** CCA plot of sites using epibenthic taxa, showing significant variables

Partial CCA was carried out to determine variance explained by each of the significant variables. When a CCA is constrained to one variable, the first eigenvalue ( $\lambda_1$ ) represents the variance explained by that variable and the second eigenvalue ( $\lambda_2$ ) represents the maximum amount of variation explained by the unconstrained axis orthogonal to the variable axis. The ratios of the two eigenvalues ( $(\lambda_1/\lambda_2)$ ) (Table 5.3) were used to describe the relative strength of the environmental variable constrained to the first axis (ter Braak, 1992). Chlorophyll A and lead (Pb) accounted for most of the variability on the canonical axes and Chromium (Cr) was less important (Table 5.3).

**Table 5.3** Strength of the relationship between species data and significant environmental variables constrained separately to the first canonical correspondence analysis (CCA) ordination axis. Strength is expressed as the eigenvalue ratio axis 1 vs. axis 2 ( $\lambda_1/\lambda_2$ )

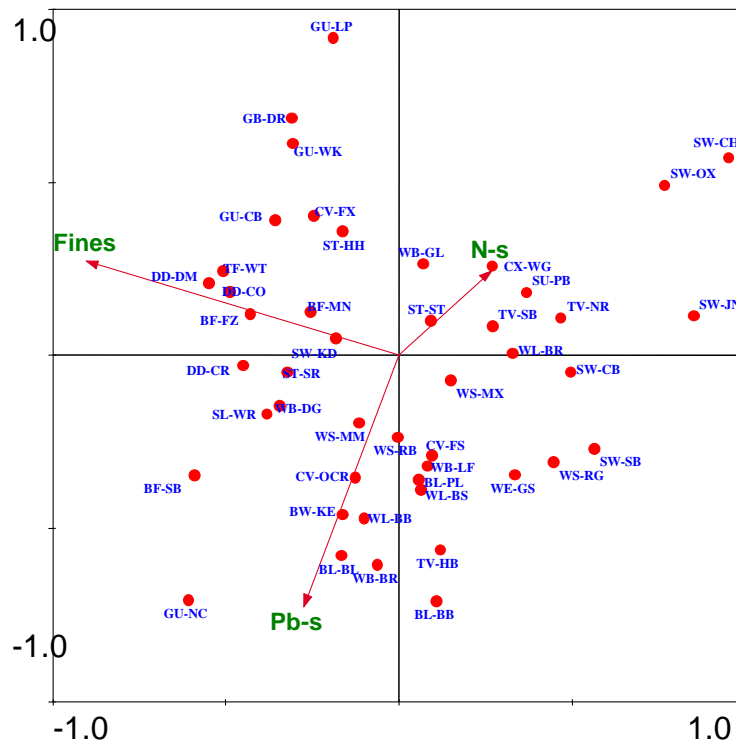
Environmental variable	$\lambda_1$	$\lambda_2$	$\lambda_1/\lambda_2$
Chl A	0.136	0.192	0.71
Pb	0.124	0.202	0.61
Cr	0.095	0.238	0.40

The ordination plot (Fig. 5.6) shows more polluted sites situated in the lower left of the diagram. A separate group of sites can be found to the right of the graph. These sites are situated close to a large sewage works and it seems that these sites are related to organic/nutrient effects, with lower chlorophyll concentrations.

#### 5.3.1.3 *Enbenthic taxa*

35 enbenthic species (Wilson, 1993) were present in the study. A gradient length of 2.252 was produced in a DCA, so the data were analysed using a unimodal technique (CCA). The first two DCA axes explained 30.5% of the species variation.

After forward selection and Bonferroni-type adjustments, three variables were significantly related to species variance, lead, nitrogen and sediment fines (Fig. 5.7). The first two axes were significant in a restricted permutation test and captures 16.9% of the variance in species data and 89.3% of this could be explained by the three environmental variables.



**Figure 5.7** CCA plot of sites (sediment data) showing forward selected variables using enbenthic data

**Table 5.4** Strength of the relationship between species data and selected environmental variables constrained separately to the first canonical correspondence analysis (CCA) ordination axis. Strength is expressed as the eigenvalue ratio axis 1 vs. axis 2 ( $\lambda_1/\lambda_2$ )

Environmental variable	$\lambda_1$	$\lambda_2$	$\lambda_1/\lambda_2$
Fines	0.223	0.349	0.64
Pb-s	0.127	0.251	0.51
N-s	0.065	0.426	0.15

The ratios of the two eigenvalues ( $\lambda_1/\lambda_2$ ) (Table 5.4) were used to describe the relative strength of the environmental variable constrained to the first axis. It can be seen that sediment fines and lead account for more species-environment variation.

### 5.3.2 GENERALISED LINEAR MODELLING (GLM)

GLM illustrated that species diversity with, i) combined taxa - declined with increasing metal concentration and increased with increasing TON (Fig. 5.8a) ii) epibenthic taxa - declined with increasing chlorophyll and lead concentrations and increased with increasing chromium levels – (Fig. 5.8b) iii) enbenthic taxa – declined with increasing lead and increased with increasing nitrogen – (Fig. 5.8c). Species diversity was also shown to decrease with increasing CCU values (Fig. 5.9).

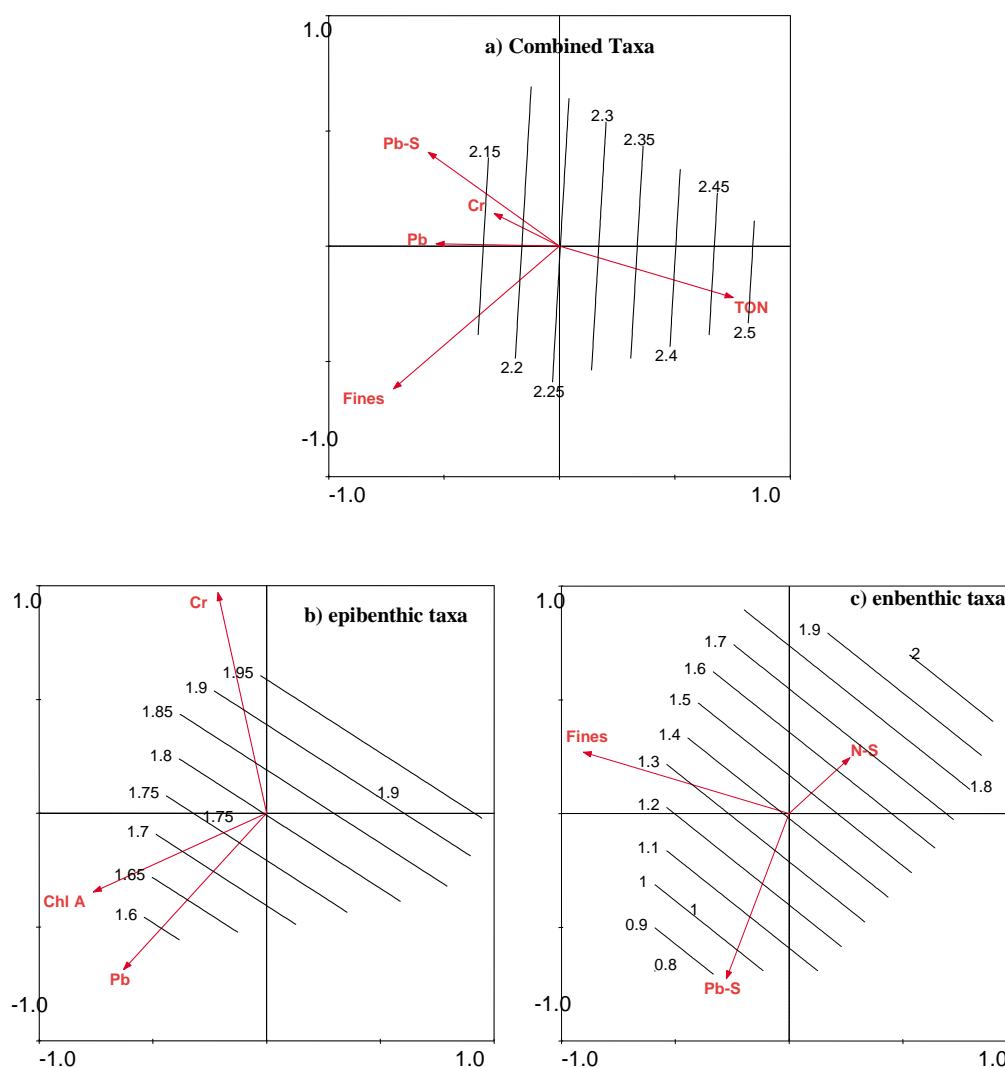
Figs. 5.10–5.12 illustrate how the abundance of individual species responds to chemical variables. Some species were seen to significantly increase their abundance in the presence of metals. *Parachironomus arcuatus* increased in abundance when the data were combined (Fig. 5.10) and also with water chemistry (Fig. 5.11), whereas *Paracladius conversus* increased with increasing levels of sediment chemistry (Fig. 5.12). Other species responded significantly to nutrients. *Cricotopus sylvestris* increased with higher levels of TON (Fig. 5.10), whereas *Parachironomus arcuatus*, as well as increasing abundance with metals also increased with higher levels of chlorophyll (Fig. 5.11). *Xenochironomus xenolabis* was seen to decrease in abundance in the presence of metals but increase with increasing levels of TON (Fig. 5.10). *Procladius choreus* (Fig. 5.10) decreased in abundance with increasing levels of both TON and metals. *Dicrotendipes nervosus* (Fig. 5.10) also decreased in the presence of TON.

When only water was considered (Fig. 5.11) the following patterns were also observed: *Cricotopus sylvestris* decreased with metals and chlorophyll; *Glyptotendipes pallens* decreased with increasing metals and chlorophyll; *Nanocladius bicolor* increased in abundance with increasing levels of chlorophyll and lead, whereas *Xenochironomus xenolabis* decreased with increasing levels of lead and chlorophyll.

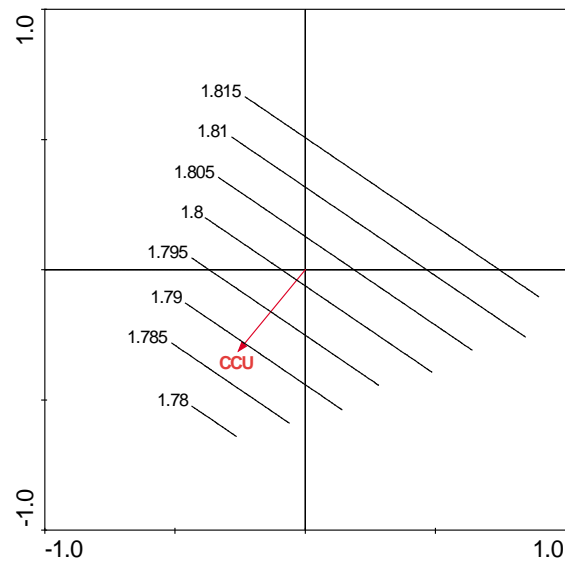
In sediment (Fig. 5.12), *Procladius choreus* decreased in abundance with increasing levels of nitrogen and lead.

In ecological terms, the species are likely to be responding to a number of factors. Metals are reducing diversity, both individually and as a function of CCU, except

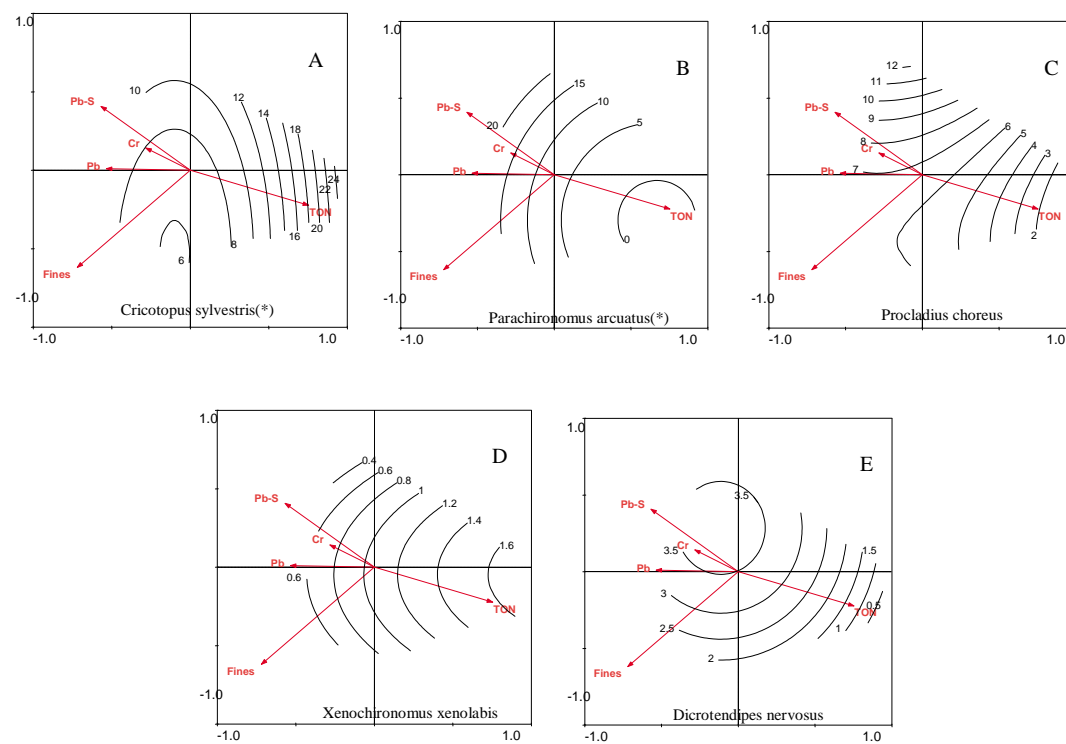
where epibenthic taxa were investigated. Here diversity increased with increasing chromium, with increasing chlorophyll reducing diversity. This may be because there is increased levels of algal activity reducing light levels, so preventing growth of macrophytes. Direct toxic effects or indirect effects via competitive release reducing trophic levels and thus diversity might also be a reason. These may be some of the reasons that affect individual species as well as the whole community.



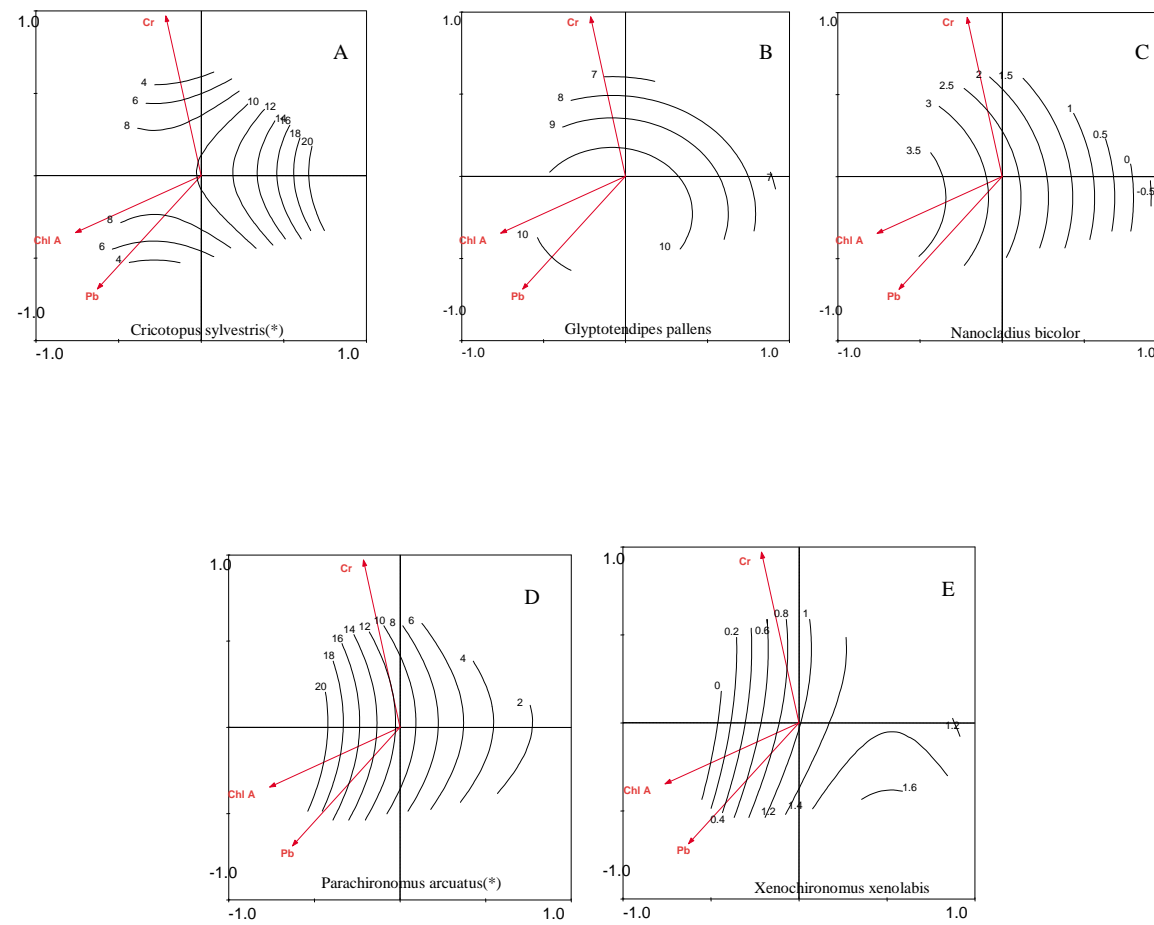
**Figure 5.8** Variation in species diversity in relation to significant environmental variables. The contour lines were generated within Canodraw for windows using GLM (ter Braak 2003). The values refer to Shannon's diversity index.  
a) combined taxa; b) epibenthic taxa and c) enbenthic taxa.



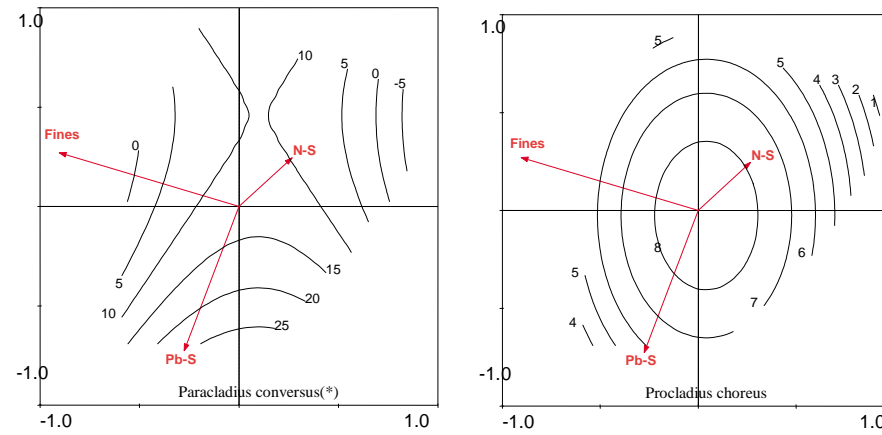
**Figure 5.9** Variation in species diversity in relation to CCU. The contour lines were generated within Canodraw for windows using GLM (ter Braak 2003). The values refer to Shannon's diversity index



**Figure 5.10** Output from the Generalized Linear Models of selected species with N2 values (combined taxa). Contours on the plots refer to predicted abundance values of each species. \* $P < 0.05$



**Figure 5.11** Output from the Generalized Linear Models of selected species with N2 values (water taxa). Contours on the plots refer to predicted abundance values of each species. \* $P < 0.05$



**Figure 5.12** Output from the Generalized Linear Models of selected species with N2 values (sediment taxa). Contours on the plots refer to predicted abundance values of each species. \* $P < 0.05$

### 5.3.3 INDICATOR TAXA

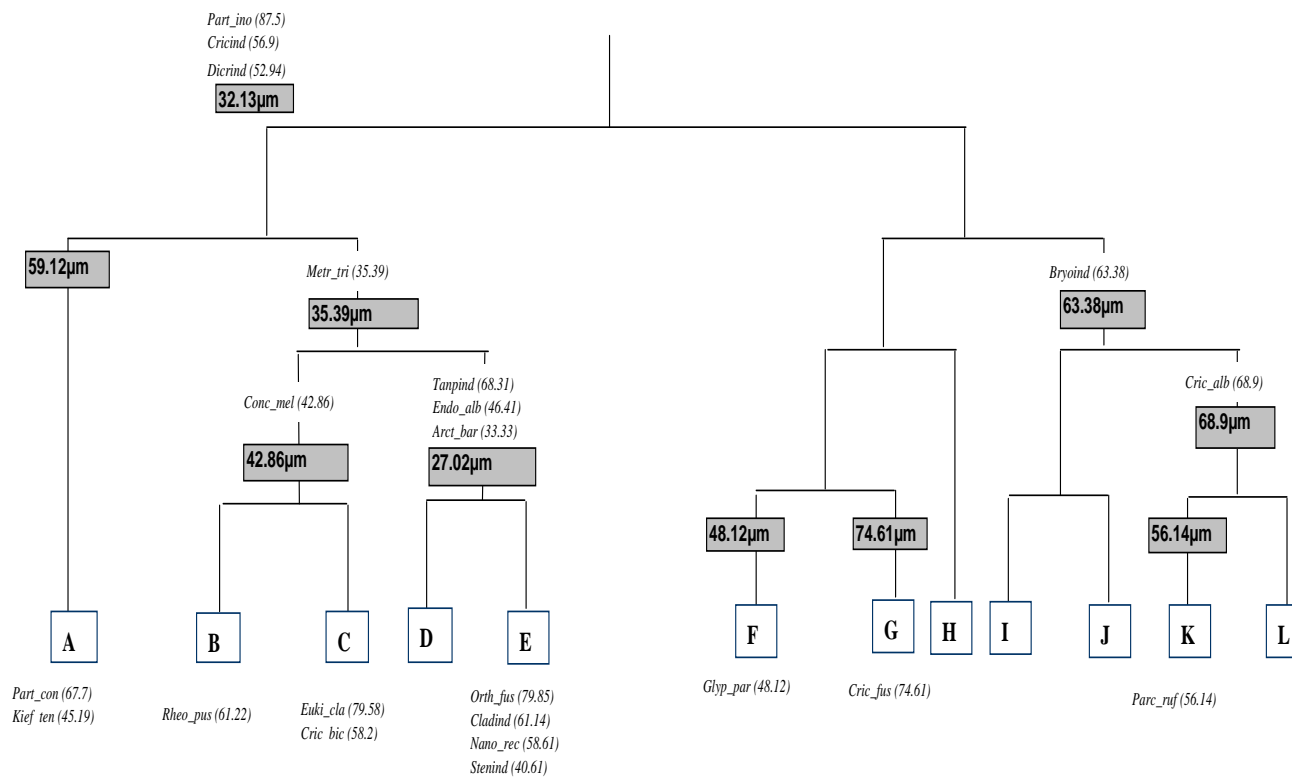
Each significant variable was used to constrain site classifications. Figures 5.13 to 5.21 illustrate the COINSPAN classifications for the combined dataset as well as for the separate water and sediment chemistry data set. Taxa assemblages had a defined mean, determined by their associated COINSPAN group. The INDVAL scores were calculated for every taxon at every COINSPAN division. The taxa were arranged in two-way tables of samples versus taxa. Taxa were placed in COINSPAN group order according to their highest INDVAL score. Species are shown with INDVAL values >25%.

#### 5.3.3.1 Water data set

The highest index value was shown for chlorophyll a was 87.5 (Table 5.5) shown by *Paratanytarsus inopertus* (Part\_ino). This species is characteristic of the first split in the classification dendrogram (group A-E) (Fig. 5.13) where chlorophyll levels are lower. Taxa characteristics of high chlorophyll levels are shown by *Bryophaenocladus sp* (Bryoind) (INDVAL value 63.38, groups I-L) and *Paratrichocladus rufiventris* (Parc\_ruf) (INDVAL value 56.14, group K) with mean chlorophyll levels of 96.65 and 93.85 respectively. *Bryophaenocladus sp* (Bryoind) and *Paratanytarsus inopertus* (Part\_ino) are characteristic eurytopic taxa in that their maximum INDVAL value occurs in higher order groups (at the first split in the COINSPAN classification) and can also be considered generalist taxa. *Paratrichocladus rufiventris* (Parc\_ruf) on the other hand, is an example of a stenotopic (specialist) species, being an indicator of only one site group (K), its indicator value increasing regularly as the number of groups increase. *Cricotopus albiforceps* (Cric\_alb), *Tanypus spp.* (Tanpind) and *Conchapelopia melanops* (Conc\_mel) are taxa that can be considered as bimodal species, i.e they could be more than one species or a generalist. Their maximum INDVAL values occur at an intermediate level of the hierarchy.

The highest maximum INDVAL value achieved for the COINSPAN classification of lead was 85.77 (Table 5.6), shown by *Bryophaenocladus* sp (Bryoind) and together with *Limnophyes* sp. (Limnind) were examples of bimodal taxa indicating high lead levels. Two bimodal species that indicate low lead levels were *Paratanytarsus inopertus* (Part\_ino) and *Tvetenia calvescens* (Tvet\_cal). Three taxa could be described as eurytopic, *Cricotopus* sp, *Dicrotendipes* sp and *Glyptotendipes paripes* (Cricind, Dicrind and Glyp\_par), while six taxa were seen to be stenotopic (Fig. 5.14).

The highest maximum INDVAL value for chromium (91.98) was shown by a stenotopic species, *Cladopelma* sp (Cladind) that indicates high chromium levels (Table 5.7). There were three taxa that showed eurytopic tendencies: *Limnophyes* sp, *Cricotopus* sp (Limnind and Cricind) that indicated high chromium levels; *Endochironomus tendens* (Endo\_ten) that indicated low chromium levels (Fig. 5.15).



**Figure 5.13** COINSPAN classification of chironomid data constrained by chlorophyll (water)

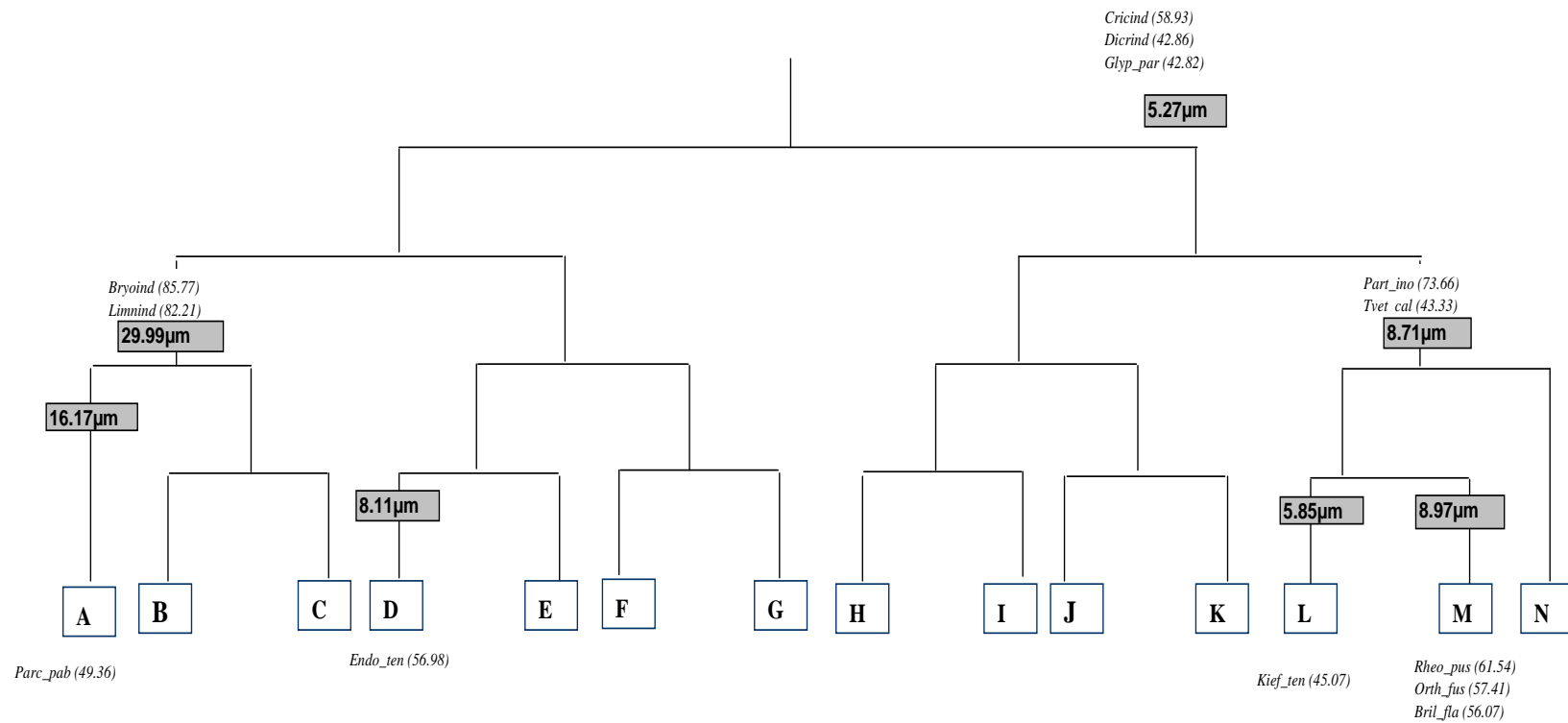
**Table 5.5** Two-way indicator table showing the species indicator power for COINSPAN clustering hierarchy. Species presence is indicated by % abundance.

?? Species passing one significance test

\*\* Species passing both significance tests

	Species	Indval	Mean Chl A	1	2	3	4	5	6	7	8	9	10	11	12
(I+J+K+L)	Bryoind	<b>63.38**</b>	96.65			0.32	1.37								
K	Parc_ruf	<b>56.14**</b>	93.85	0.06						0.08				<b>0.7</b>	
F	Glyp_par	<b>48.12**</b>	80.05	1.72	3.68	0.21	2.33		<b>15.23</b>	1.17	4.86				
(K+L)	Cric_alb	<b>68.9**</b>	69.37					2.77		11.42					
A	Part_con	<b>67.7**</b>	59.12	<b>16.29</b>		1.96	0.69								
	Kief_ten	<b>45.19**</b>		<b>0.95</b>	0.73		0.04								
G	Cric_fus	<b>74.61**</b>	55.11							<b>18.07</b>		0.09			
B	Rheo_pus	<b>61.22**</b>	40.32		<b>0.61</b>	0.15	0.16	0.07							
(A+B+C+D+E)	Part_ino	<b>87.5**</b>	32.13	<b>6.13</b>	<b>0.54</b>										
	Cricind	<b>56.9**</b>		<b>7.18</b>	<b>2.89</b>										
	Dicrind	<b>52.94**</b>		<b>0.58</b>											
E	Orth_fus	<b>79.85**</b>	31.9			0.31	1.09	<b>2.74</b>							
	Cladind	<b>61.14**</b>		0.11			0.93	<b>5.41</b>							
	Nano_rec	<b>58.61**</b>					0.31	<b>2.09</b>				0.23			
	Stenind	<b>40.61??</b>		0.17			0.16	<b>0.39</b>					0.06		
(D+E)	Tanpind	<b>68.31**</b>	27.02	0.09		3.3		<b>1.8</b>	0.21						
	Endo_alb	<b>46.41**</b>				1.42			0.11						
	Arct_bar	<b>33.33**</b>				0.46									
(B+C+D+E)	Metr_tri	<b>35.39**</b>	23.83		<b>0.31</b>		<b>0.03</b>								
(B+C)	Conc_mel	<b>42.86**</b>	21.09		<b>0.12</b>										
C	Euki_cla	<b>79.58**</b>	13.4	0.06		<b>12.12</b>									
	Cric_bic	<b>58.2**</b>				<b>2.77</b>		0.07							

Cluster	Sites
A	TV-NR, WB-GL, ST-HH, WL-BR
B	SW-CH, SU-PB
C	SW-KD, SW-SB, SW-CB, SW-JN, SW-OX
D	CV-FX, WE-GS, CX-WG
E	TV-SB, CV-OCR, CV-FS
F	GU-LP, DD-CO
G	WS-RB, BF-SB, BF-FZ, GU-WK
H	ST-ST, BL-PL, WL-BS
I	TV-HB, WS-MX, WB-BR, ST-SR, WL-BB, BW-KE, GU-CB, SL-WR, DD-CR, DD-DM
J	WS-RG, WS-MM, BL-BL, TF-WT
K	WB-LF, BF-MN, GU-NC
L	WB-FG, GB-DR



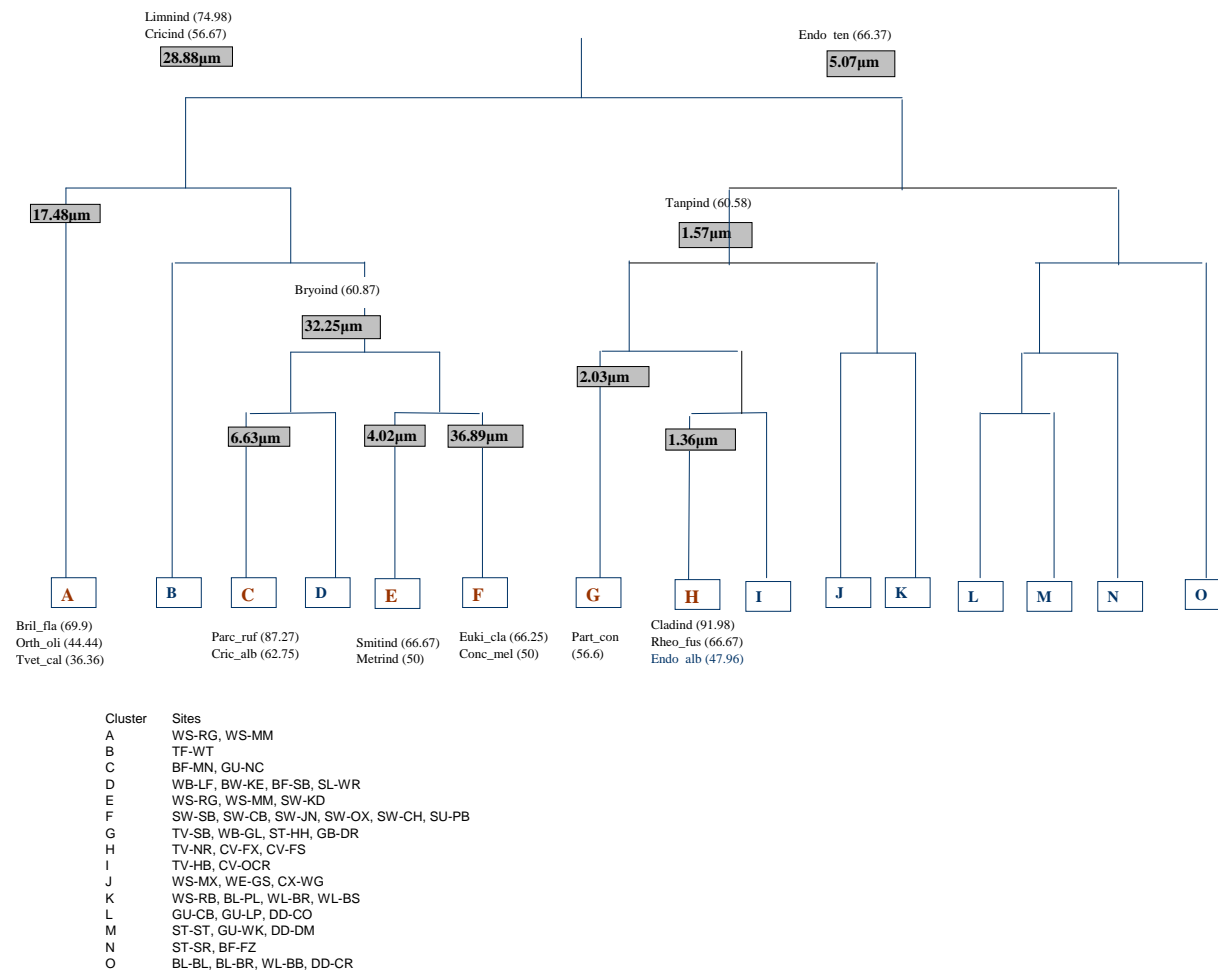
**Figure 5.14** COINSPAN classification of chironomid data constrained by lead (water)

**5.6** Two-way indicator table showing the species indicator power for COINSPAN clustering hierarchy. Species presence is indicated by % abundance.

?? Species passing one significance test  
 \*\* Species passing both significance tests

	Species	Indval	Mean Pb	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(A+B+C)	Bryoind	<b>85.77**</b>	29.99	<b>2.05</b>	0.37												
	Limnind	<b>82.21**</b>		<b>14.4</b>	1.84	0.51	0.51										
M	Rheo_pus	<b>61.54**</b>	8.97								0.06			0.24		<b>0.41</b>	
	Bril_fla	<b>56.07**</b>			0.13		0.49					0.12	0.08	0.06		<b>0.61</b>	
(L+M+N)	Part_ino	<b>73.66**</b>	8.71	0.18	2.32	6.88	<b>10.95</b>										
	Tvet_cal	<b>43.33**</b>			0.27		<b>0.8</b>										
(H+I+J+K+)	Cricind	<b>58.93**</b>	5.27	1.99	<b>7.89</b>												
	Dicrind	<b>42.86**</b>			<b>0.5</b>												
	Glyp_par	<b>42.82**</b>		0.55	<b>2.09</b>												
A	Parc_pab	<b>49.36??</b>	16.17	16.17	<b>17.15</b>			0.61									
D	Endo_ten	<b>56.98??</b>	8.11		0.13	0.06	<b>6.97</b>	1.03	1.21	0.18	2.58		0.8	0.3			
L	Kief_ten	<b>45.07??</b>	5.85					1.32		0.09	0.13	0.82			2.36		0.42
M	Orth_fus	<b>57.41??</b>	8.97				0.73	0.59								<b>0.2</b>	

Cluster	Site
A	TV-HB, GU-NC
B	WB-BR, ST-SR, WL-BB, BF-MN, SL-WR
C	WB-LF, BL-BL, BL-BB, BW-KE, BF-SB
D	CV-FS, WB-DG
E	CV-OCR, GU-WK, DD-CR, DD-DM
F	ST-ST, GU-CB, DD-CO
G	WS-MM, WS-RB, BF-FZ, TF-WT
H	TV-NR, WS-MX, WE-GS, CX-WG
I	CV-FX, WS-RG
J	BL-PL, WL-BR, WL-BS, GU-LP, GB-DR
K	SW-JN, SW-OX, SW-CH, SU-PB
L	SW-CB, WB-GL, ST-HH
M	TV-SB, SW-SB
N	SW-KD



**Figure 5.15** COINSPAN classification of chironomid data constrained by chromium (water)

**Table 5.7** Two-way indicator table showing the species indicator power for COINSPAN clustering hierarchy. Species presence is indicated by % abundance.

?? Species passing one significance test

\*\* Species passing both significance tests

Species	Indval	Mean Cr	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O		
Euki_cla	66.25**	36.89	10.17										0.12						
Conc_mel	50??																		
Bryoind	60.87??	32.25	2.03		1.34	3.08		0.3	0.08										
Limnind	74.98**	28.88	9.63	1.74															
Cricind	56.67**		10.22	1.36															
Bril_fla	69.9**	17.48	0.56	0.09	0.07	0.03													
Orth_oli	44.44??																		
Tvet_cal	36.36??																		
Parc_ruf	87.27**	6.63			0.97	0.07				0.06									
Cric_alb	62.75**		12.2		16.59	0.28									2.38				
Endo_ten	66.37**	5.07	0.03	1.44															
Smitind	66.67**	4.02	0.52																
Metvind	50**				0.24	0.07	1.29	0.13				0.59	0.12						
Part_con	56.67??	2.03	0.37			0.07			19.51				1.07	2.14	3.7	1.46	0.31		
Tanvind	60.58**	1.57	1.48																
Cladind	91.98**	1.36											4.89	0.44					
Rheo_fus	66.67??												0.17						
Endo_alb	47.96??												0.37	1.42	0.09	0.39			

### 5.3.4 BEST SUBSET REGRESSION

Table 5.14 shows the results of the 2- and 3-predictor models. CCU models function best with a combination of species and individual metals, when compared against chironomid tribes or metals on their own. For example, in the 2-predictor model, combinations of species or species and metals had two or three times larger  $r^2$  values respectively as opposed to metals on their own. Similar patterns were observed in the 3-predictor models, where a combination of chironomid tribes and metals had much smaller  $r^2$  values when compared to species combinations or species combinations and metals.

**Table 5.14** Best subsets models

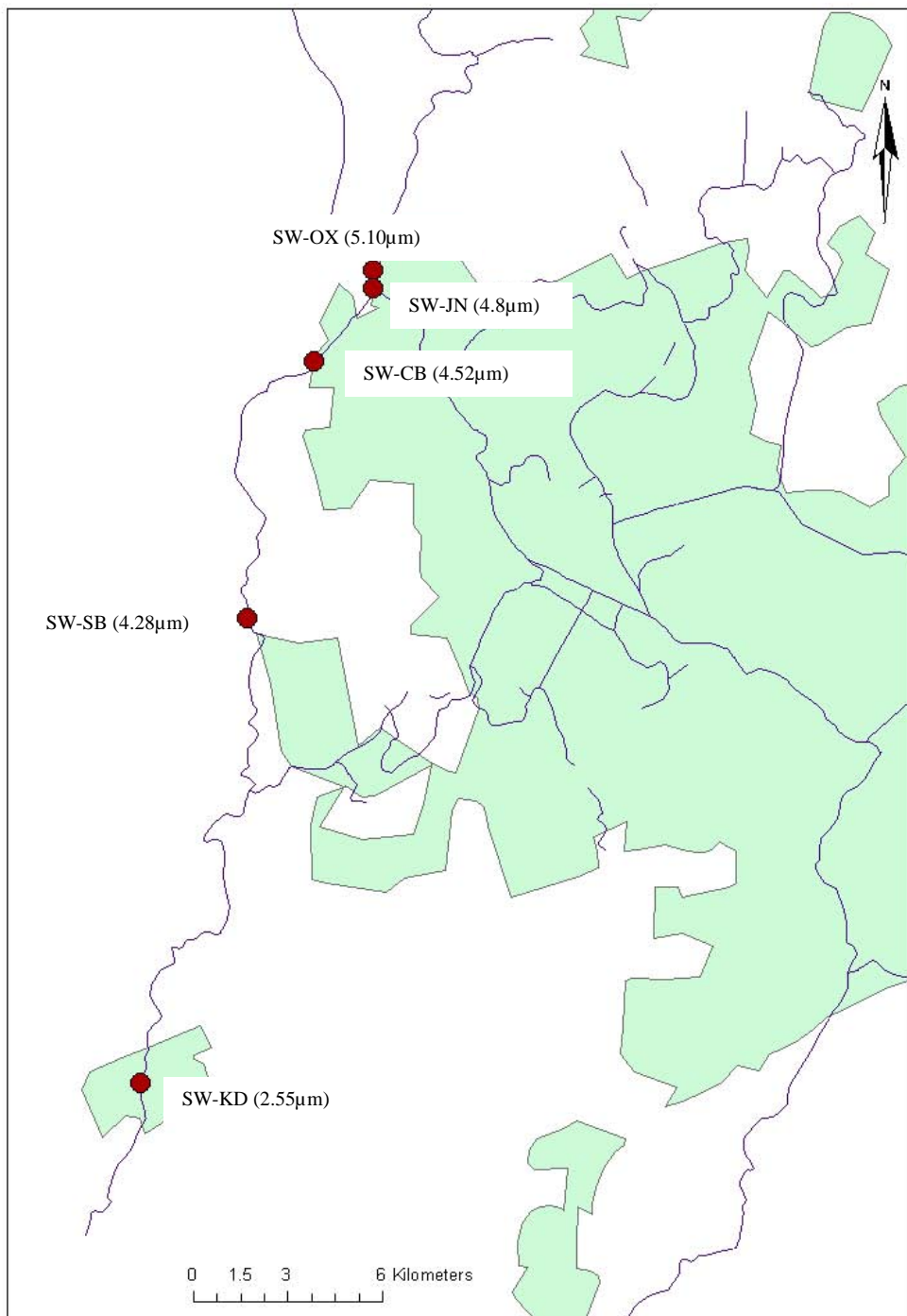
2-model			3 model		
Dependent variable	Predictors in model	$r^2$ (adj)	Dependent variable	Predictors in model	$r^2$ (adj)
CCU	Cu + Fe	11.4	CCU	Pentaneurini + Cu + Pb	18.1
	Zn + Fe	10.8		Pentaneurini + Pb + Ni	16.4
	Nano_bic + Limnind	27.1		Nano_bic + Parc_ruf + Part_ino	29.3
	Nano_bic + Parc_ruf	28.6		Nano_bic + Parc_ruf + Part_con	29.3
	Nano_bic + Cu	38.7		Nano_bic + Part_ino + Cu	39
	Nano_bic + Cr	33.2		Nano_bic + Pb + Cu	38.5

Best subsets regression identifies the best-fitting regression models that can be constructed with the predictor variables you specify. Best subsets regression is an efficient way to identify models that achieve your goals with as few predictors as possible. Subset models may actually estimate the regression coefficients and predict future responses with smaller variance than the full model using all predictors.

## **5.4 DISCUSSION**

### **5.4.1 MAIN ENVIRONMENTAL FACTORS AFFECTING ASSEMBLAGES**

With all chemical variables and species combined the first axis of the CCA ordination reflects nutrient enrichment (Fig. 5.5). A grouping of sites to the right of the ordination graph (on the Staffordshire & Worcester Canal, SW) shows that there is a nutrient effect from a large sewage works. Nitrogen seems to be the limiting nutrient, with the dominant effect coming via sediment bound nitrogen. This can be seen in Fig. 5.7, where sediment nitrogen is the primary variable, whereas chlorophyll a is seen to be dominant when only the water variables are considered (Fig. 5.6). A previous study by Wilson (1993) at the same sites revealed similar patterns in terms of the impact from the effluent discharge into the canal. There was a direct impact near the discharge followed by an improvement in the canal environment down its length. For example, that there is a gradual fall in TON, ammonia, O-P, phosphorus and nitrogen (Fig. 5.16) from SW-OX, to OX-JN, OX-CB, OX-SB and finally at SW-KD (Table 2.2).



**Fig. 5.16** Sites on the Staffs & Worc Canal showing falling levels of Nitrogen from the sewage outfall at SW-OX

As recovery takes place from the source of organic enrichment, it would be expected that algae or macrophytes might increase which utilize the available nutrients. However, there is a general lack of aquatic macrophytes in the Staffordshire and

Worcestershire Canal, most likely due to the high boat traffic, favouring the development of planktonic algal blooms. This is borne out by increasing chlorophyll levels from the outfall downstream to SW-KD. When the sediment chemistry is taken into account, it can be seen that nitrogen levels also progressively falls downstream from the outfall.

The patterns noted above are also seen in the distribution of species. *Cricotopus sylvestris* and *Eukiefferiella claripennis* progressively reduce in abundance from the outfall towards Kidderminster (SW-KD) (Table 5.15), a similar pattern was also observed in the study by Wilson (1993). A similar pattern was also seen in *Paratanytarsus lauterborni* where Wilson noted an increase in abundance between SW-OX (near the outfall) and the next site down (SW-JN) before abundance fell again. It is possible that these changes in species abundance reflected a change in the canal as a consequence of the reduction in the environmental effects of the sewage, i.e. self-purification.

**Table 5.15** Species abundance

	SW-KD	SW-SB	SW-CB	SW-JN	SW-OX
Cric_syl	8.60	10.07	17.44	20.13	33.81
Euki_cla	0.00	0.50	2.40	7.69	27.40

#### **5.4.2 INDICATOR TAXA REFLECTING SIGNIFICANT ENVIRONMENTAL VARIABLES**

The use of INDVAL has previously been shown to be more sensitive than TWINSpan in identifying indicator species as it provides flexible and precise information on the environmental preferences of chironomid species (Dufrêne & Legendre, 1997). The use of COINSPAN allows INDVAL to be constrained to single significant variables. The species classifications obtained here are useful for bioassessment because the species are subdivided into precise assemblages.

The identification of indicator taxa using INDVAL proved to be a useful as a tool in the possible future determination of the ecological potential of artificial water bodies.

Furthermore, the importance of metals revealed by ordination and the identification of indicator taxa could prove to be useful in the ecological assessment of urban canals across the UK. However, if importantly, chironomids are ignored by the WFD, artificial water bodies such as canals will not be adequately monitored.

The use of a single taxonomic group as indicators of the quality of ecosystems has been criticised (Landres et al., 1988; Niemi et al., 1997; Prendergast et al., 1993). Selection of bioindicators for aquatic health assessment needs a scale-sensitive survey and analysis of the distribution and selection of habitat (Fairweather, 1999; Karr, 1999; Norris and Thoms, 1999; Hansen et al., 1999; Pedroli et al., 2002).

This study illustrates that the broad range of habitats and sensitivity to environmental changes makes chironomids good indicator types for canal assessments. By using tools such as COINSPAN that classifies sites according to a single significant variable species with clear indicator groups can be identified. This has been successfully shown with chironomids in lakes (Ruse, 2002), where alkalinity separated COINSPAN classes and twenty-two taxa were found to be significant indicators for the species for an enhanced lake classification scheme.

#### **5.4.3 RESPONSE OF CHIRONOMID TAXA TO TOTAL METAL LOADING**

In North American streams, CCU proved to be useful for assessing the impact of metals on benthic communities (Clements et al. 2000). This thus presented a way to link ecotoxicological data with ecological assessment. In the UK, work by Hirst et al. (2002) found the concept of CCU also worked. CCU scores were selected over individual metals as predictors of diatom assemblages and significantly with invertebrate richness. In this study the intended advantage of the CCU scores expressing toxicity relative to thresholds were apparent. Zinc contributed more to total metal concentration, but invertebrate richness and abundance were related more to copper. This was because copper was ascribed a lower criterion value (= threshold) therefore a greater contribution to potential toxicity. Despite lead being the single

largest contributor to potential toxicity, it had little effect on either invertebrates or diatoms. When lead was removed from the CCU calculations it was found that there was a weakening of the relationship between CCU scores and diatoms but an improved relationship between CCU and macroinvertebrates. This suggested that lead exerted a toxic effect on diatoms but not the invertebrates. In the present study CCU was seen to affect diversity (Fig. 5.9) when GLM modelling was carried out, where diversity decreased with increasing CCU levels. With best subset regression, CCU could be reasonably predicted by using indicator taxa and individual metals. However, this worked best with copper which contributed little to the total metal concentration (Fig. 3.9a) and a slightly greater contribution to potential toxicity (Fig. 3.9b).

Hirst et al. (2002) suggested that in rivers CCU scores should be refined to take into account variations in acidity. Certain metals such as manganese and especially aluminium are not modified to take into account varying toxicity with pH. The effects of acidity on canals may be of less concern, but similar refinements may be needed in the future

The use of best subset regression is an efficient way to identify models that achieve the goal of predicting CCU with as few predictors as possible – these models may actually estimate the regression coefficients and predict future responses with smaller variance than the full model using all predictors. Also a small number of species can be used to predict CCU, although with a lower  $r^2$  than when combined with metals.

## **5.5 SUMMARY**

This chapter has demonstrated that the CPET method is an effective means to monitor the biodiversity of canals. The collection of pupal exuviae integrates both marginal and deep-water habitats from a wide area, which also integrates pupal emergence. Many taxa that were found inhabit the sediment, a habitat normally difficult to sample. Chironomid taxa assemblages were able to differentiate between canal sites

of different chemistry. Indicator species assemblages were identified that are associated with different COINSPAN groups constrained by each of the significant potential polluting variables determined by CCA analysis.

An effective bioassessment tool should be characterised by a minimum effort to extract the greatest amount of information possible. The methods under consideration here achieved this by utilising species response along environmental gradients, using all the species data including abundance and by using reference sites. The methods were able to detect impacts with clear differentiation between impacted and unimpacted sites. The methods also separated different impact types and some measure of total ecological change at each site was also determined.

## **CHAPTER 6**

# **FUNCTIONAL ECOLOGY OF URBAN CANALS IN THE WEST MIDLANDS**

### **APPROACH**

Body size distributions are presented for chironomid species found within urban canals. Pupal exuviae were ordinated against chemical variables and key taxa used to establish whether there were significant differences in body length within species at different sites. Ordination of size classes against environmental variables found that metals were significant in determining distribution of body size. The relationship between functional feeding groups and metals was also investigated. It was found that predators were positively correlated to zinc and negatively correlated to CCU, whereas grazers were negatively correlated with lead.

### **6.1 INTRODUCTION**

There is a wealth of studies that examine the links between species diversity and environmental stress (Mousavi et al., 2003; Ricciardi et al., 2009) but this does not say anything about how pollution impacts ecological function. A major downfall of using such indices is that all species have the same equal weight irrespective of their ecological function. Therefore diversity indices do not account for the biological differences among species associated with life history or physiology (Solow and Polasky, 1994). Functional diversity should be a measure of the functional differences among taxa in a community (Petchey and Gaston, 2002).

Another approach to assess environmental impacts is the use of ecological functions of species (Doledec et al., 1999; Stratzner et al., 2001). These are biological traits that reflect the adaptation of species to environmental disturbances (Townsend and

Hildrew, 1994). Typical traits include maximal size, shape of the body and feeding strategies. Invertebrates are good for monitoring change in an aquatic system in that they have relatively short lifespans and have strategies that enable them to adapt to environmental gradients (Rosenberg and Resh, 1993).

There is a close relationship between the benthos and sediment at a microhabitat scale, therefore it is important to specify the habitat requirements at a community or species level (Buffagni et al., 1995). During growth and development, the spatial niche of an organism can change, as conditions become more suitable to its size (Osbourne and Herricks, 1987), modifications to its dietary needs or habitat (sediment) quality changes. The distribution of body sizes can also be useful when measuring community patterns. An investigation will be carried out to determine whether body size analysis can be used in bioassessment. The measurement of water and sediment characteristics allow for the evaluation of the roles of such variables, in explaining changes in body patterns.

A number of studies have been carried out on river systems that receive elevated amounts of metal and their effects on biotic communities and to a lesser extent, algae (Surber, 1959; Yasuno et al., 1985; Wilson, 1988; Clements et al., 2000; Ruse and Davison 2000; Ruse et al., 2000; Burton et al., 2001; Hirst et al., 2002; Drakare et al., 2003). Because many urban canals sites will be impacted by a variety of metals, a cumulative measure of total metal concentration will be required. The approach taken here follows that of the U.S EPA, water criteria documents of 1986 and updated in 2002.

The aims of this chapter are to investigate whether:

- I) Traits such as body length can be used as a bioassessment tool;
- II) Chironomid functional groups respond to metal pollution in a systematic and predictable manner.

## 6.2 METHODS

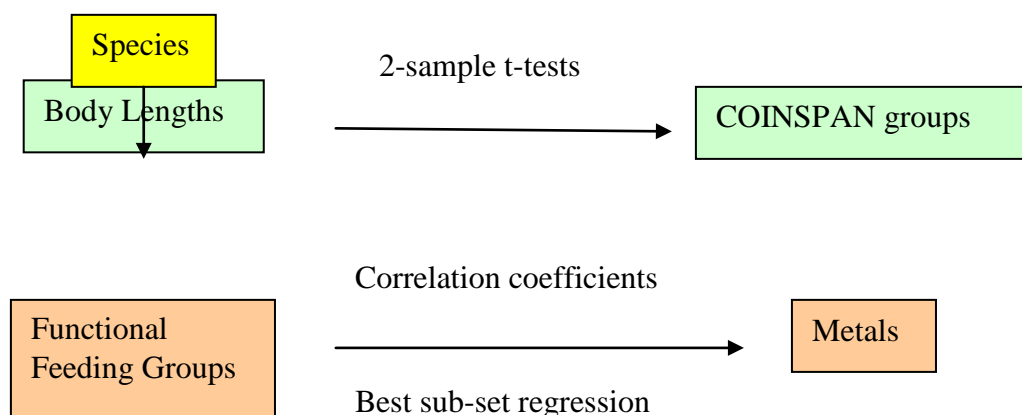
### 6.2.1 BODY SIZE AND FUNCTIONAL FEEDING GROUPS

All pupal exuviae collected were measured under the microscope with a stage micrometer. Functional feeding groups were assigned according to ecological and habitat data listed in Wilson and Ruse (2005).

### 6.2.2 STATISTICAL ANALYSIS

Variation in mean length was examined using t-tests. Key taxa (established from the previous chapter) were used to establish whether there were any significant differences in body length between species at different sites impacted by water or sediment pollution. COINSPAN analysis (Carleton et al., 1996) was used to investigate whether individual species showed significant changes in mean body length; Mean body length of species between COINSPAN groups of significant variables were then compared (Chapter 5). CCA analysis was also used to ordinate size classes against chemical variables (Fig.6.1).

Relationships between feeding groups and metals were investigated with Pearson's correlation coefficients.

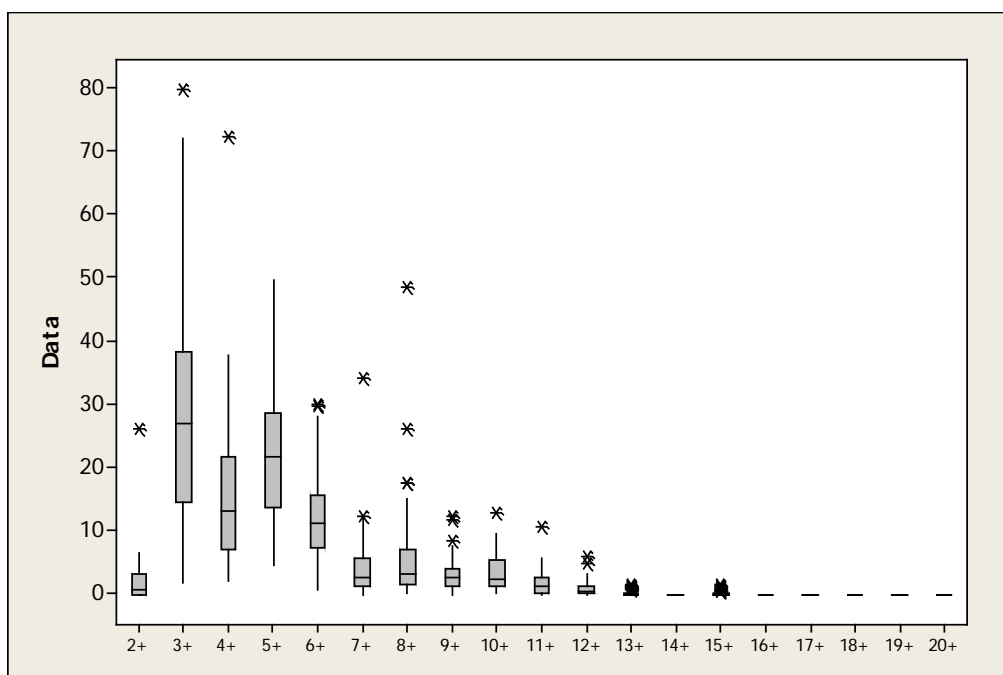


**Figure 6.1** Analysis steps taken in this chapter

## 6.3 RESULTS

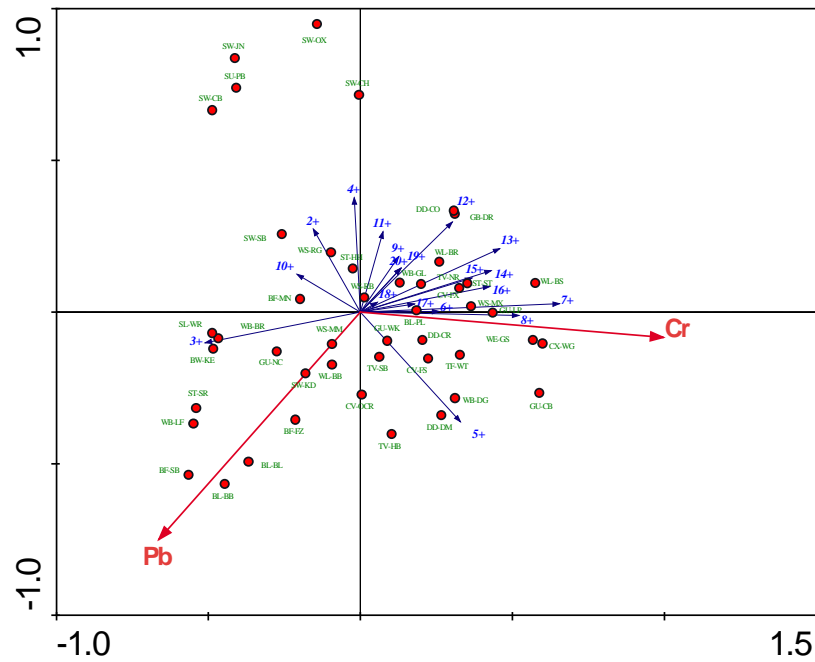
### 6.3.1 BODY SIZE

The distribution of size classes found in this study is shown in Fig. 6.2 It can be seen that most are small bodied between 3 mm and 6 mm.



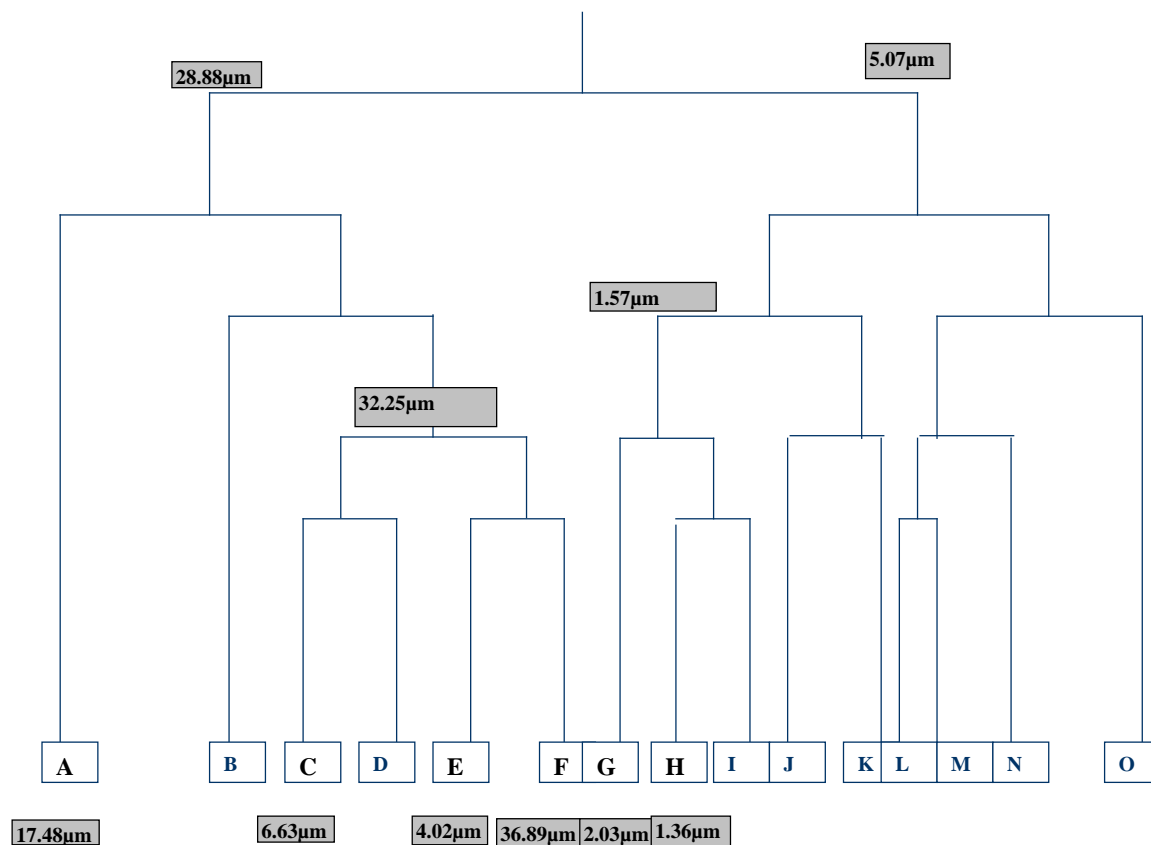
**Figure 6.2** Distribution of size classes

Significant chemical variables found by CCA ordination were used to assess body size distribution. Ordination of size classes against chemical variables found that metals were significant (from Monte-Carlo permutations) in determining distribution of body sizes within the 46 sample sites (Fig. 6.3).

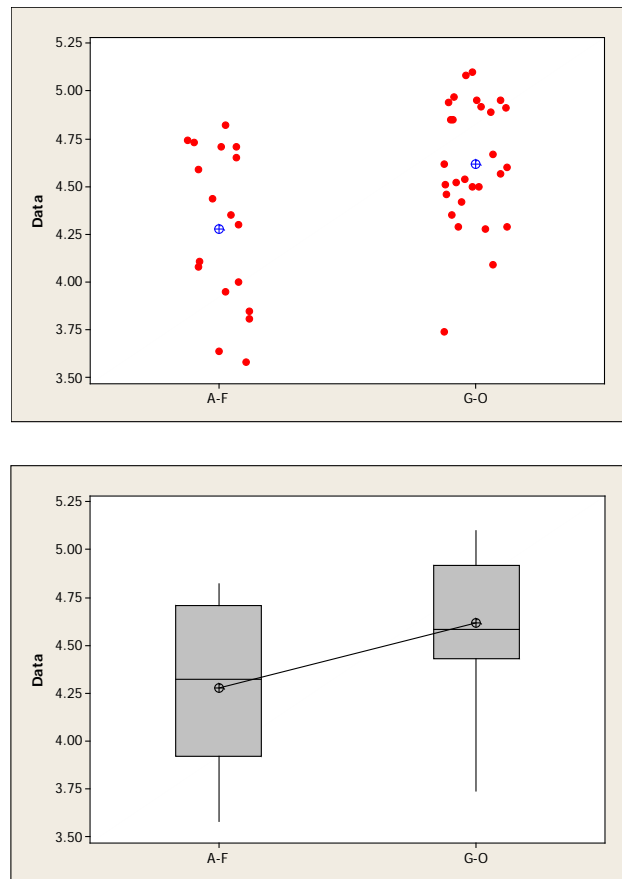


**Figure 6.3** CCA body size distribution

Significant variables from CCA analysis (Chapter 5) were used and isolation of indicator species via INDVAL was also chosen to assess body size differences. Using chromium (water) as an example, COINSPAN groups with high and low levels of the metal were compared (Fig. 6.4). It was seen that *Cricotopus sylvestris* has significantly larger body size at low chromium levels (Fig. 6.5).

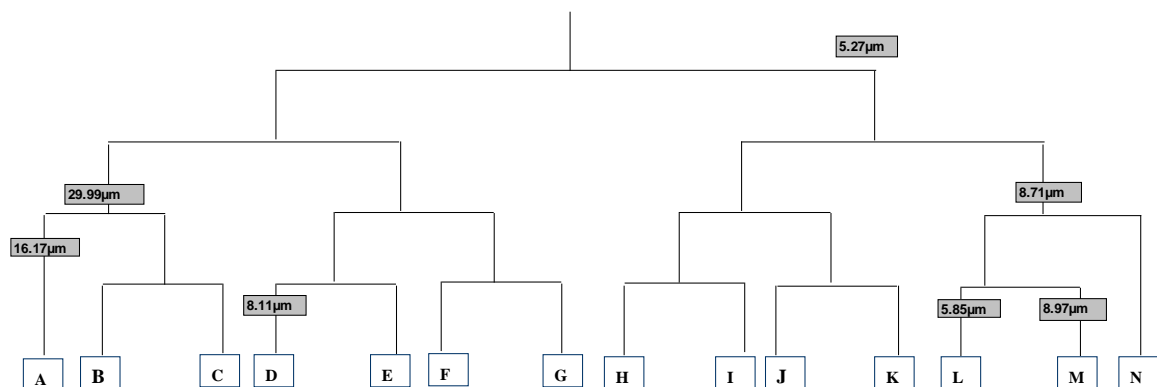


**Figure 6.4** COINSPAN classification (*Cricotopus sylvestris*) for Chromium

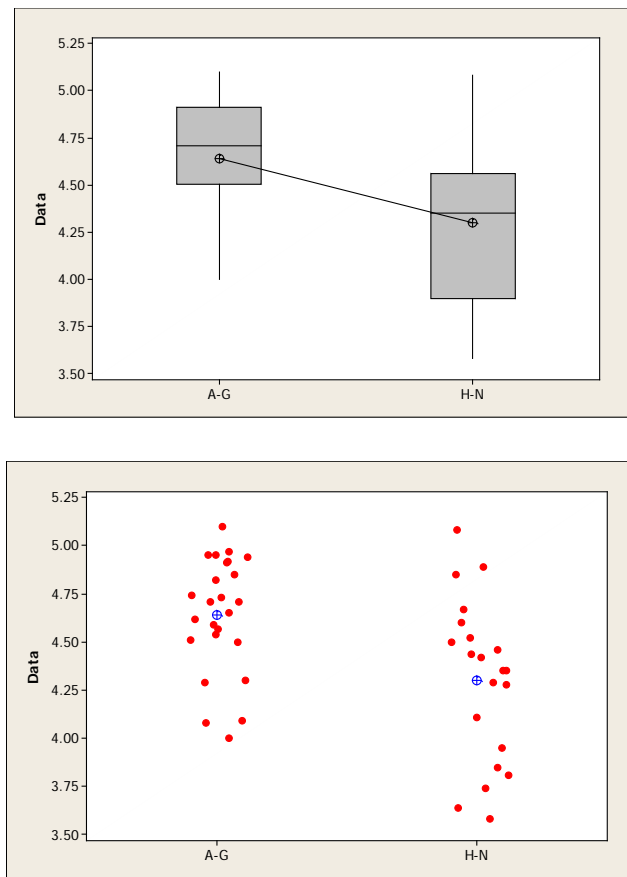


**Figure 6.5** Size difference of *Cricotopus sylvestris* between COINSPAN groups A/F and G/O ( $p=0.006$ )

It was also found that *Cricotopus sylvestris* was significantly larger at higher levels of lead (water) (Figs. 6.6 and 6.7). Figs. 6.8 and 6.9 show that individuals of both *Cricotopus sylvestris* and *Parachironomus arcuatus* were larger at higher chlorophyll levels.



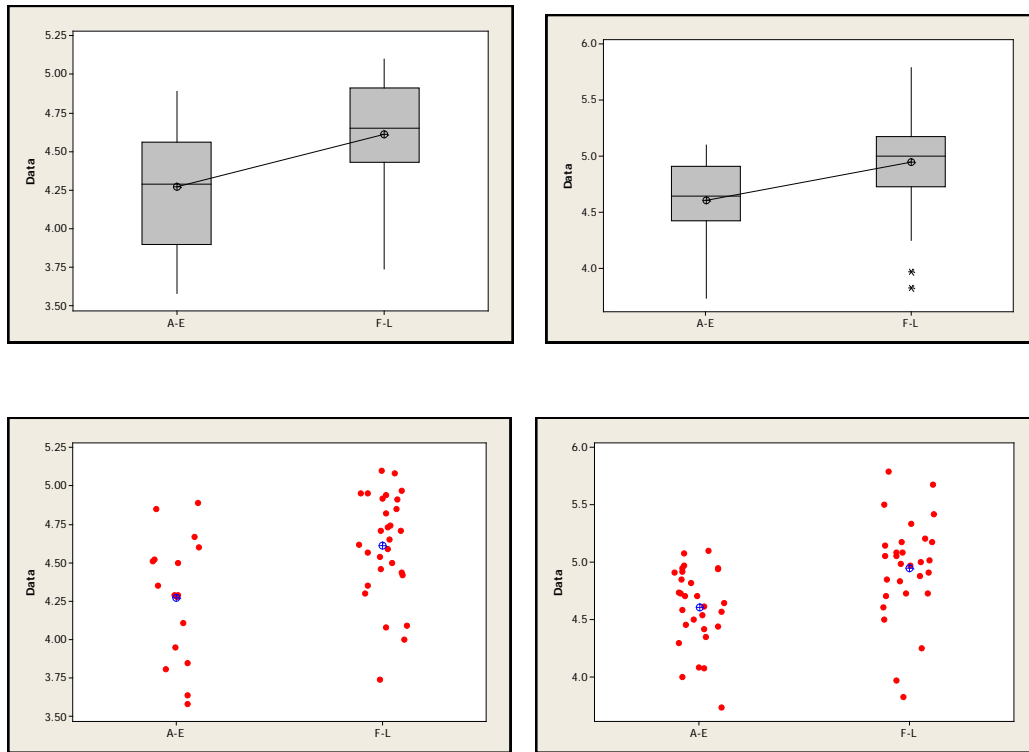
**Figure 6.6** COINSPAN classification (*Cricotopus sylvestris*) for lead (water)



**Figure 6.7** Size difference of *Cricotopus sylvestris* between COINSPAN groups A-G and H-N for lead (water\_ (p=0.004)



**Figure 6.8** COINSPAN classification (*Cricotopus sylvestris*) for chlorophyll (water)



(a) *Cricotopus sylvestris*

(b) *Parachironomus arcuatus*

**Figure 6.9** Size difference of (a) *Cricotopus sylvestris* and (b) *Parachironomus arcuatus* between COINSPAN groups A-E and F-L ( $p=0.0077$ )

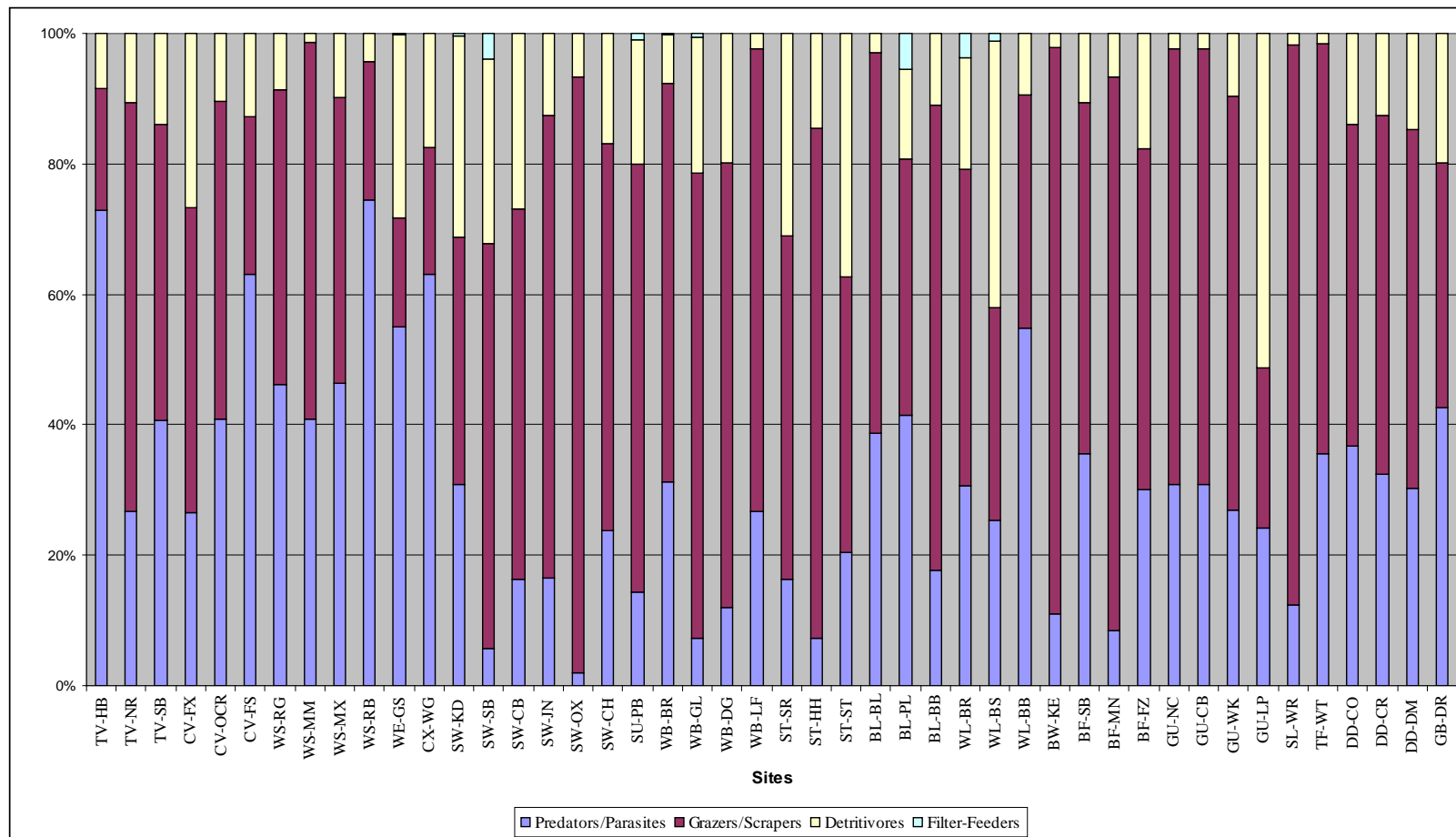
### 6.3.2 FUNCTIONAL GROUPS

The relationship between functional feeding groups and metals was investigated using Pearson's correlation coefficients (Table 6.1). Predators were found to be positively correlated to zinc and negatively correlated to CCU, whereas grazers were negatively correlated with lead.

**Table 6.1** Correlation coefficients (\*\* $p < 0.001$ , \* $p < 0.005$ )

	Predators	Grazers
Zn	(+)0.025**	
Pb		(-)0.008**
CCU	(-)0.04*	

The distribution of feeding forms varies considerably by site (Fig. 6.10). The chironomid assemblages at the study sites are dominated by grazer/scrapers and predators, with very few filter feeders and detritivores.



**Figure 6.10** Distribution of functional feeding groups

In a best sub-set regression of CCU against the pool of functional groups the most powerful predictive model was obtained when using a 3-predictor rather than a 2-predictor model (Table 6.2). The 3-predictor model selected predators together with copper and lead.

**Table 6.2** Best subset models

<b>2- model</b>			<b>3- model</b>		
Dependent variable	Predictors in model	$r^2(\text{adj})$	Dependent variable	Predictors in model	$r^2(\text{adj})$
CCU	Predators+Fe	14.6	CCU	Predators+Cu+Pb	37.1

## 6.4 DISCUSSION

### 6.4.1 BODY SIZE

Unravelling why the distribution of size classes differs between sites is a complex one. Many factors are likely to influence the mean body length of individual chironomid species, including habitat, the amount of macrophytes, water and sediment chemistry, that all in turn can affect larval feeding. Predation has been shown to affect the distribution of different sizes of aquatic macroinvertebrates (Hildrew et al., 1980; Flecker and Allan, 1984; Teague et al., 1985; Whale 1992). Competitive release may also affect the distribution of size classes. This occurs when one or more competing species are removed from a site, so releasing the remaining species from one or more factors that limited either population size or as in this case individual size. For example, it was found that the populations of hydropsychids in certain American rivers increased due to decreasing populations of zebra mussels (Battle et al., 2007). It was suspected that because both are filter-feeding invertebrates, some of the differences in populations found were due to direct or indirect responses to changes in the populations of the zebra mussels. Food quality and quantity can also have an affect. It has been shown for example, in acid streams (Ledger and Hildrew, 2001) that there is an apparent absence of specialist herbivorous grazers. The absence of herbivores was shown to be a consequence of the low quality and/or quantity of biofilms in acid streams.

When analysing patterns of species' responses to sewage enrichment in fish deBruyn et al. (2002) found that there was a size-structured food web. They found that only the largest size classes increased with sewage enrichment. There were also better correlations between small and medium-sized fish and local site characteristics rather than with sewage enrichment. Small and medium-sized fish species were mostly associated with low current, irrespective of weed cover or sewage enrichment. Medium-sized benthivores and suckers covaried with weed cover but in opposite directions: benthivore densities were highest at weedy sites, whereas suckers were associated with relatively bare sites. Weed cover was only weakly correlated with

productivity, so the influence of weed cover on fish densities appeared to be the provision of physical structure to the habitat. There was also an interaction between site characteristics and productivity. At low weed cover, there was dominance by suckers, whereas at high weed cover, piscivores dominated. This was possibly due to the fact that the most dominant piscivores in the system were esocids (Pikes), which are ambush predators typically associated with macrophyte beds.

Overall, the larger benthivore fish had higher densities at enriched sites at all levels of weed cover, but small and medium benthivore fish did not respond to sewage enrichment. These patterns provided support for the hypothesis that exploitation (top-down control) was more important than resource abundance in determining benthivore densities. During growth and development, the niche space of an organism can change, as conditions become more suitable to its size (Osbourne and Herricks, 1987), modifications to its dietary needs or habitat quality changes. In the present study the difference in mean body size at different sites is likely to be a combination of several factors. One of the primary factors is likely to be availability of food. *Cricotopus sylvestris*, a tube-dweller, is a grazer of plants and algae and it was seen that at sites with higher levels of chlorophyll was significantly larger when compared to sites with lower levels. Similar patterns were observed in epilithic communities of lake littorals (Harrison and Hildrew, 2002). They found peaks of density occurring chiefly beneath deciduous trees in spring and summer. This suggests that there was greater resource availability compared to sites with lower levels of chlorophyll. A similar pattern was seen with *Parachironomus arcuatus*, where the mean body length was larger at higher levels of chlorophyll. This species is a detritivore and could also be utilizing the greater resource available with higher chlorophyll concentrations. The response of *Cricotopus sylvestris* to chromium and lead differed; the species were significantly larger at sites with higher lead levels but smaller at sites with higher levels of chromium, suggesting lead tolerance and chromium intolerance.

Differences in size may be attributed to trophic cascades where predators in the food chain suppress the abundance of their prey, thereby releasing the next lower trophic level from predation or herbivory (if the intermediate level is an herbivore).

It is possible that a greater adaptive response to higher levels of metal allows certain species to increase in size and so able to compete better than other species. It is also possible that these species are poor competitors that grow in the absence of competition (competitive release) when less metal-tolerant species (superior competitors) are removed or that the species are more generalist in their food. It is known that some aquatic insects can adapt to higher metals levels in rivers by accumulating the metals within their bodies, by using a range of pathways, for example from solution or from food (Luoma, 1983). Population differentiation caused by local adaptation is a basic principle of modern evolutionary biology. Adaptation has been defined as a trait that enhances an individual's fitness and that arose historically as a result from natural selection (Admundson, 1996; Lauder, 1996). The effects of pollution may intensify selective pressures compared to that caused under natural circumstances (Shaw, 1999).

An increased tolerance to metals within a few generations has been demonstrated in several chironomid species. It has been shown in experiments (Postma et al., 1995a,b) that increased tolerance to cadmium in *Chironomus riparius* showed that there was a reduction in the adverse effect on larval growth rate. Reduced fitness under cleaner conditions with lower metal concentrations has also been shown, that has been explained as being a cost associated with being tolerant (Holloway et al, 1990). Other organisms, such as *Collembola*, have shown high mortality under cleaner conditions (Posthuma et al., 1993). One reason suggested is the possible lack of metabolically available essential metals such as zinc (Posthuma et al., 1992). Increased zinc accumulation has also been found in metal-adapted chironomids (Postma et al., 1995a,b). In this case, a causal relationship with high control mortality was less likely, because mortality remained high when cadmium-tolerant midges were supplied with additional zinc. These experiments under control conditions, demonstrate an increase in larval development time observed in some tolerant chironomid populations could be partly due to an increased need for essential metals such as zinc. These findings for chironomids question the 'costs of tolerance' of reduced fitness of metal adapted populations reared in a clean environment. The most likely explanation is a shortage of zinc and so an indirect effect of the tolerance mechanism instead of a direct consequence of the extra energy invested in maintaining a tolerance mechanism.

It seems possible then that the size differences seen in certain species in this study represents a response to metal adaptation as well as possibly other factors as habitat, predator pressure or selective release from predator pressure. Whatever the cause, the predictable size of certain indicator species in relation to pollutant loads suggests that they have greater potential for the bioassessment of impacted waterbodies.

#### **6.4.2 FUNCTIONAL GROUPS**

In terms of feeding strategies, the survey sites were dominated by grazers. Despite the fact that at many of the sites there were no macrophytes present, the chironomid grazers were most likely utilizing the bank reinforcements for algae. These results suggest that this type of canal habitat negatively affect the functional organization of benthic invertebrates (collector-gatherers, predators, and scrapers) (Tadeusz ,2003).

This study is the first to assess both the effects of metal contamination and toxicity on chironomid assemblages within canal ecosystems by means of CCU scores. The results show that CCU scores can be predicted when selected together with other predictors in 2- and 3- predictor regression models. Studies conducted in rivers have shown that CCU scores are significant when assessing metal toxicity (Clements et al., 2000; Hirst et al., 2002). However this was not apparent in the current study, where individual metals were more significant.

Where filter-feeders were present, this could have been a result of less polluted conditions allowing the development of phytoplankton. For example, on the Staffs & Worc Canal, no filter-feeders were present below the sewage outfall at SW-OX, only becoming present at the two furthest sites at SW-SB and SW-KD. Grazer/Scrapers tend to develop where there is abundant macrophyte growth allowing algae to develop on their surfaces to be utilized by chironomid grazers. In the present study it was found that sites were often dominated by Grazers where in fact no macrophytes were

present. A probable explanation is that algae were utilizing the hard surfaces of the bank reinforcements often present at urban sites. This can be seen at such sites as BW-KE, WB-LF and GU-NC where macrophytes were totally absent.

## **6.5 SUMMARY**

Metal concentrations were seen to affect mean body size of certain species, possibly as a result of a combination of metal adaptation, habitat, predator pressure or selective release from predator pressure. The results are very positive but before they can be used predictively more work is needed in order to establish the exact mechanisms involved.

It can be concluded that the chironomid functional groups in this study do respond to individual metal concentrations. However, more work is needed to ascertain whether CCU scores can prove useful for assessing metal contamination in canals, however. Individual metals were selected over CCU as a predictor of chironomid assemblages and CCU only negatively correlated with predator abundance.

## **CHAPTER 7**

### **CALIBRATION AND TESTING OF INFERENCE MODELS**

#### **APPROACH**

Variations in chironomid assemblages are related to changes in measured environmental variables using multivariate techniques. Canonical Correspondence Analysis (CCA) indicated that five variables (Chapter 5) contributed significantly to explaining patterns of chironomid variation (TON, sediment fines, chromium, lead and sediment lead). Three variables were significant in explaining patterns of epibenthic taxa with water chemistry (chromium chlorophyll and lead) and three variables were significant in explaining patterns of enbenthic taxa with sediment chemistry (sediment fines, lead and nitrogen).

Chironomid-based inference models were generated for the reconstruction of the significant variables. The strengths of these models indicate that it would be possible to reliably infer trends in TON from the combined data set ( $r^2_{\text{jack}} = 0.75$ , RMSEP = 2.38). To a lesser extent, chromium ( $r^2_{\text{jack}} = 0.54$ , RMSEP = 0.25) and chlorophyll ( $r^2_{\text{jack}} = 0.53$ , RMSEP = 1.8) could also be reliably inferred.

## 7.1 INTRODUCTION

The idea of calibrating and inferring ecological data came initially from the need to infer and reconstruct past environmental conditions using subfossils (Birks et al., 1990; Birks, 1995, 1998). The aim of these transfer functions is to express an environmental variable as a function of biological data. This transfer function is constructed via calibration, frequently using complicated modelling techniques and large training sets (ter Braak and Prentice 1988). The basic premise for quantitative environmental reconstruction is that one or more environmental variables,  $X$  is required to be reconstructed from fossil biological data,  $Y$  that consists of  $m$  taxa in  $t$  samples. Therefore, to estimate  $X$  the responses of the same taxa today have to be modelled in relation to the environmental variables of interest. In palaeoecological terms, this involves a modern training set of  $m$  taxa at  $n$  sites ( $Y$ ) which are studied as assemblages from surface sediments and associated with a set of environmental data ( $X$ ) that includes the target variable for retrodiction. The modern relationships between  $X$  and  $Y$  are statistically modelled with the resulting function used as a transfer function to transform the fossil data into estimates of past environmental conditions ( $X$ ) (Smol, 2002). These same principles can be applied to infer chemical conditions from sites where this information is lacking. In other words sites with known chemical conditions and taxa can be used to infer the conditions in sites where the chemistry has not been measured using only the biological variables.

For the development of a robust chironomid-based inference model, the knowledge of the autecological preferences of individual species is essential and this requires high quality environmental data (Birks, 1998). In order to obtain a representative average, it is necessary to take numerous environmental samples over the course of a year (Bennion and Smith, 2000) and couple this with targeted and appropriate biological samples. The classification and assessment system needs to be based on quantitative changes and differences in the chironomid community that match different ends of an environmental gradient (Brodersen and Lindegaard, 1999). Weighted averaging (WA) models (Birks et al., 1990; ter Braak and Juggins, 1993) are possible ways to establish this relationship. The WA methods are based on species optima and tolerances calculated via WA regression across a given environmental gradient. These models

have been used many times to establish palaeoenvironmental relationships using diatoms, chironomids, pollen and chrysophytes and develop transfer functions for salinity, nutrients, conductivity, temperature and oxygen (Bradshaw and Anderson, 2001; Brooks and Birks, 2001; Brooks et al., 2001; Larocque and Hall, 2004; Lotter et al., 1998; Ponader et al., 2005; Reed, 1998; Wilson et al., 1996). However, functions have not been developed for contemporary problems that are difficult to sample, such as canals.

The importance to the regulatory bodies of the UK of robust transfer functions needs to be stressed as more and more of routine monitoring of waterbodies is being progressively reduced. Moreover, being able to estimate certain water quality variables from biota such as chironomids would be a very cost effective method of being able to still chemically monitor canals as well as lakes and rivers.

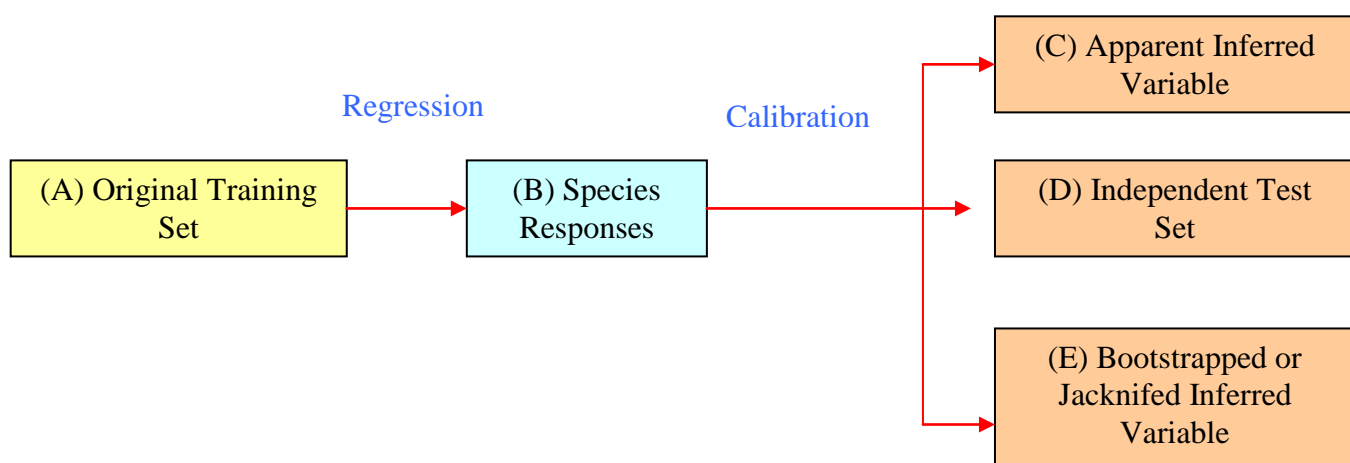
The aim of this chapter is therefore to:

- Assess the applicability and predictive ability of inference models developed using significant variables found by CCA in Chapter 5.

## 7.2 METHODS

### 7.2.1 TRANSFER FUNCTIONS

To test the ability of selected environmental variables to infer water chemistry from new sites, a selection of weighted averaging tests (transfer functions), were performed on the forty-six sample sites. Data created from Chapter 5 were used in the creation of transfer models. Weighted averaging, weighted averaging partial least squares and partial least squares regression were performed on the dataset. Transfer functions were developed using the programme Calibrate, version 1.4 (Juggins, 2003). Both unimodal weighted averaging (WA) regression and calibration models were tested and their predictive capabilities compared. A  $WA_{(tol)}$  option was used (with both classical and inverse deshrinking) to downweight taxa with high tolerances (Birks et al., 1990). To improve estimates of species optima in the final WA predictor weighted average partial least squares (WA-PLS) were also applied (ter Braak and Juggins, 1993). The  $r^2$ , root mean square error of prediction (RMSEP), root mean square error  $RMSE_{jack}$ , and maximum bias of the models were calculated by “leave-one-out” jack-knifing cross validation procedures (ter Braak and Juggins, 1993). The best “minimum adequate model” was those with the highest coefficient of determination ( $r^2_{jack}$ ), lowest RMSEP and low mean and maximum bias (Birks, 1998).



**Figure 7.1** Typical steps used in developing and assessing the predictive ability of an inference model

Fig. 7.1 illustrates the steps that are used to produce transfer functions after determining which of the measured environmental variables influence species distribution (A) and modelling the taxa to the environmental gradients (B). The final calibration step in the development of the transfer function usually involves statistical techniques such as weighted averaging regression and calibration, or Partial Least Squares methods. These techniques use the information provided in the response curves (optimum and tolerance) of the species (Fig. 7.1(B)) and relates the species and their abundances to the environmental variable of interest.

The predictive ability of the transfer functions can be addressed in one of three ways (Fig. 7.1C-E). The first explores how a species assemblage can infer an environmental variable from the training set. The coefficient of determination resulting from this is the ‘apparent’  $r^2$  (Fig. 7.1C). However, this over overestimates the robustness of the inference model because it uses the same samples to estimate species parameters and to test the strength of the transfer function. Cross-validation is a technique that can resolve this and produce a more realistic estimate of the robustness of the transfer function. This technique uses a second set of independent samples to determine how well the resulting transfer function can infer an environmental variable (Fig. 7.1D). Although statistically robust this approach is a labour intensive procedure, as more sites need to be sampled and new samples need processing. There are, however, other approaches that can use the same data but also provide more realistic error estimates, known as bootstrapping and jackknifing (‘leave-one-out’ substitution) (Fig. 7.1D). These create new training sets from the original (Birks, 1995). Jackknifing creates a new dataset from the original by excluding one of the samples. This one sample forms the independent test sample to determine the inference model. This sample is returned and another removed and the inferences run again. This is done repeatedly until all samples are removed one by one and errors determined for the transfer function. The coefficient of determination that results from this is known as  $r^2_{\text{jackknifed}}$  and is always lower than the more optimistic  $r^2_{\text{apparent}}$ .

Bootstrapping is similar to jackknifing but is a more complex error estimation technique. It also creates a subset from the original dataset but instead of removing

one sample at a time with no replacement, bootstrapping removes samples with replacement.

The strength of the transfer can be determined by the coefficient of determination ( $r^2$ ) of bootstrapped or jackknifed regression, as well as by the root mean squared error (RMSE). These give an indication of how well an environmental variable can be inferred from a species assemblage.

## 7.2 RESULTS

### 7.2.1 WEIGHTED-AVERAGING MODELS

#### 7.2.1.1 Epibenthic taxa and water chemistry

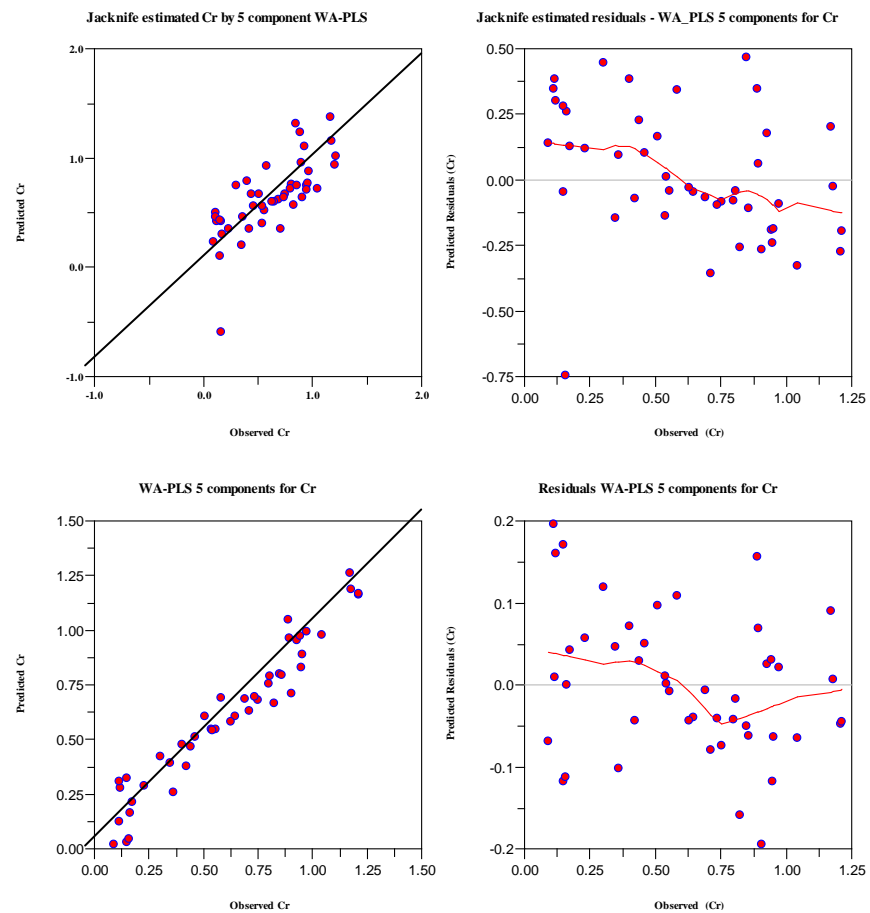
Among all the models tested, the five-component WA-PLS model best represented Chromium-chironomid relationship (Table 7.1). The model predicts chromium values that were reasonably closely related to the actual observed values (Fig. 7.1A), with an  $r^2_{\text{jack}}$  of 0.54.

**Table 7.1** Performance statistics for significant water environmental variables inference models

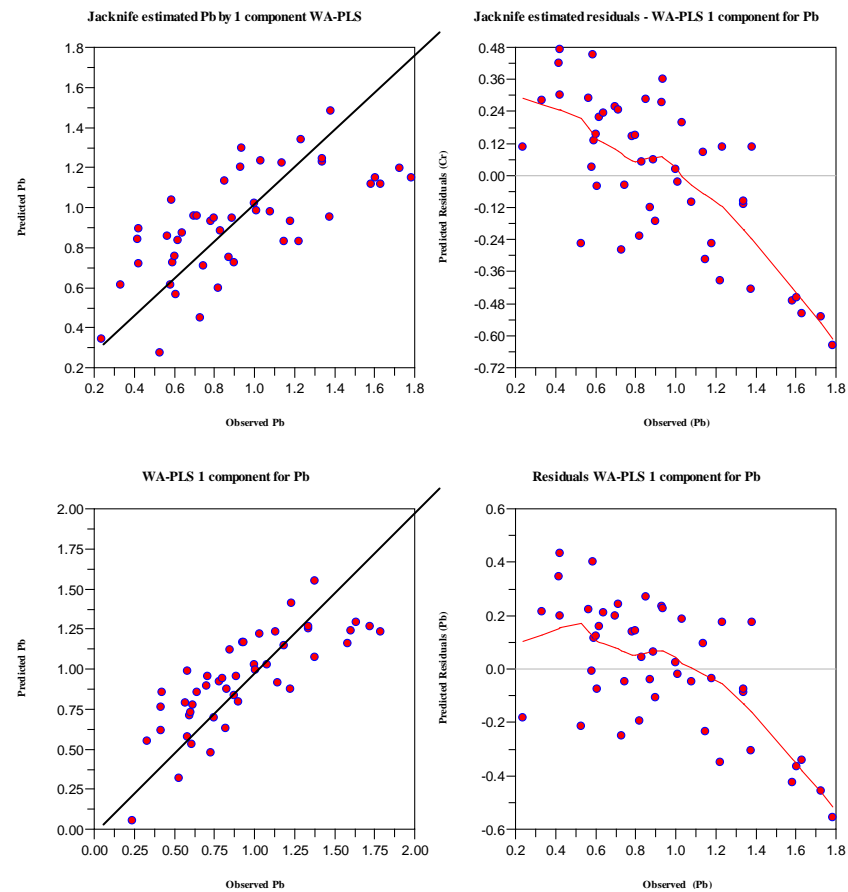
	Method	Variable	$r^2$	RMSEP
<b>Water</b>	WAPLS-5components	Cr	0.54	0.25
	WAPLS-2components	Chl A	0.53	1.8
	WAPLS-1component	Pb	0.45	0.28

The residual plots (Fig. 7.2) shows that the models tended to underestimate Cr values above about 60  $\mu\text{g/l}$ ; Pb values above about 1.1  $\mu\text{g/l}$  and Chl A values above about 9.6  $\mu\text{g/l}$ .

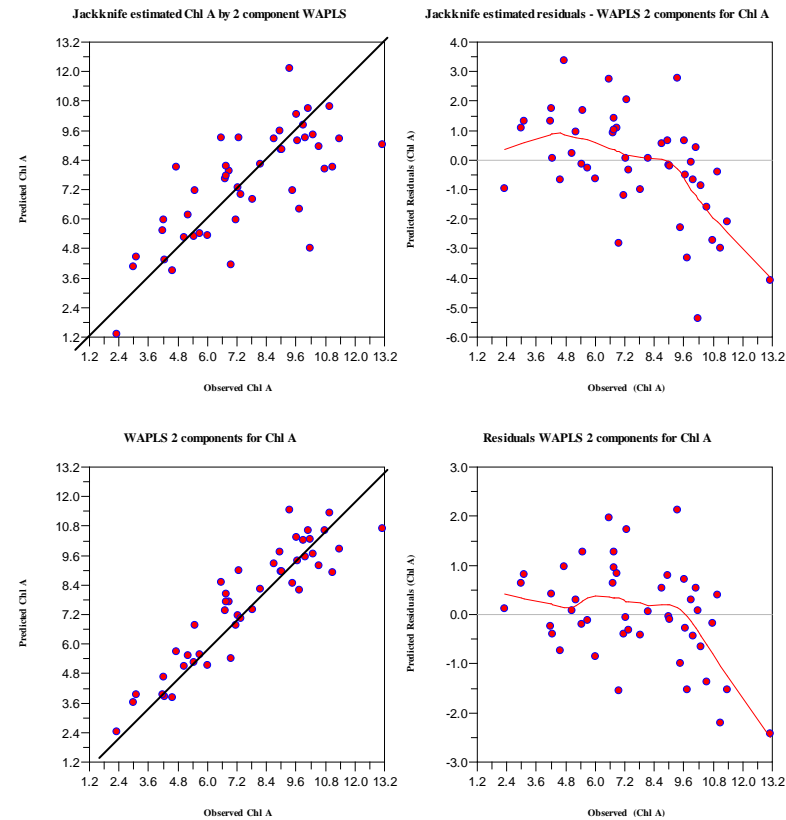
(A)



(B)



(C)



**Figure 7.2** (Water Chemistry Data Set). Plots of WA-PLS inference models showing chironomid inferred value of (A) Chromium - Cr, (B) Lead - Pb and (C) Chlorophyll - Chl A. The diagonal line is a 1:1 line. The solid line shows a LOESS scatter plot smoother (span = 0.45).

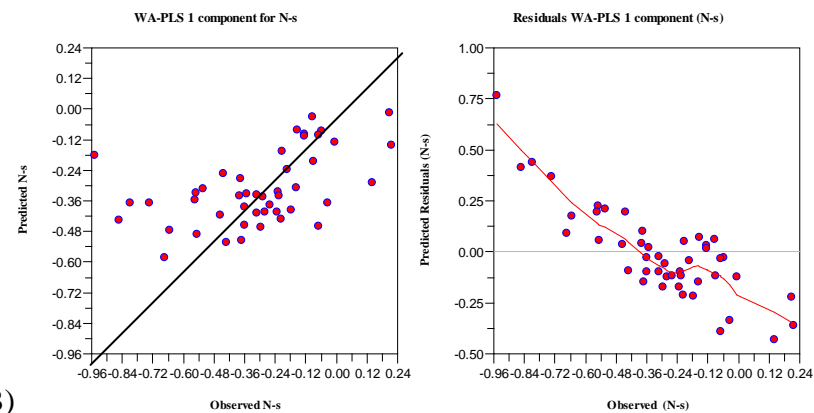
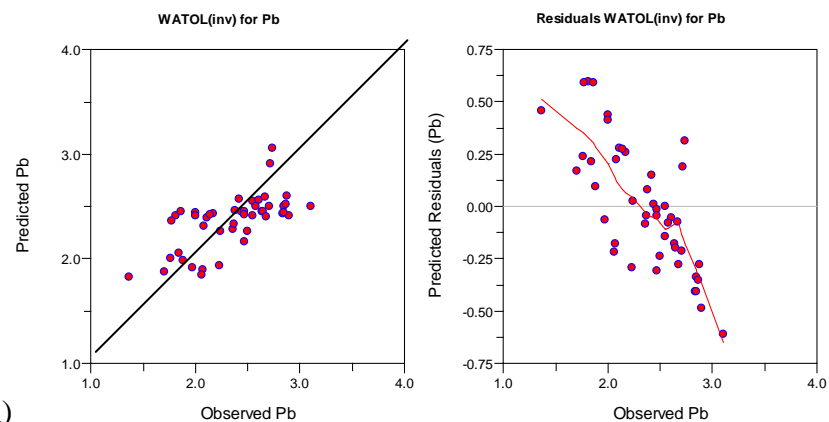
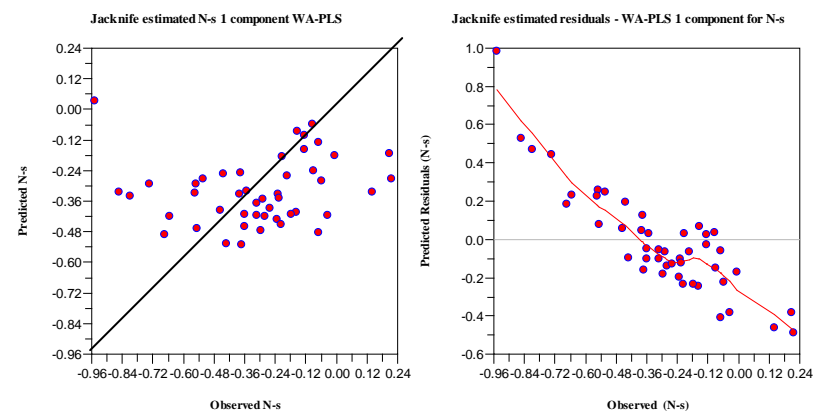
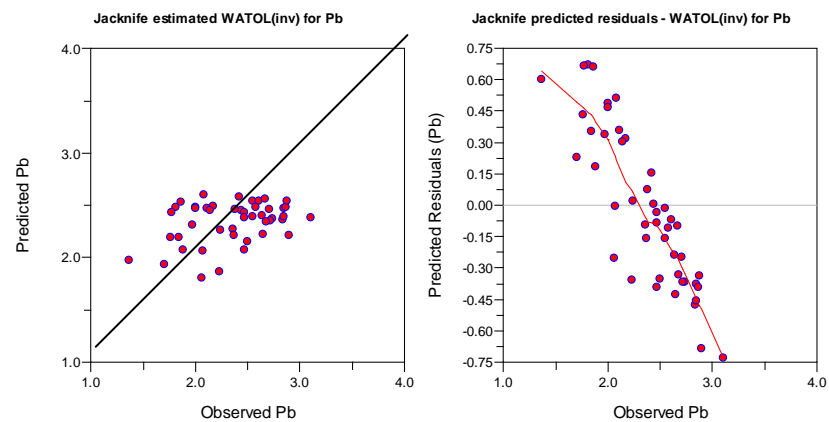
### 7.2.1.2 *Enbenthic taxa and sediment chemistry*

Among all the models tested, the WATOL<sub>inv</sub> model was the best model representing sediments for fines with an  $r^2$  of 0.47 (Table 7.2).

**Table 7.2** Performance statistics for significant sediment environmental variables inference models

	Method	Variable	$r^2$	RMSEP
<b>Sediment</b>	WATOL <sub>inv</sub>	fines	0.47	1.47
	WATOL <sub>inv</sub>	Pb-s	0.12	0.37
	WAPLS-1 component	N-s	0.02	0.27

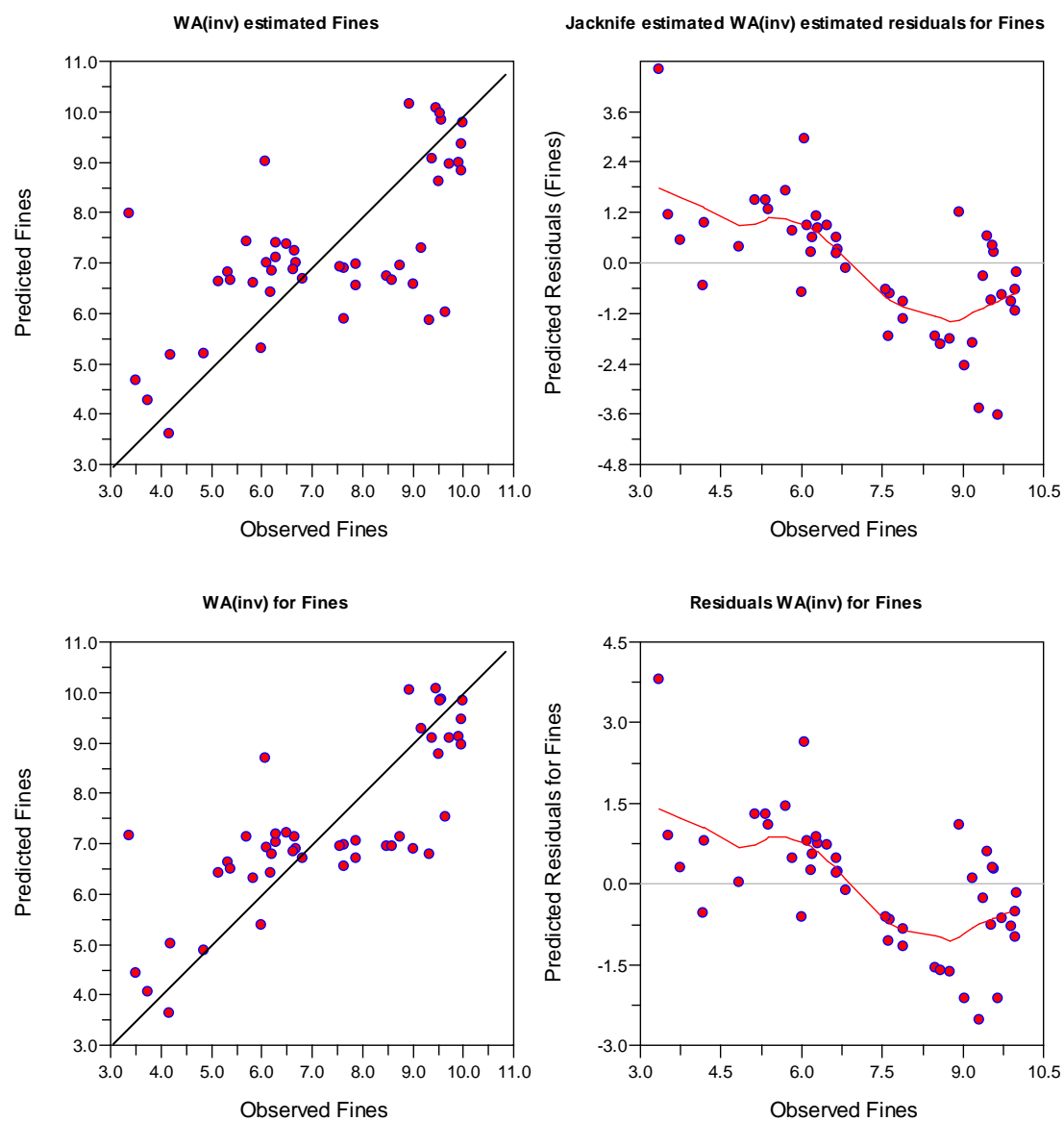
The residuals (Fig 7.3) show that the models underestimated values of Pb over about 375 mg/kg, N over about 0.3mg/kg.



(A)

(B)

(C)



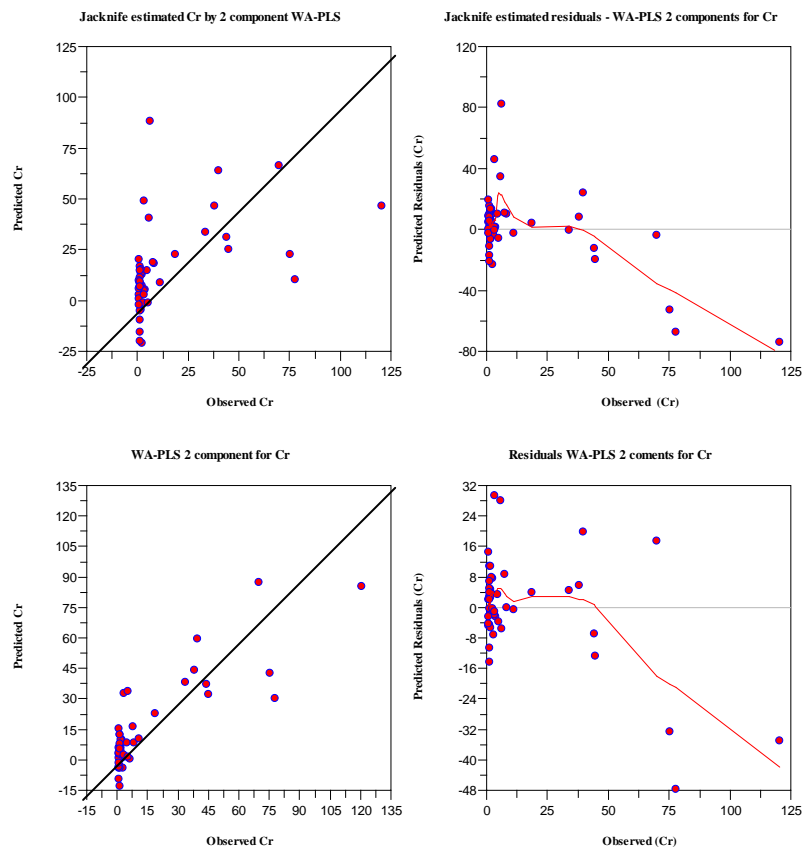
**Fig. 7.3** (Sediment chemistry data set). Plots of WA-PLS inference models showing chironomid inferred value of (A) Lead - Pb, (B) Nitrogen - N and (C) Fines. The diagonal line is a 1:1 line. The solid line shows a LOESS scatter plot smoother (span = 0.45).

### 7.2.1.3 Combined taxa and chemistry

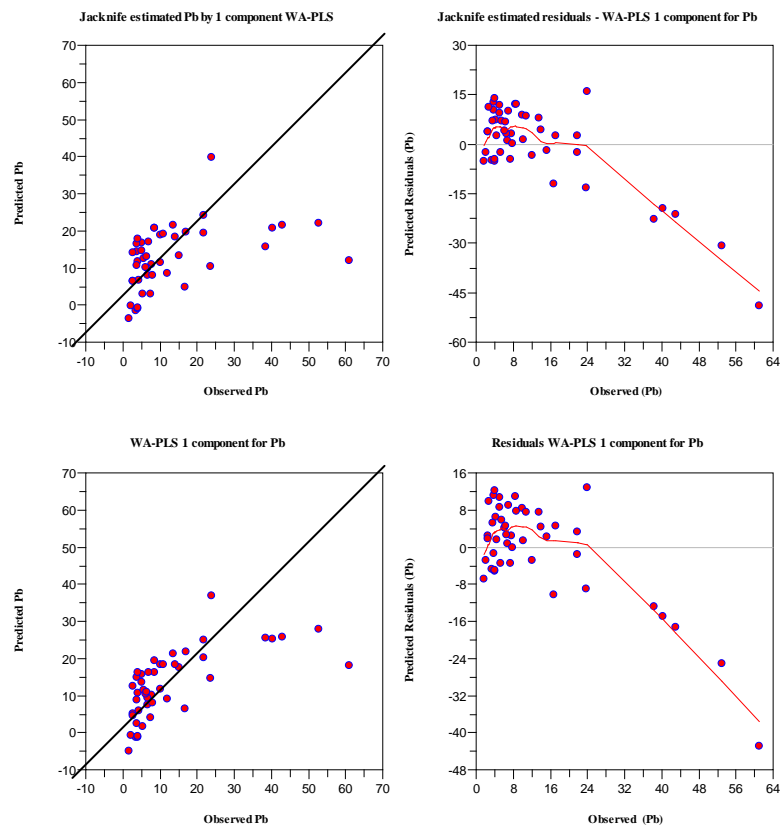
**Table 7.3** Performance statistics for significant combined environmental variables inference models

	Method	Variable	$r^2$	RMSEP
<b>Combined</b>	WAPLS-2components	TON	0.75	2.38
	WAPLS-1component	fines	0.39	22.14
	WAPLS-2components	Cr	0.23	24.52
	WAPLS-1component	Pb	0.19	12.48
	WAPLS-1component	Pb-s	0.07	266.54

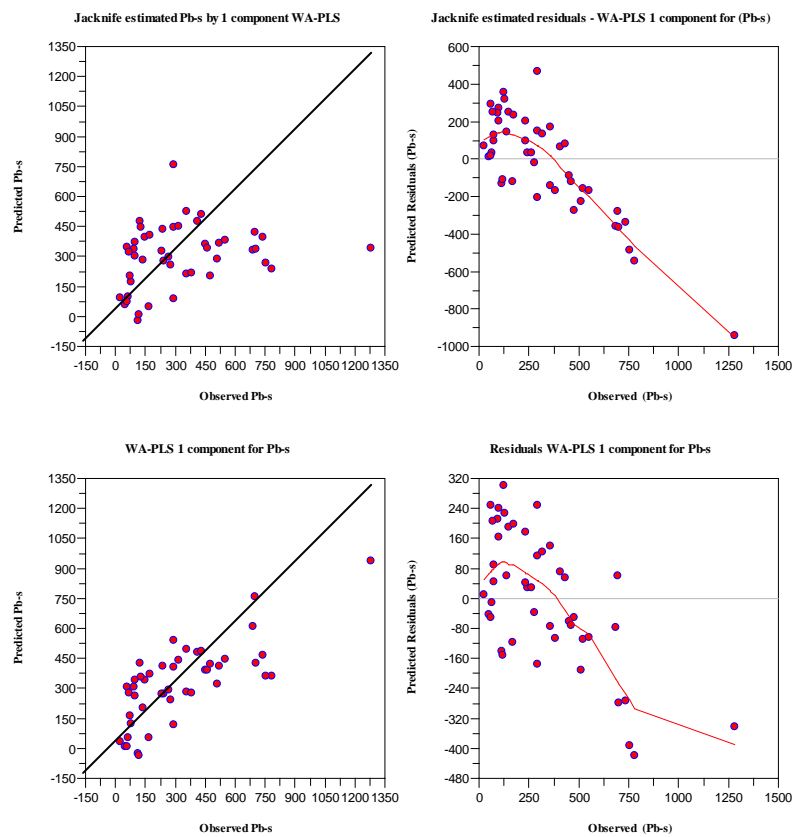
Among the models tested (Table 7.3), a 2-component WA-PLS for TON was the best model to represent the chironomid-water relationship. The model predicts TON values that are closely related to the actual observed values with an  $r^2_{\text{jack}}$  of 0.75. The models tended to underestimate Pb values above about 24  $\mu\text{g/l}$  (Fig. 7.12b); Cr above about 50  $\mu\text{g/l}$ ; TON values above 4  $\mu\text{g/l}$  and sediment fines above 50% (Fig. 7.4)



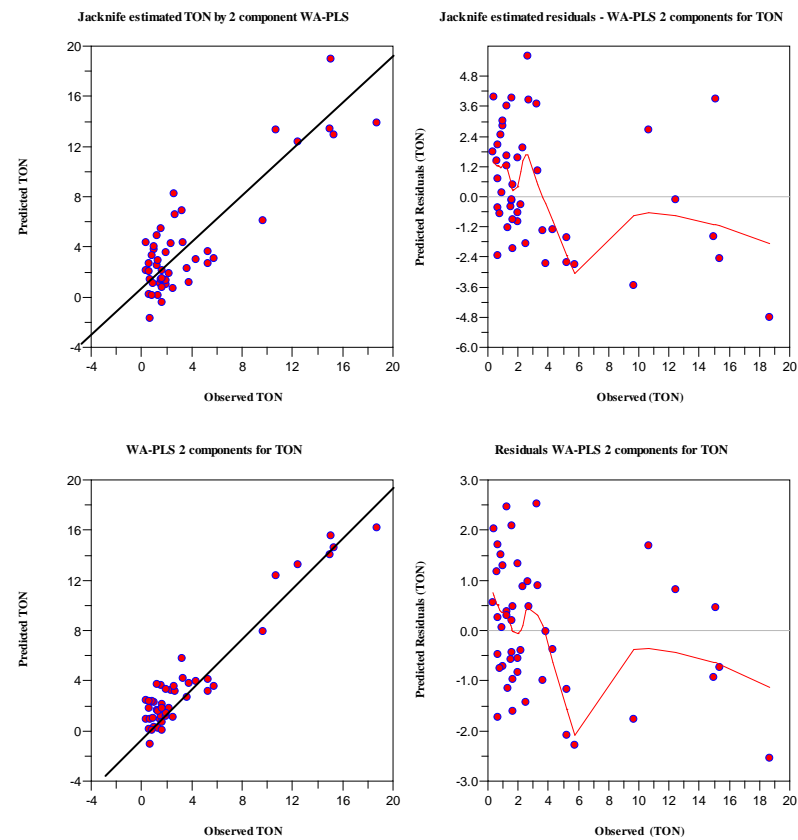
(A)



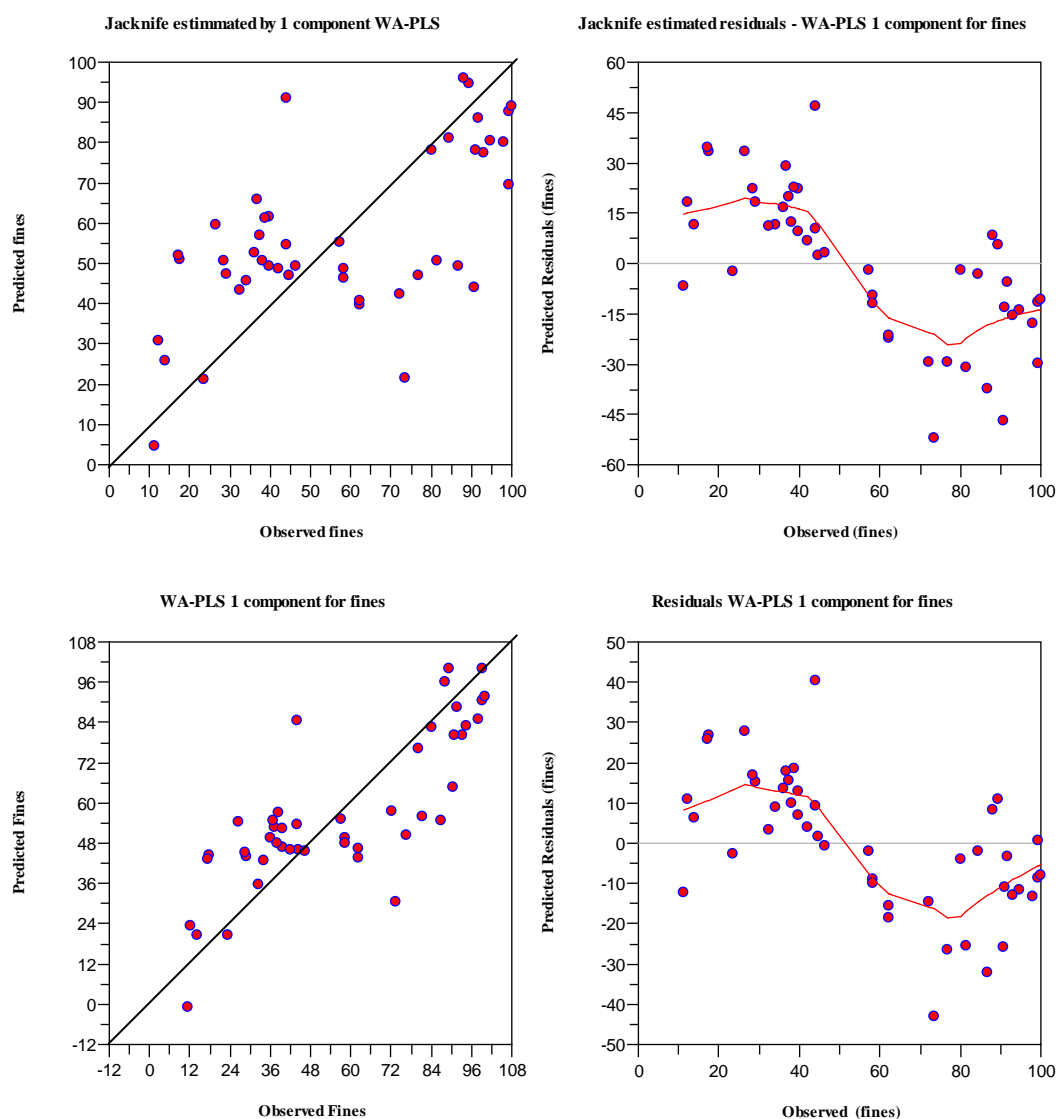
(B)



(C)



(D)



(E)

**Fig 7.4** (combined data set). Plots of WA-PLS inference models showing chironomid inferred value of (A) Chromium, (B) Lead and (C) Lead (sed), (D) TON and (E) Fines. The diagonal line is a 1:1 line. The solid line shows a LOESS scatter plot smoother (span = 0.45).

## 7.3 DISCUSSION

### 7.3.1 Transfer functions

When the data set was split into separate water and sediment chemistry data, the water chemistry model had a higher prediction accuracy than the sediment chemistry model. Although the geographical range of the study was relatively small the variable gradients in all the models were relatively large. For example, dissolved Pb ranged from 1.73µg/l to 61.02µg/l, sediment lead from 23.3mg/kg to 1280mg/kg, chlorophyll from 5.36µg/l to 171.63µg/l; TON from 0.33µg/l to 18.7µg/l and sediment nitrogen from 0.11mg/kg to 1.65mg/kg. Although there was a relatively wide range for some of the variable concentrations, model performance could also certainly be improved by the addition of further sites over a wider geographical range, as well as taking in more rural sites.

The best model was found in the combined dataset, with a 2-component WAPLS model for TON, with a high  $r^2$  and low RMSEP. Using only water chemistry, however, all three determinants (chromium, chlorophyll and lead) performed better overall. However, there is the possibility that the 5-component model was over-fitted. There was a greater than 5% improvement in  $r^2_{adj}$  values, the RMSEP were less so despite the 5-component model having the lowest RMSEP. The sediment data did not perform well at all. The apparently low  $r^2$  values are most likely a result of a smaller range of sediment parameters and not just heavily polluted sites. This has been found to be the case in paleolimnological studies where increasing the number of sites improved transfer function results. For example, a study of salinity inference models from diatom assemblages (Wilson et al., 1996), showed an improvement in transfer function predictability with the addition of more lakes. The low number of sites, the length of variable gradient and uneven distribution along these gradients may also be responsible for odd-shaped residual curves. The TON model (Fig. 7.4D) is an example of this.

Other studies that have used chironomids for inference models have examined reconstructing past environments such as nutrient status (Brodersen and Lindegaard, 1999), oxygen (Quinlan and Smol, 2001) and temperature (Korhola et al., 2002). In their study of shallow lake communities in Denmark Brodersen and Lindegaard (1999) found a strong correlation between chironomid data and chlorophyll that they used to create a weighted averaging (WA) model to infer lake trophic state. Their best model was a simple WA model using inverse deshrinking that had an  $r^2_{\text{jack}}$  of 0.67. Using the same parameters in the current study produced a model with a slightly lower  $r^2_{\text{jack}}$  of 0.51. This was most likely to have been lower because of the lack of macrophytes at many of the canal sites.

### **7.3.2 Implications for biomonitoring and model limitations**

This successful demonstration of the use of chironomids to infer chemical conditions in canals shows their potential to support the implementation of the WFD. The predictive ability of the models is likely to be improved by the addition of further sites over a wider geographical range and the addition of further species. Other studies have emphasized the importance of including many taxa in inference models, for the benefit of improving RMSE of prediction. In a study to assess the reliability of salinity inference models from diatom assemblages Wilson et al. (1996) found that as more lakes were included in the model, the numbers of taxa present in the transfer function increased and more consistent estimates of taxa optima and tolerances to salinity were generated.

Another reason why the above models may not have reconstructed the chemical variables as accurately as might be expected is possibly due to the fact that multiple stressors are acting on the canal ecosystems. Levels of boat traffic and the corresponding effects on macrophyte growth, turbidity and oxygen are likely to be the principal complicating factors. Studies in lakes have found similar problems (Langton et al., 2006; Scheffer et al., 1993; Jones and Sayer, 2003). The stressors influencing nutrient levels in these cases were likely to be summer hypoxia, plants and fish.

## **CHAPTER 8**

### **APPLICATION OF MODELS – ASSESSING THE ECOLOGICAL POTENTIAL OF URBAN CANALS**

#### **APPROACH**

This study has shown that the use of EQR derived from 46 sites using chironomid pupal exuviae has proved to be effective. Although the study sites were confined to a relatively small geographical area, it has shown that there is potential for the development of a tool to be implemented into the macroinvertebrate requirement of the WFD and define ecological status through comparison of observed to reference EQR. Eighty-eight taxa were found in the study and revealed species that are sensitive to a range of impacts presented by the urban canals studied.

## 8.1 INTRODUCTION

The previous chapters have considered the relationship between biology and physico-chemistry. The significant variables found will be used here to suggest suitable standards for creating EQR boundaries.

The Water Framework Directive (WFD) stipulates ecological quality assessment against near-natural reference conditions specific to each type of water body (Birk & Hering, 2009). In rivers, fish, benthic invertebrates, macrophytes and benthic algae are assessed. Results are given in relation to reference conditions and expressed as numbers between 0 (worst status) and 1 (near reference status). This is the Ecological Quality Ratio (EQR). The EQR range is split into five classes (high, good, moderate, poor and bad). Individual nations can develop new methods or modify their own national assessment methods, but the quality classification at the European level is harmonized by intercalibration (Heiskanen et al., 2004). This guarantees consistent quality classifications, while still allowing member countries to have diverse assessment methods. The WFD permits Member States to identify and designate Heavily Modified Water Bodies (HMWB) and Artificial Water Bodies (AWB). Under the EC Water Framework Directive (WFD) Member States must develop methods to classify the ecological status of their surface water bodies (WFD, 2000). Canals are defined as an AWB; therefore, to fulfil UK obligations in the assessment of the ecological potential of canals, the UK needs to introduce a method to classify their ecological status. The WFD develops the concept of ecological potential in AWBs, as opposed to ecological status in natural water bodies to allow their continued beneficial uses such as navigation and recreation.

Sites are classified using the Ecological Quality Ratios (EQR). The calculation of an EQR requires that the observed value and the value that would be expected for that element under reference conditions. In the case of canals Maximum Ecological Potential (MEP) is the reference state against which observed values are assessed.

The application of chironomid exuviae in the assessment of canals was first proposed by Ruse (1998), where he developed a scoring system and key to classify canal water quality. Later, the technique was revised and a tool developed to define reference states of lakes so that anthropogenic impacts could be measured and classified by their ecological quality (Ruse, 2002, 2008), and where the sensitivity of chironomid species to acidification and nutrient enrichment were determined. Scores for each impact were used to compare with reference scores modelled from impact-independent characteristics of a lake to provide an ecological quality ratio (EQR).

This chapter will use the sensitivity of chironomid taxa to certain environmental variables to produce a score for each impact and comparisons made to a reference score to provide an ecological quality ratio (EQR) for each of the forty-six canal sites that comprise this study.

This chapter aims to:

- i) Outline procedures for identifying reference sites for chironomids in urban canals;
- ii) Describe a rationale for setting boundaries between each of the five ecological status classes: high, good, moderate, poor and bad;
- iii) Describe a site-specific approach for defining ecological quality ratios (EQR).

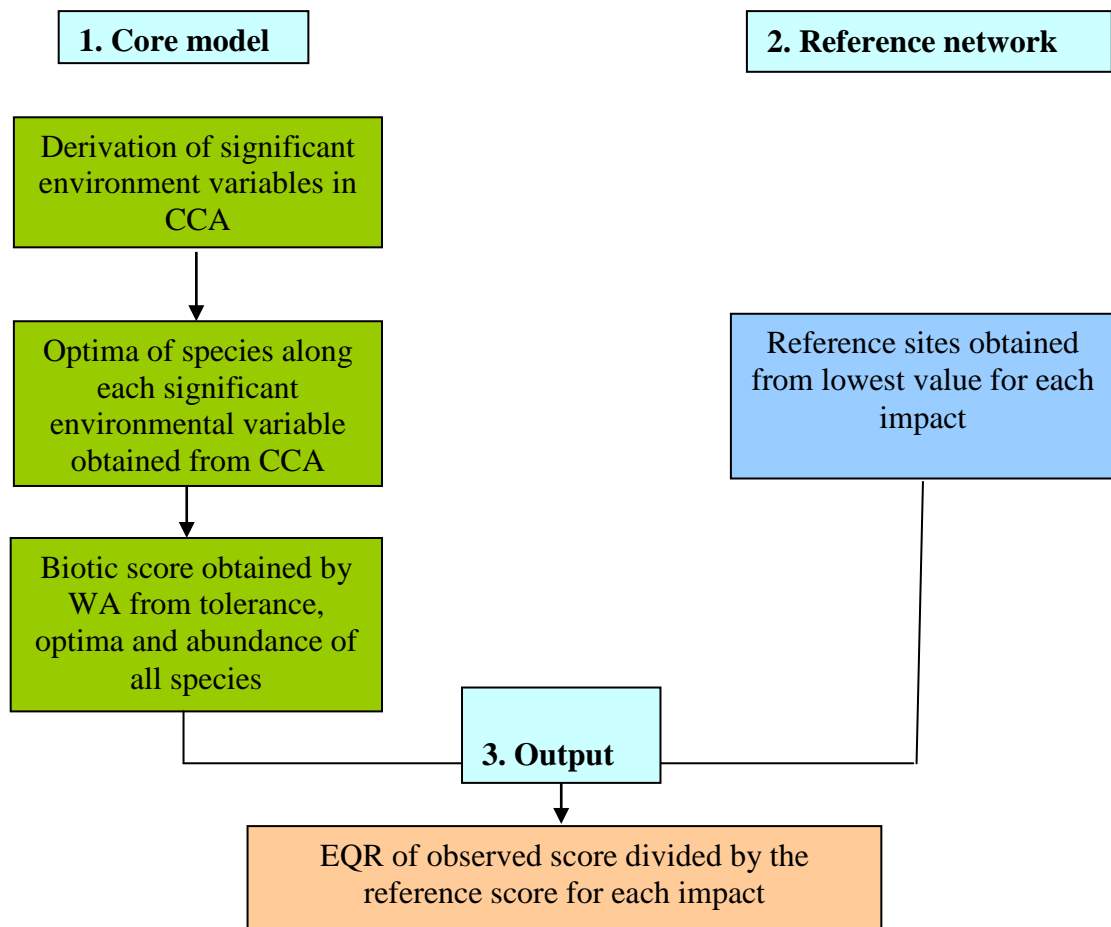
## 8.2 METHODS

### 8.2.1 ECOLOGICAL QUALITY RATIO (EQR)

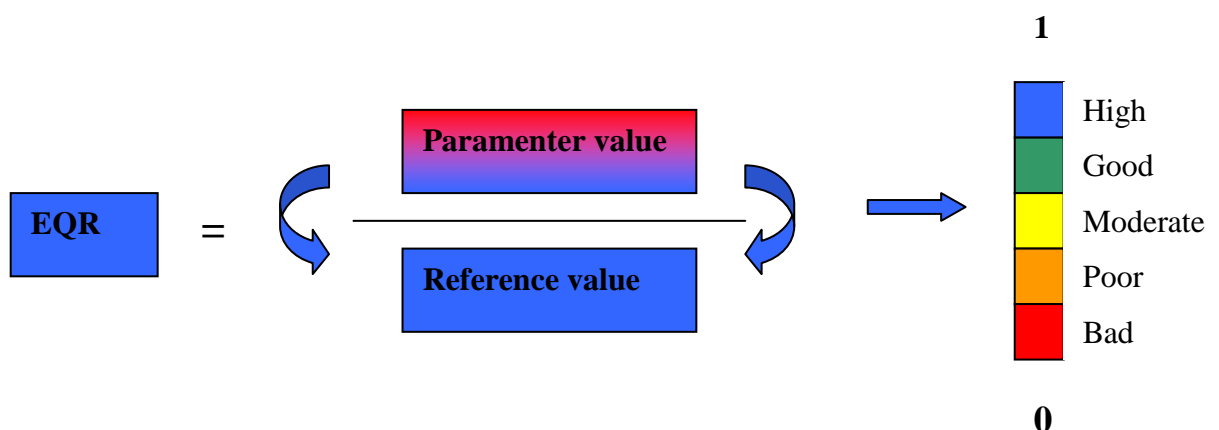
The steps taken to produce the EQRs are shown in Fig. 8.1 and 8.2. A weighted-averaging formula was used to calculate a biotic score for each significant variable produced from a CCA, to take advantage of the relative abundance and tolerance (niche breadth) for all taxa.

$$\text{Score} = \frac{\sum \text{abundance} * \text{optimum} * (2 - \text{tolerance})}{\sum \text{abundance} * (2 - \text{tolerance})}$$

The sum of products of % species abundance multiplied by the species optimum and a measure of niche breadth (2-tolerance) for all taxa collected was divided by the sum of products of abundance and niche breadth. This formula is constructed so that the narrower the species niche breadth the greater weight will be attached to its optimum value. Tolerances for all species and all impacts analysed were always less than 2.0 standard deviation units so '2-tolerance' produced the highest value for the narrowest niche breadth. Site-specific reference conditions were derived for each of the impacts studied, for the full data set as well as separate reference conditions for the sediment and water data sets. Sites were selected on the basis of the relative proportions of impact-sensitive and tolerant species as determined through COINSPAN (Chapter 5). An Ecological Quality Ratio (EQR) of the observed score divided by the reference score for each impact at each site had to be formulated to take account negative scores that could be produced by the negative optima of sensitive species. This was achieved by adding the maximum negative score to each site; this was then divided by the reference score. The resulting EQR was rescaled to range from 0-1 by dividing it by the maximum EQR. EQR classes were plotted and were based on five equal partitions of 0.2 to reflect the five quality bands required by the WFD. These were colour coded as follows: red (Bad), orange (poor), green (moderate), light blue (good) and dark blue (High).



**Figure 8.1** Schematic diagram showing the steps used to derive Ecological Quality Ratios (EQR). The sections are the core model (1), the reference network (2) and the output (3). Arrows show the progression of analysis



**Figure 8.2** Graphical representation of the concept of the Ecological Quality Ratio (EQR)

## 8.3 RESULTS

### 8.3.1 SPECIES WA-OPTIMA AND TOLERANCES

The different WA calibration models were used to infer water and sediment quality based on the significant variables as deduced from the models created in Chapter 6. This included the following:

combined taxa and chemistry;

epibenthic taxa and water chemistry;

enbenthic taxa and sediment chemistry.

Each will be examined in turn.

#### *8.3.1.1 Combined taxa and chemistry*

Using the full data set, five variables were found to be significant (TON, sediment fines, Pb-s, Pb, and Cr). In a constrained analysis with the significant variables analysed separately, the ratio of eigenvalues for the first and second axes were: 0.74, 0.65, 0.55, 0.48 and 0.42 respectively. Species optima were calculated and the results (Table 8.1) show the species with effective numbers of occurrences (Hill's N2) >10. Species apparent WA Cr optima ranged from 0.68 to 71.15; Pb from 2.38 to 41.44; Chl A from 6.69 to 116.05; Pb-s from 45.27 to 981.12, and TON from 0.34 to 15.46.

**Table 8.1** Species optima - combined taxa and chemistry (N2>10)

N2	Cr		Pb		Chl A		Pb-s		TON	
	Code	Optimum	code	Optimum	code	Optimum	Name	Optimum	Name	Optimum
<b>23.35</b>	Cric_syl	14.59	Cric_syl	8.89	Cric_syl	59.99	Cric_syl	271.44	Cric_syl	5.37982
<b>10.24</b>	Cricind	20.44	Cricind	6.62	Cricind	45.08	Cricind	304.38	Cricind	8.77521
<b>10.33</b>	Cryp_sup	8.75	Cryp_sup	8.30	Cryp_sup	39.95	Cryp_sup	336.12	Cryp_sup	4.05059
<b>23.73</b>	Dicr_ner	12.03	Dicr_ner	14.99	Dicr_ner	59.19	Dicr_ner	332.45	Dicr_ner	3.01933
<b>25.22</b>	Glyp_pal	17.08	Glyp_pal	13.18	Glyp_pal	65.76	Glyp_pal	265.69	Glyp_pal	3.90619
<b>13.57</b>	Glypind	8.34	Glypind	19.24	Glypind	88.71	Glypind	285.41	Glypind	3.03823
<b>16.34</b>	Nano_bic	16.68	Nano_bic	17.67	Nano_bic	74.35	Nano_bic	259.56	Nano_bic	2.46732
<b>11.73</b>	Para_cnv	15.75	Para_cnv	14.89	Para_cnv	83.08	Para_cnv	381.08	Para_cnv	2.56884
<b>24.70</b>	Parc_arc	9.85	Parc_arc	14.12	Parc_arc	82.88	Parc_arc	418.91	Parc_arc	1.75343
<b>16.46</b>	Parl_con	20.11	Parl_con	21.20	Parl_con	74.16	Parl_con	343.54	Parl_con	2.25115
<b>27.56</b>	Proc_cho	7.68	Proc_cho	11.99	Proc_cho	67.01	Proc_cho	360.14	Proc_cho	2.44037
<b>11.48</b>	Prod_oli	6.92	Prod_oli	11.12	Prod_oli	44.30	Prod_oli	230.09	Prod_oli	4.39629
<b>17.71</b>	Xwno_xen	18.07	Xwno_xen	13.16	Xwno_xen	41.06	Xwno_xen	264.57	Xwno_xen	6.23371

#### *8.3.1.2 Epibenthic taxa and water chemistry*

In a water only calculation, Chlorophyll (Chl A), Lead (Pb) and Chromium (Cr) were shown by CCA analysis to be the most significant variables ( $p < 0.005$ ) influencing chironomid distribution in the 46 canal sites. In a constrained analysis with the three significant variables analysed separately, the ratio of eigenvalues for the first and second axes were 0.71, 0.61 and 0.40 respectively. Thus Chl A and Pb and to a lesser extent, Cr appeared to be appropriate variables for the development of reliable inference models. Species apparent WA Chl A optima ranged from 2.89 to 10.62; Pb from 0.43 to 1.52 and Cr from 0.20 to 1.01. Species optima were calculated and the results (Table 9.2) show the species with effective numbers of occurrences  $N_2 > 10$ .

**Table 8.2** Species optima - epibenthic taxa and water chemistry (N2>10)

N2	Chl A Code      Optimum	Pb Code      Optimum	Cr Code      Optimum
<b>12.02</b>	Bryoind      9.22	Bryoind      1.29	Bryoind      0.41
<b>36.54</b>	Cric_syl      7.38	Cric_syl      0.85	Cric_syl      0.60
<b>15.29</b>	Cricind      6.36	Cricind      0.73	Cricind      0.54
<b>15.14</b>	Deme_ruf      8.17	Deme_ruf      0.95	Deme_ruf      0.67
<b>34.03</b>	Dicr_ner      7.45	Dicr_ner      0.98	Dicr_ner      0.63
<b>18.15</b>	Endo_ten      7.58	Endo_ten      0.83	Endo_ten      0.84
<b>36.41</b>	Glyp_pal      7.66	Glyp_pal      0.93	Glyp_pal      0.60
<b>12.35</b>	Glyp_par      6.79	Glyp_par      0.65	Glyp_par      0.80
<b>27.02</b>	Glypind      8.27	Glypind      1.03	Glypind      0.63
<b>19.55</b>	Limnind      8.71	Limnind      1.20	Limnind      0.41
<b>27.17</b>	Nano_bic      8.08	Nano_bic      0.97	Nano_bic      0.65
<b>35.59</b>	Parc_arc      8.41	Parc_arc      0.97	Parc_arc      0.65
<b>17.78</b>	Part_ino      6.06	Part_ino      0.78	Part_ino      0.53
<b>23.64</b>	Xwno_xen      6.42	Xwno_xen      0.93	Xwno_xen      0.57

#### *8.3.1.3 Enbenthic taxa and sediment chemistry*

CCA with forward selection identified 3 environmental variables that significantly ( $P < 0.005$ ) explained the variance in chironomid species composition. These were Pb, N and sediment fines. Species apparent WA optima ranged from 1.87 to 2.74 (Pb); 0.64 to  $-0.07$  (N) and sediment fines from 4.25 to 9.71. The results (Table 9.3) and show the species with effective numbers of occurrences  $>10$ .

**Table 8.3** Species optima - enbenthic taxa and sediment chemistry (N2>10)

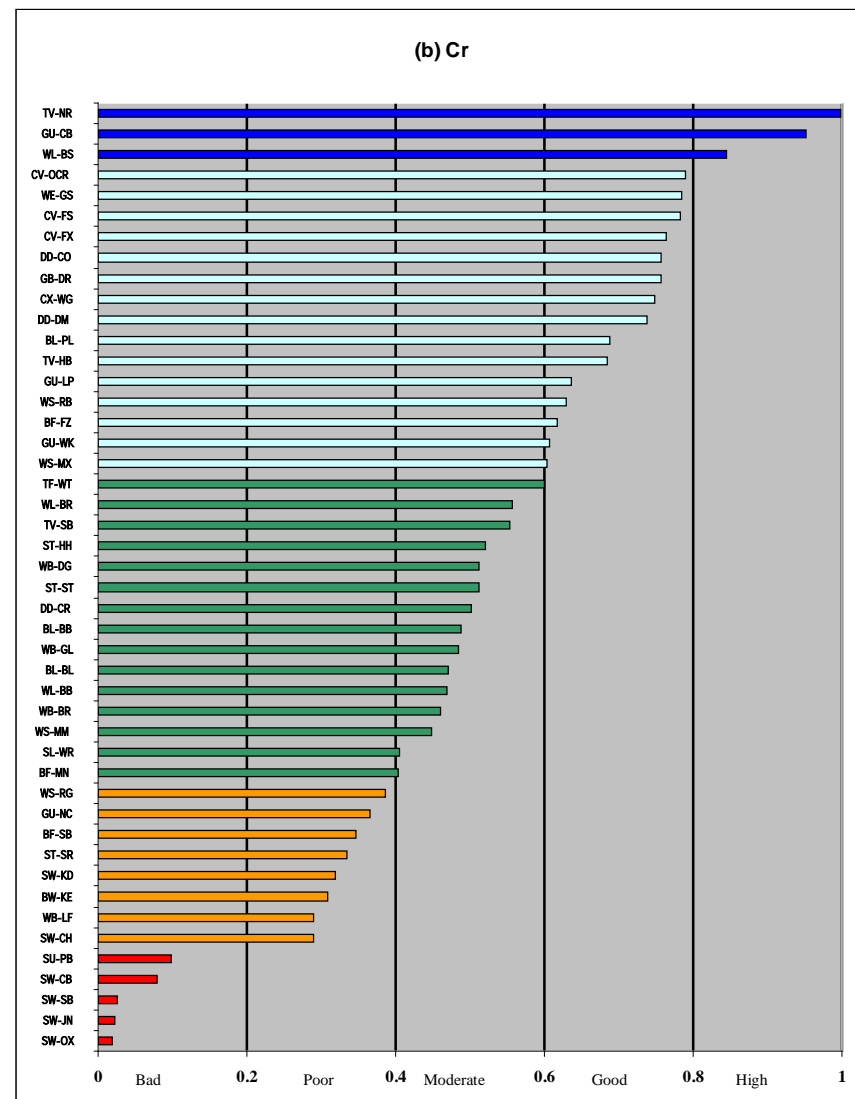
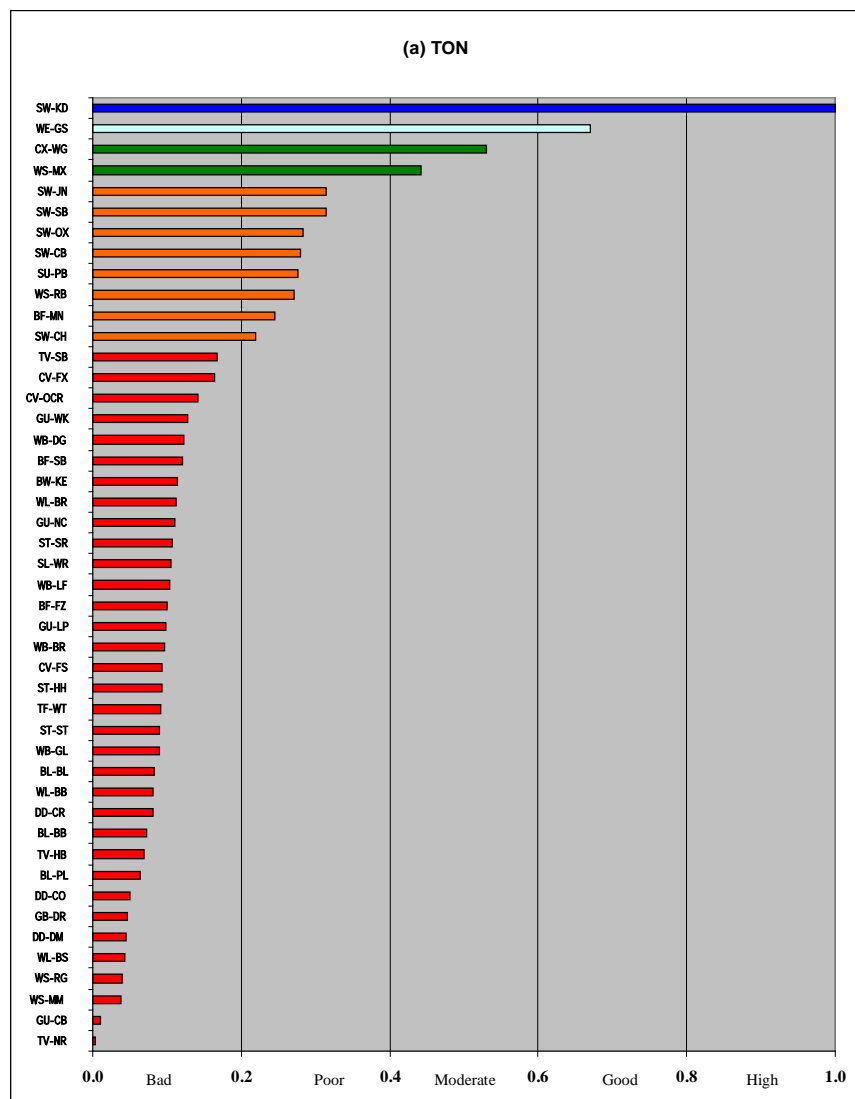
N2	Pb Name      Optimum	Cr Code      Optimum	fines Name      Optimum	N Name      Optimum
<b>11.88</b>	Cryp_sup      2.23	Cryp_sup      0.13	Cryp_sup      7.64	Cryp_sup      -0.24267
<b>10.16</b>	Einf_pag      2.23	Einf_pag      0.12	Einf_pag      7.21	Einf_pag      -0.37981
<b>10.64</b>	Para_cnv      2.42	Para_cnv      0.10	Para_cnv      8.81	Para_cnv      -0.42073
<b>16.17</b>	Parl_con      2.47	Parl_con      0.09	Parl_con      6.99	Parl_con      -0.36364
<b>26.18</b>	Proc_cho      2.38	Proc_cho      0.12	Proc_cho      7.27	Proc_cho      -0.31109
<b>11.28</b>	Prod_oli      2.21	Prod_oli      0.13	Prod_oli      7.61	Prod_oli      -0.3529

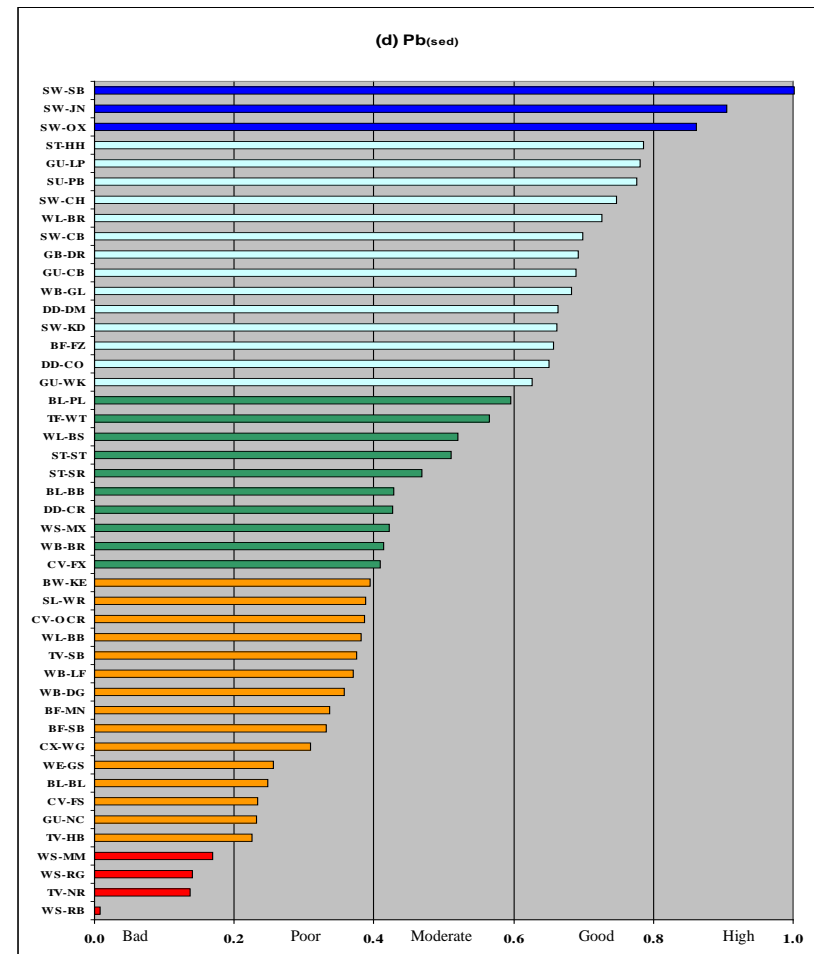
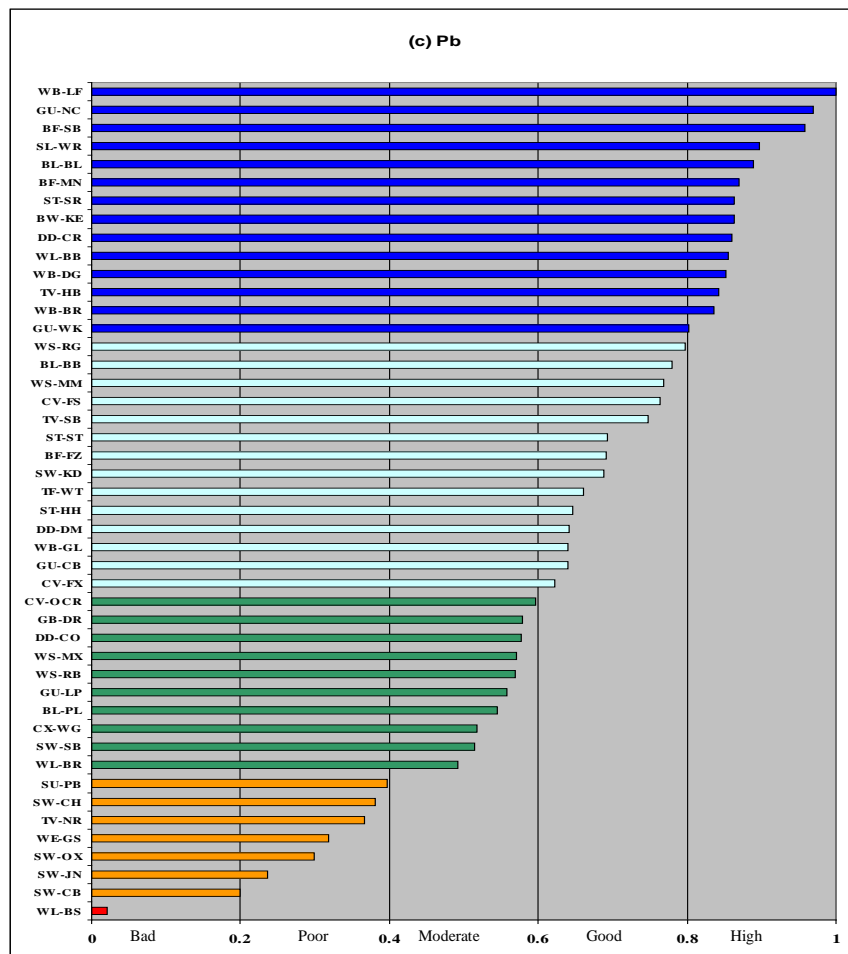
## 8.3.2 REFERENCE SCORE AND EQR

### 8.3.2.1 Combined results

Ten sites were chosen as reference conditions (see methods) in terms of impact by TON on the basis of their component-sensitive chironomid species. The mean TON value for these were  $10 \mu\text{g}^{-1}$ . The EQR results are shown in Fig 8.3(a). Three sites were chosen as reference condition for anthropogenic chromium impact. The mean chromium level for these sites was  $2.87 \mu\text{g}^{-1}$  (Fig 8.3b). Five sites were chosen as reference conditions for lead and the mean value of lead was  $2.79 \mu\text{g}^{-1}$  (Fig. 8.3c). Four sites were chosen as reference conditions for sediment lead. Mean lead concentration for these sites were was  $66.3 \text{ mg}^{-\text{kg}}$ . EQR results are shown in Fig. 8.3d.

It can be seen that there is a better spread of sites across the EQR boundaries for the significant metal variables when compared to TON. As this study has been concerned with urban sites, this distribution is perhaps to be expected. Sites were chosen so that there would be a gradient of metal concentrations, whereas there would be mainly eutrophic conditions at most sites. This is reflected in the number of sites that were of moderate ecological potential or below. For TON just 2 sites out of 46 were Good or High ecological potential. This compares with 39%, 61% and 37% of Good or High ecological potential for Cr, Pb and  $\text{Pb}_{(\text{sed})}$ .



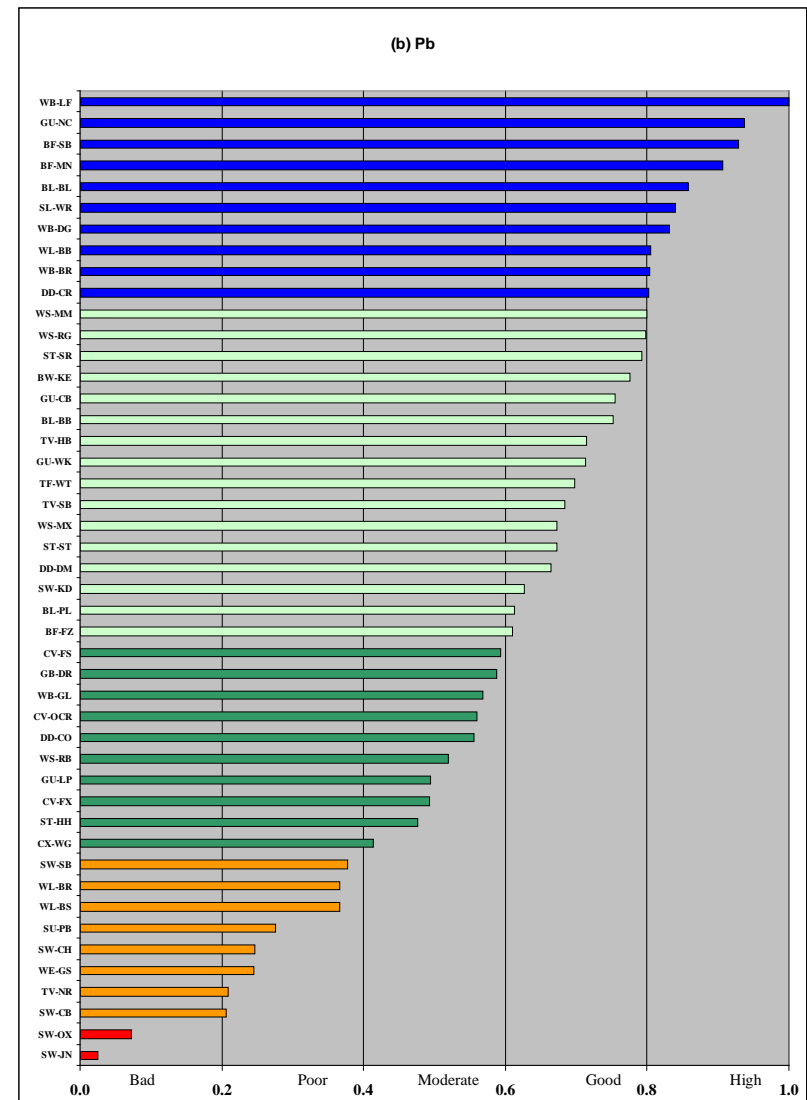
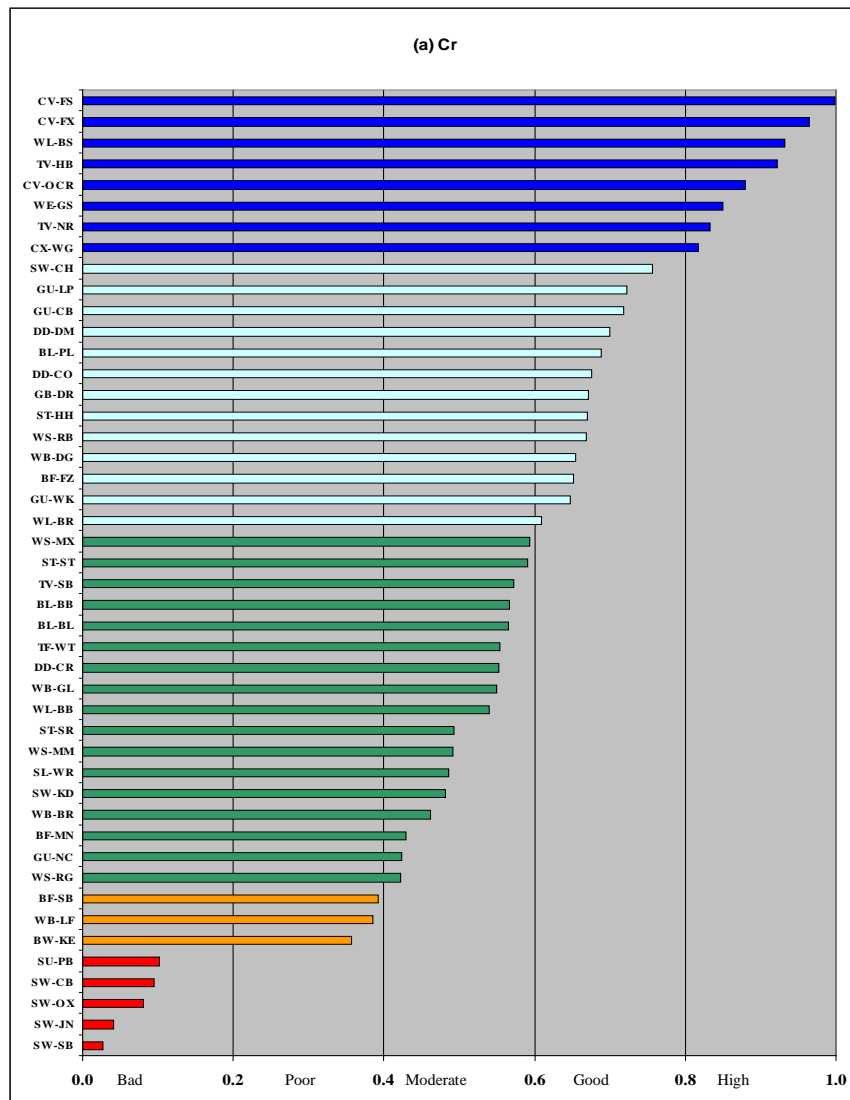


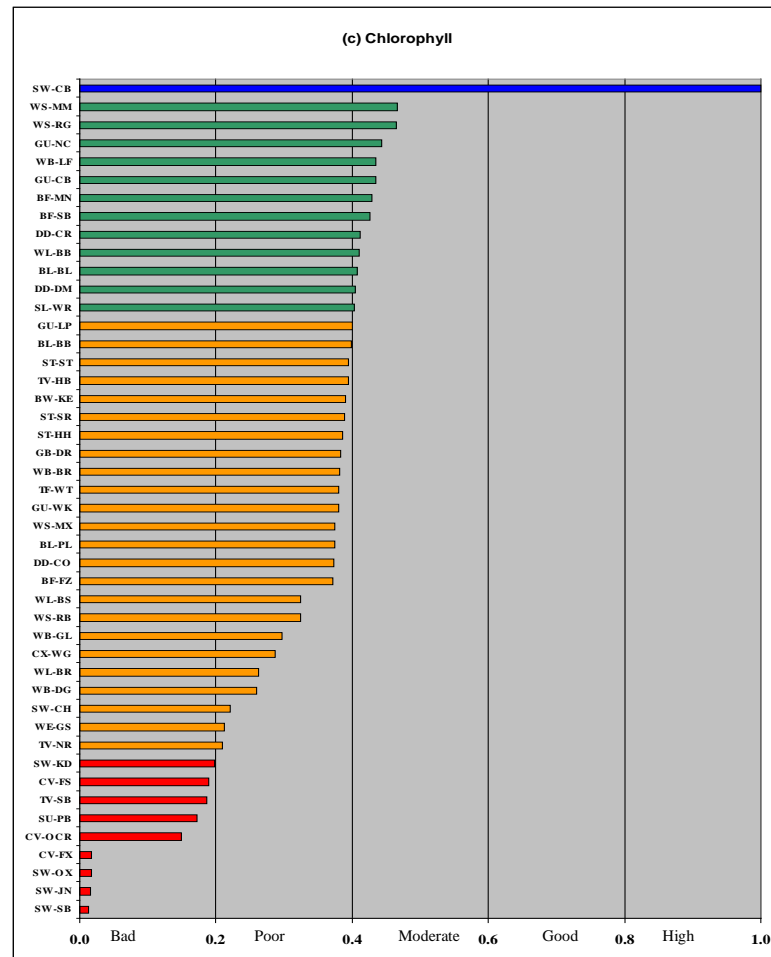
**Figure 8.3** EQR for combined dataset. EQR classes are based on five equal partitions, of 0.2. Sites banded red were Bad, orange were Poor, green were Moderate, light blue were Good and dark blue were of High status. Status is given for a) TON; b) Cr; c) Pb and d) Pb<sub>(sed)</sub>

#### 8.3.2.2 *Epibenthic taxa and water chemistry*

Three sites were chosen as reference conditions for chromium. The mean concentration for these sites was  $1.36\mu\text{g}^{-1}$  and EQR results are presented in Fig. 8.4(a). Four sites were used as reference conditions for lead, with mean concentrations of  $2.83\mu\text{g}^{-1}$  (Fig. 8.4b). Five sites were chosen as reference conditions for chlorophyll with a mean concentration of  $13.4\mu\text{g}^{-1}$  (Fig. 8.4c).

The water chemistry showed similar patterns to that of the combined dataset. 17% of sites for Chromium (Fig. 8.4a) were Poor/Bad and 46% of sites were Good/High; 22% of sites for lead (Fig. 8.4b) were Poor/Bad and 57% of sites were Good/High; 72% of sites for chlorophyll were Poor/Bad and 2% of sites were Good/High.



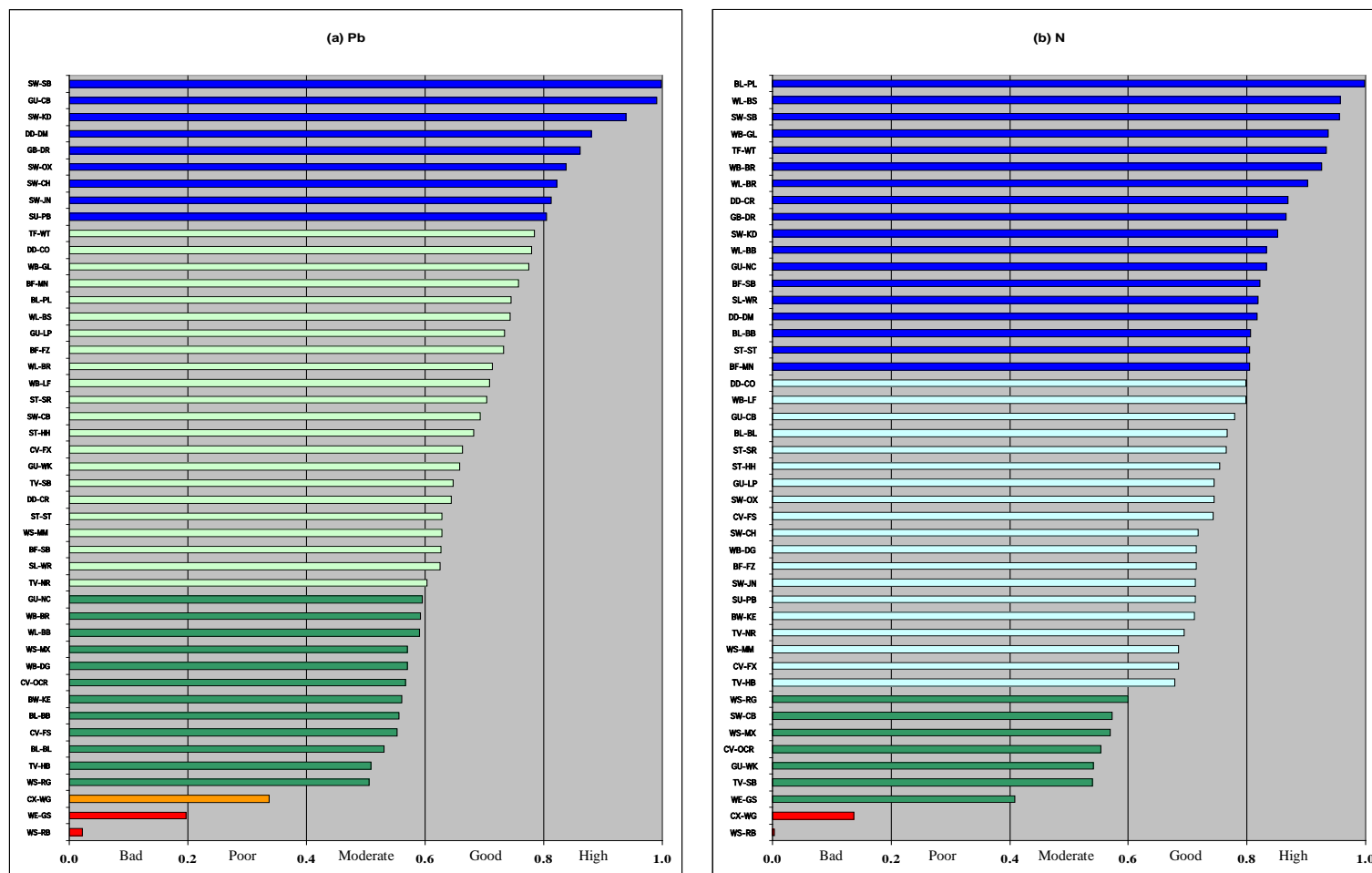


**Figure 8.4** EQR for water chemistry dataset. EQR classes are based on five equal partitions, of 0.2. Sites banded red were Bad, orange were Poor, green were Moderate, light blue were Good and dark blue were of High status. Status is given for a) Cr; b) Pb and c) Chlorophyll

#### 8.3.2.3 *Enbenthic taxa and sediment chemistry*

Five sites were used as reference conditions for lead with a mean concentration of  $86.96\text{mg}^{-\text{kg}}$ . EQR results are presented in Fig 8.5(a). Four sites were used a reference condition for nitrogen with a mean concentration of  $0.4\text{mg}^{-\text{kg}}$  and EQR results are presented in Fig. 8.5(b).

The situation for sediment was slightly different in that there were higher proportions of Good/High potential sites. For nitrogen there were 80% and 67% for lead.



**Figure 8.5** EQR for sediment chemistry dataset. EQR classes are based on five equal partitions, of 0.2. Sites banded red were Bad, orange were Poor, green were Moderate, light blue were Good and dark blue were of High status. Status is given for a) Lead; and b) Nitrogen

## **8.4 DISCUSSION**

The results showed that most sites were badly impacted by eutrophication, as indicated by Figs. 8.3a and 8.4c. This was unsurprising given the study area being mostly urban in nature. Metals on the other hand showed a more even distribution of EQRs. This is an indication of the metal gradients present and also shows how the past history of the canal sites has affected these sites.

### **8.4.1 SELECTION OF REFERENCE SITES AND INTERPRETING ECOLOGICAL POTENTIAL**

Within the WFD there is a requirement that type-specific biological reference conditions be established where the biota is that which is ‘normally associated with that type under undisturbed conditions and show no, or only very minor evidence of distortion’ (Annex V, Table 1.2). An interpretation of reference conditions has been given as ‘a state in the present or in the past corresponding to very low pressure, without the effects of major industrialization, urbanisation and intensification of agriculture and with only very minor modification of physiochemistry, hydromorphology and biology’ (Wallin et al., 2005). Within lakes baseline conditions can be achieved using subfossils. Bennion et al. (2004) used subfossil diatoms to infer nutrient conditions over 150 years. Subfossil chironomid head capsules have also been used to infer a variety of past environmental conditions (Brooks et al., 2001; Brodersen and Lindegaard, 1998; Larocque and Hall, 2004; Lotter et al., 1998; Reed, 1998). The use of chironomid subfossils is unavailable in canals and for lakes this would be too time consuming and expensive as a workable bioassessment tool within the water industry.

Within canals the WFD states that artificial and heavily modified water bodies should attain high ecological potential as opposed to status. Defining Maximum Ecological Potential for canals is problematic due to the internal pressures relating to the management of canals as sustainable navigable waterways. However, there are a number of options that can be used:

- i) *Comparison with other natural water bodies* – With this option is the concept of comparing an AWM/HMWB to the closest equivalent natural waterbody type. For example, reservoirs and gravel pits have an obvious analogue with lakes as they are both areas of standing water, although differing in other attributes. For canals, finding a similar analogue is difficult. They have attributes common to both rivers and lakes (Murphy et al., 1995). Their morphology is closer to that of large, slow flowing river than lakes. Those canals with very little or no boat traffic are more similar to a series of interlinked ponds, becoming more river-like with increasing boat traffic and its associated increases in water movement. If the conditions of a lowland river associated with High ecological status forms the basis for canal MEP (Maximum Ecological Potential), then even sites of high ecological quality would fail this standard. To exclude the effects of boat traffic would likely lead to canals failing to achieve GEP, without massive unsustainable expenditure.
- ii) *Comparison with untrafficked canals* – In this approach canal sites would be compared with sites on disused canals. The outcomes would be similar to option (i) where canals with more than very light boat traffic would be penalised.
- iii) *Comparison with the best sites at a given level of use* – with this option the best biology for a given level of boat use could be defined. Here boat traffic is considered a permitted source of environmental variation. This would enable the sustainable management of canals.

In a study by Murphy et al. (1995) it was shown that there was a critical level of boat movements (2000 per year) where a significant impact was seen on the macrophytes present in canals. In this study there were too few data to assess this, but this will be investigated in a follow-up study. In this project and similar studies that have been carried out on lakes (Ruse, 2002) the focus was on low level physicochemical impacts coupled with an assessment of the biology.

#### **8.4.2 SETTING BOUNDARIES FOR EQR**

True divisions that define boundaries between EQR to produce the required five classes of ecological status do not exist. Because all EQRs were to be scaled between 0-1, it was possible to divide the bands into five bands of 0.2 width. This appeared to give good classification of canals sites. In most of the EQR there was a relatively even spread of sites within the middle three classes. Few sites tended to occupy either the 'Bad' or 'High' ecological status. The WFD states that the purpose of expressing results as an EQR is to ensure comparability between different assessment methods in order to provide a common scale of ecological quality.

The classification of Ecological Status (or Potential for canals) requires the consideration of the status of the biological and physicochemical quality elements with overall status being the poorer of the two groups. For artificial and heavily modified waterbodies the specific physicochemical elements to be considered are defined as transparency, thermal conditions, oxygenation, salinity, nutrient condition and other pollutants. Annex VIII (indicative List of Main Pollutants) include two sub-elements that have particular relevance to canals:

- 'Materials in suspension' (this will directly reflect boat traffic, as well as suspended solids from sewage and runoff) and
- 'Metals and their compounds' (which may accumulate in canal sediments where there is a history of pollution from industrial point sources).

The physicochemical elements are considered to support the biology and Environmental Quality standards need to be proposed that are compatible with this supporting relationship.

Within this study there was emphasis placed on obtaining metals data for both water and sediment, but there was insufficient data to undertake a meaningful analysis of the relationship between the biology and boat traffic data.

Chironomid pupal exuviae using CPET to set EQR boundaries offer a good, easy and effective method of monitoring and assessment of canals. This may be used by itself or as an extension to other monitoring techniques. The advantage of EQR over measuring chemistry is primarily one of cost. Chemistry sampling is expensive, as monthly sampling is required and a range of chemical determinants are normally taken at each site, each with its own cost implication.

#### **8.4.3 A SITE-SPECIFIC APPROACH FOR DEFINING ECOLOGICAL QUALITY RATIOS**

The results in the setting of EQRs have shown some interesting patterns and illustrate the importance of eutrophication within urban canals. The data show for both the combined and water data, nutrients to be of Bad or Poor status (Potential). These data possibly suggest that nutrients effects were mainly associated with water rather than in the sediment. Within the sediment the majority of sites were of High or Good ecological status (Potential).

Work on lakes using the CPET method has revealed that EQRs could be assessed based on their nutrient and anthropogenic acidification (Ruse, 2002). Other biological elements are also being used to evaluate ecological status of lakes. Diatoms are being used to assess lakes using alkalinity to distinguish lake types. Reference sites were established using a combination of palaeoecological techniques and expert judgement (Kelly et al., 2008).

For canals, Wilby (2008) has developed a classification tool based on macroinvertebrates and macrophytes. This has considered boat traffic to be a characteristic of the canal system and therefore a source of environmental variation. It was considered that, the Maximum Ecological Potential (MEP) was determined by the best available biology for any specified level of boat traffic. To identify reference sites, different approaches were taken for macrophytes and macroinvertebrates. This was due to difficulties in linking biological data to impact data. For invertebrates, it

was possible to screen by impact data, whereas for macrophytes it was necessary to identify sites with the best biology for a given level of boat traffic. Different metrics were developed for macrophytes and invertebrates in order to reflect different pressures (nutrient enrichment, boat traffic, channel modification etc).

The data indicate that as boat traffic increased so the flora and fauna changed from that typical of a series of well vegetated ponds to that of large turbid lowland rivers.

To optimise a method for ecological assessment the ecology of a particular taxonomic group under consideration should be considered. There are a number of modelling considerations that could be problematic:

- i) *The number of species* – In the case of chironomids this is not a problem as there are many species (612) that have been identified from Britain (Wilson and Ruse, 2005). This compares to low macrophyte species, especially at low nutrient levels (Haury, 1996; Dodkins et al., 2005).
- ii) *Natural variation* – Macrophytes in rivers suffer from high natural variation insofar as spate disturbance produces a high natural variation in species composition. This can lead to environmentally similar sites that may have a different species composition (Dodkins et al., 2005). There is also the added problem of small-scale physical habitat variability (French, 1996). These problems do not arise for chironomids in canals.
- iii) *Survey methodology* – Because the CPET sample is passively collected, chironomid emergence is integrated from the previous 1-2 days. This method has been shown to be more efficient at collecting profundal species than by grab sampling (Raunio et al., 2007). Macrophytes suffer because survey information is dependent on the observational abilities of the surveyor in the field (Dodkins et al., 2005). For canals the PYSM methodology (Biggs et al., 2000) suffers by the hit-and-miss nature of sampling, i.e. being able to sample enough microhabitats such as sediment by dredging or sweep netting marginal vegetation.

## CHAPTER 9

### CONCLUSIONS AND SUMMARY OF METHOD

The overall objective of this thesis was to develop a classification tool for urban canals using chironomid pupal exuviae. Under this objective, the key aims of this study were:

- I      Classify chironomid assemblages in canals using pupal exuviae;
- II     Establish species distributions, ecology and function;
- III    Determine indicator taxa suitable for classifying canal water and sediment;
- IV    Analyse potential boundaries between the WFDs five ecological classes and develop a method to calculate an ecological quality ratio (EQR).

The findings of the study in relation to these four objectives are discussed in this chapter. The limitations of the project are also discussed and suggestions made for further research are also advanced. Also, the main improvements that have been introduced in the latest development of the tool are also discussed.

The tool can be summarised as follows:

1. Collect pupal exuviae from behind obstacles such as lock gates or among vegetation.
2. Make three sampling visits from April until October.
3. Identify and count species from subsamples of around 200 exuviae.
4. Amalgamate the species counts before square-root transformation.
5. Environmental data transformed to normal distribution when necessary.
6. DCA of chironomid data confirmed suitability of unimodal methods. CCA selected significant explanatory variables.
7. Species optima obtained by single variable CCA
8. Overall canal biotic score calculated using weighted-averaging.

9. Reference sites were obtained on the basis of their component sensitive species and low mean impact variable.
10. Produced a ratio of observed to reference biotic scores to assess status.
11. To take account of negative scores that could be achieved by the negative optima of sensitive species, the maximum negative score was added to each site and dividing by the reference score.
12. The resulting EQR was rescaled to range from 0-1 by dividing it by the maximum EQR.
13. EQR classes were plotted and were based on five equal partitions of 0.2 to reflect the five quality bands required by the WFD.

## **9.1 CHARACTERISE AND INVESTIGATE VARIABILITY OF METALS IN THE WATER AND SEDIMENT OF URBAN CANALS**

The heavy metal concentrations of 46 canal sites were characterised by applying a Critical Criterion Unit (CCU). Patterns of metal contamination were determined by cluster analysis on the basis of CCU. The results showed that the CCU can be used as a proxy for individual metal concentrations and that the combined measure of toxicity can be used to classify canals successfully.

Spatial analysis trends for sediment metals at 46 sites in the West Midlands canal network were determined using a metal pollution index (MPI). The MPI was based on the concentrations of Cu, Zn, Cd, Pb, Cr and Ni, normalized to reference sites. The MPI was designed to vary between 0 for pristine sites and 100 for extremely impacted sites. A general classification of the pollution levels, in relation to metals, was achieved and the most heavily impacted sites identified. It was shown that sediment quality guidelines can be effectively applied as a screening tool when identifying, ranking and prioritising metals which are of most concern by comparing their concentrations to ERL and ERM values. However, because the mean quotient is used, the contribution of individual metals to the overall toxicity of the sample may be diminished. A metal with a very high concentration in a sample containing metals with low concentrations will see the overall toxic effect reduced.

## **9.2 CLASSIFY CHIRONOMID ASSEMBLAGES IN CANALS USING PUPAL EXUVIAE**

Forty-six sites on canals in the English Midlands were sampled using the Chironomid Pupal Exuviae Technique (CPET). Species data were associated with water and sediment chemistry at each site. Separate CCA analyses were carried out on (i) all species and all chemical variables, (Pb (water and sediment), TON and fines were the best variables discriminating between sites) (ii) epibenthic species and water chemistry Cr, Pb & Chlorophyll) (iii) inbenthic (sediment dwelling) species and sediment chemistry (Fines, Pb & N. Biological classifications constrained by each of the significant variables were used to calculate indicator species scores and reveal species assemblages. Using chironomid exuviae was found to be an effective tool to determine water and sediment quality within urban canals.

It was demonstrated that the CPET method was an effective means to monitor the biodiversity of canals. The collection of pupal skins integrates both marginal and deep-water habitats from a wide area, which also integrates pupal emergence. Many taxa that were found to inhabit the sediment, a habitat normally difficult to sample. Chironomid taxa assemblages were able to differentiate between canal sites of different chemistry. Indicator species assemblages were identified that are associated with different COINSPAN groups constrained by each of the significant potential polluting variables determined by CCA analysis.

An effective bioassessment tool should be characterised by a minimum effort to extract the greatest amount of information possible. The methods under consideration in this study achieved this by utilising species response along environmental gradients, using all the species data including abundance and by using reference sites. The methods were able to detect impacts with clear differentiation between impacted and unimpacted sites. The methods also separated different impact types and some measure of total ecological change at each site was also determined.

### **9.3 UNDERSTAND THE DISTRIBUTION OF SPECIES, THEIR ECOLOGY AND FUNCTION**

Body size distributions were presented for chironomid species found within urban canals. Pupal exuviae were ordinated against chemical variables and key taxa used to establish whether there were significant differences in body length within species at different sites. Ordination of size classes against environmental variables found that metals were significant in determining distribution of body size. The relationship between functional feeding groups and metals was also investigated. It was found that predators were positively correlated to zinc and negatively correlated to CCU, whereas grazers were negatively correlated with lead. Metal concentrations were seen to affect mean body size of certain species, possibly as a result of a combination of metal adaptation, habitat, predator pressure or selective release from predator pressure. The results are very positive but before they can be used predictively more work is needed to establish the exact mechanisms involved.

### **9.4 FIND INDICATOR TAXA THAT ARE SUITABLE TO CLASSIFY CANALS IN TERMS OF WATER AND SEDIMENT CHEMISTRY**

Variations in chironomid assemblages were related to changes in measured environmental variables using multivariate techniques. Canonical Correspondence Analysis (CCA) indicated that five variables contributed significantly to explaining patterns of chironomid variation (TON, sediment fines, chromium, lead and sediment lead). Three variables were significant in explaining patterns of epibenthic taxa with water chemistry (chromium chlorophyll and lead) and three variables were significant in explaining patterns of enbenthic taxa with sediment chemistry (sediment fines, lead and nitrogen).

Chironomid-based inference models were generated for the reconstruction of the significant variables. The strengths of these models indicate that it would be possible to reliably infer trends in TON from the combined data set ( $r^2_{\text{jack}} = 0.75$ , RMSEP =

2.38). To a lesser extent, chromium ( $r^2_{\text{jack}} = 0.54$ , RMSEP = 0.25) and chlorophyll ( $r^2_{\text{jack}} = 0.53$ , RMSEP = 1.8) could also be reliably inferred.

## **9.5 ANALYZE POTENTIAL BOUNDARIES BETWEEN WFD CLASSES AND DEVELOP A METHOD TO CALCULATE ECOLOGICAL QUALITY RATIOS THAT WILL SUPPORT THE OBJECTIVES OF THE WFD**

This study has shown that the use of EQR derived from 46 sites using chironomid pupal exuviae has proved to be effective. Although the study sites were confined to a relatively small geographical area, it has nonetheless shown that there is potential for the development of this tool for it to be implemented into the macroinvertebrate requirement of the WFD and define ecological status through comparison of observed to reference EQR. Eighty-eight taxa were found from the sample sites. This investigation has revealed Chironomidae to contain species that are sensitive to a range of impacts presented by the urban canals studied.

## **9.6 LIMITATIONS OF THIS STUDY AND OPPORTUNITIES FOR FURTHER RESEARCH**

This study has concentrated on canals within a mainly urban context extending across a small geographical range. The next stage in order to develop this work further will be to extend the range of sites across the UK and Scotland. The main objective of the WFD is to produce a tool to classify the ecological potential of canals that takes into account sustainable use of these waterways in terms of navigation and boat traffic levels. Therefore, more data will be required in order to determine reference conditions for different levels of boat traffic.

There is also the requirement to define reference conditions and Maximum Ecological Potential (MEP). This is problematic for canals as they are subject to a variety of external and internal pressures. External pressures include nutrient enrichment and sediment contamination from waterside industries. Internal pressures relate to the management of canals as navigable waterways. A possible approach might be to

compare sites with the highest quality at a given level of use. This could then lead to the prospect of sustainable management of canals by providing targets and assessing performance relative to these.

The WFD also requires that there are estimates of confidence and precision in the results of monitoring programmes. In other words, how close are repeated measurements and are they of the same quality? Further development of the tool could also address what variation is there in the contingency of which months the samples are collected, what variation is there between subsampling and sample size and what is the confidence of a canal classification based on its observed EQR.

Finally the tool will require external validation across Europe and intercalibration of class boundaries will also be required.

Since this study was completed, the above points, apart from intercalibration, have been addressed. The resulting report can be viewed in the appendix.

## **9.7 IMPROVEMENTS INTRODUCED IN THE LATEST DEVELOPMENT OF THE TOOL**

From the report in the appendix, it can be seen that there have been improvements made. These can be summarised as follows:

1. Wider geographic context encompassing England, Wales and Scotland.
2. Boat traffic taken under consideration.
3. Improvements in selecting reference sites.
4. EQR boundaries.
5. Uncertainty estimations of a) spatial and operator sampling variability b) seasonal variability c) sample size d) confidence of class.

### **9.7.1 WIDER GEOGRAPHIC CONTEXT**

One of the most important improvements in the tool is the increase in the number of sample sites from forty-six to one hundred and sixty seven. These sites represented a wide range of canal types from disused to heavily trafficked; urban and rural sites and a full geographic range from South West England to Wales and Scotland.

### **9.7.2 BOAT TRAFFIC**

An important criterion for the development of the revised tool was that boat traffic be taken into consideration. This was to allow the canals' continued beneficial sustainable use for navigation and recreation. A system was therefore required that defined Maximum Ecological Potential (MEP) for any specified level of boat traffic.

### **9.7.3 REFERENCE SITES**

It was important to allow boat traffic to be considered when selecting reference sites as well as in the final tool. It was decided to make use of the best contemporary data, which took usage into account. As far as possible, sites were chosen on the basis of low value of the parameter under consideration.

### **9.7.4 EQR BOUNDARIES**

There is a problem in assigning EQR boundaries, in that no true boundaries exist in nature. Also, there is no justification in placing the good/moderate boundary on any point along the ecological potential gradient. It was therefore decided to consider the crossover point of sensitive and tolerant taxa, which reflected the structural and ecophysiological changes of the chironomid communities. This was the point at which the tolerant taxa become more abundant than sensitive taxa. The use of presence/absence data was found to be as good as other WA calculations. This is an

advantage in terms of the time taken to process samples in that abundance is not required.

### **9.7.5 UNCERTAINTY ESTIMATIONS**

Estimates of the level of confidence and precision of the results provided by the monitoring programmes are required by the WFD. Estimates of uncertainty were made which gave confidence of the canal classification was of high precision.

The tool after improvements can be summarised as follows:

1. Total oxidised nitrogen (TON) was found to be the best variable for assessing the nutrient status in canals.
2. Boat traffic is a characteristic of the canal system and this was taken into account when developing the tool.
3. Reference sites were defined by the proportion of sensitive and tolerant species, away from significant urban influences and low levels of TON. Using site-specific predictions of expected impact scores enabled EQRs (Ecological Quality Ratios) to be calculated for all samples.
4. Site-specific reference conditions were developed that took into account levels of boat traffic. This avoided splitting the dataset into separate boat traffic classes.
5. EQR boundaries were calculated using the standard deviation of 'fits' for tolerant taxa when the tolerant and sensitive taxa were plotted.
6. Spatio-temporal variability of samples was estimated and used to calculate uncertainty associated with EQR predictions.
7. Converting the measure of temporal variability into estimates of uncertainty suggests that there is no bias to certain seasons and that confidence of canal classification was of high precision.

There were other slight differences in techniques that were used between the thesis and report. In the report I have used the somewhat unusual data transformation of taking the fourth root of the data, as opposed to square root in the thesis. Fourth root gave better normality to the data and is best thought of as the equivalent to a log transformation that treats zero values more conveniently.

In the report, I used 3-tolerance and not 2-tolerance as in the thesis in order to obtain impact scores. This was simply because the tolerances (niche breadth) were less than 3.0 SD units.

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# APPENDIX I:

## Taxa codes

Code	Name and author	Code	Name and author
ABLA_LON	Ablabesmyia longistyla Fitt	ORTH_EUO	Orthocladus (Euorthocladus) spp
ABLA_MON	Ablabesmyia monilis (Linnaeus)	ORTHss	Orthocladus spp
ABLA_YIA	Ablabesmyia spp	PARC_ARC	Parachironomus arcuatus (Goetghebuer)
ACRI_LUC	Acricotopus lucens (Zetterstedt)	PARC_BIA	Parachironomus biannulatus (Staeger)
APSE_TRI	Apsectrotanypus trifascipennis (Zetterstedt)	PARC	Parachironomus spp
ARCT	Arctopelopia spp	PARD_LMA	Paracladopelma spp
BRIL_BIF	Brilia bifida Kieffer	PARD_NGA	Paracladopelma nigrifulva (Goetghebuer)
BRIL_FLA	Brilia flavifrons Johannsen	PARD_CAMg	Paracladopelma campolabis (Kieffer) gp
BRYO	Bryophaenocladus spp	PARH	Paraphaenocladus spp
CARD_FUS	Cardiocladius fuscus Kieffer	PARK_FEN	Parakiefferiella fennica Tuiskunen
CHIR_PLU	Chironomus plumosus (Linnaeus)	PARK	Parakiefferiella spp
CHIR	Chironomus spp	PARL_CON	Paracladius conversus (Walker)
CLAD	Cladopelma spp	PARM	Paramerina spp
CLAT	Cladotanytarsus spp	PART_TLL	Paratanytarsus tenellulus (Goetghebuer)
CLIN_NER	Clinotanypus nervosus (Meigen)	PART	Paratanytarsus spp
CONC_MEL	Conchapelopia melanops (Meigen)	PATD	Paratendipes spp
CONC	Conchapelopia spp	PATR_RUV	Paratrachocladus rufiventris (Meigen)
CORY_URA	Corynoneura spp	PATR	Paratrachocladus spp
CRIC(C)	Cricotopus s.g Cricotopus	PHAE	Phaenospetra spp
CRIC(I)	Cricotopus s.g Isocladus	POLY_NUB	Polypedium nubens (Edwards)
CRIC_BIC	Cricotopus (C.) bicinctus (Meigen)	POLY	Polypedium spp
CRIC_INT	Cricotopus (I.) intersectus (Staeger)	POLYSORg	Polypedium (Pent) sordens (Wulp) gp
CRIC_SYL	Cricotopus (I.) sylvestris Fabricius	POTT_TIA	Potthastia spp
CRYP_MUS	Cryptochironomus spp	PROC_HOL	Procladius (Holo) spp
CRYPOBRg	Cryptochironomus obreptans gp	PROC_SUS	Procladius spp
CRYT	Cryptotendipes	PROD_OLI	Prodiamesa olivaea (Meigen)
DEME_RUF	Demeijerea rufipes (Linnaeus)	PSEC_BPS	Psectrocladius (ss) barbatipes Kieffer
DEMI	Demicryptochironomus	PSEC_OBV	Psectrocladius (Allo) obivus (Walker)
DICR_NER	Dicrotendipes (Lim) nervosus (Staeger)	PSEC_OCT	Psectrocladius (ss) octomaculatus Wulker
DICR_NOT	Dicrotendipes (Lim) notatus (Meigen)	PSEC_ss	Psectrocladius (ss) spp
DICR_TRI	Dicrotendipes (Lim) tritonus (Kieffer)	PSET_VAR	Psectrotanypus varius (Fabricius)
DICR	Dicrotendipes spp	PSEU_PRA	Pseudochironomus prasinatus (Staeger)
EINF_PAG	Einfeldia pagana (Meigen)	RHEO_ss	Rheocricotopus spp
ENDO	Endochironomus spp	RHET	Rheotanytarsus spp
EPOI_FLA	Epoicocladus flavens (Malloch)	SMIT	Smittia spp
EUKI_CLA	Eukiefferiella claripennis (Lund)	STEM_BAU	Stempellina bausei (Kieffer)
EUKI	Eukiefferiella spp	STEM	Stempellina spp
GLYP_cau	Glyptotendipes (Caulochironomus)	STEN	Stenochironomus spp
GLYP_ss	Glyptotendipes spp	STIC	Stictochironomus spp
HARN_CUR	Harnischia curtilamellata (Malloch)	SYNE	Synendotendipes spp
HARN_HIA	Harnischia spp	SYNO_SEM	Synorthocladus spp
HETE	Heterotrissocladus spp	TANP_PUN	Tanytus punctipennis Meigen
HETT_API	Heterotanytarsus apicalis (Kieffer)	TANP	Tanytus spp
KIEF_TEN	Kiefferulus tendipediformis (Goetghebuer)	TANY_BRU	Tanytarsus brundini Lindeberg
LAUT_AGR	Lauterborniella agrayloides (Kieffer)	TANY_PCS	Tanytarsus pallidicornis (Walker)
LIMN	Limnophyes spp	TANY_PT2	Tanytarsus (part 2)
MACR_NEB	Macropelopia nebulosa Meigen	TANY_PT3	Tanytarsus (part 3)
METR	Metriocnemus spp	TANY_SUS	Tanytarsus spp
MICC_TEN	Microchironomus tener (Kieffer)	TANY_SYL	Tanytarsus sylvaticus (Wulp)
MICR_ATR	Micropsectra atrofasciata (Kieffer)	TANYMEDg	Tanytarsus medius gp Reiss & Fittkau
MICR	Micropsectra spp	THIE	Thienemanniella spp
MICT	Microtendipes spp	THIY	Thienemannimyia spp
NANO_BAL	Nanocladius balticus Palmen	TVET_DIS	Tvetenia discoloripes (Goetghebuer)
NANO	Nanocladius spp	TVET	Tvetenia spp
NEOZ	Neozavrelia spp	XENO_XEN	Xenochironomus xenolabis (Kieffer)
ODON_FUL	Odontomesa fulva (Kieffer)	XENP	Xenopelopia spp
ORTH_EUD	Orthocladus (Eudactylocladus) spp	ZAVR	Zavrelimyia spp

## APPENDIX II

### *Site descriptions*

#### **Walsall Canal**

The Walsall Canal begins at Ryders Green Junction, leaving the Wednesbury Canal and heads 7 miles through Walsall town centre. The canal starts by falling down a series of eight locks, 7 in a straight flight within a space of only 600 yards. An old guillotine gate can be seen between locks 4 and 5 over a disused arm that went into the old Nelson Iron foundry basin.

The last of the 8 locks (1/4 mile below the main flight) crosses over the River Tame. Going past the former Danks branch the canal, passes another junction, the Toll End Branch. This branch connects with the Birmingham Main Line. Just further along, the canal meets the Doe Bank Junction, the entrance to the Tame Valley canal.

After passing Moxley, the canal passes through reclaimed industrial land, curving to the northeast. Beyond the M6 motorway, the canal passes through Walsall town centre to Walsall Junction from where the Wyrley and Essington Canal heads north.

#### **Wyrley & Essington/Cannock Extension Canals**

The Wyrley and Essington Canal linked the mines of the South Staffordshire coalfields and the claypits of Great Wyrley with the Black Country and Birmingham. It was closed to navigation in 1954. The canal also links the Birmingham Main Line Navigations to the Coventry Canal. From the Wyrley & Essington Canal the Cannock Extension is only navigable for a mile or so, travelling dead-straight northwards across Wyrley Common. A boatyard at Norton Canes marks the end of the navigation.

## **Staffordshire And Worcestershire Canal**

This canal starts at Stourport where it joins the River Severn and makes its way 46 miles to join the Trent and Mersey Canal. The stretch surveyed lies between Kidderminster and Coven Heath. In between the canal skirts Wolverhampton, into which the Barnhurst sewage works discharges. Just north of the discharge the Shropshire Union Canal joins the system. The sewage works provides most of the water for both canals. The fact that there is a discharge into both canals, as well as their location at the summit level, allows both north- and southwards flows.

## **COVENTRY CANAL**

The canal was primarily built to transport coal from the pits at Bedworth, Coventry and Nuneaton to the rest of the Midlands and across the country. The Coventry Canal Company was formed in 1768 and the canal was started late that year. A year later the company ran out of money and it was seventeen years before it was finally finished in 1789. In the North it starts at the junction with the Trent and Mersey Canal and winds its way through semi-rural surroundings. It forms a junction with the Litchfield (Wyrley & Essington) Canal. At Fazeley it forms another junction with the Birmingham and Fazeley Canal at Fazeley. After Fazeley, the canal crosses the River Tame on an aqueduct, skirting around the town of Tamworth. After joining with the Oxford Canal at Hawkesbury Junction, the canal then becomes urban in nature as it enters Coventry.

## **The Shropshire Union Canal**

This canal runs about 60 miles from the edge of urban Wolverhampton to the River Mersey at Ellesmere Port, mostly through rural countryside. It was one of the last canals to be built.

## **TAME VALLEY CANAL**

The Tame Valley Canal was a late addition to the Birmingham Canal Navigations. It is typified by high embankments and deep cuttings. It crosses over the M5 motorway on a dramatic aqueduct before dropping through the 13 Perry Barr Locks and winding its way beneath the Gravelly Hill Motorway Interchange (Spaghetti Junction). The canal runs for 8.5 miles from Tame Valley Junction to Perry Barr Bottom lock.

## **BIRMINGHAM CANAL NAVIGATIONS**

Birmingham Canal Navigations (BCN) is a network of canals linking Birmingham, England to Wolverhampton via the eastern part of the Black Country. At its working peak, there were about 160 miles (257 km) of canals; today just over 100 miles (160 km) are navigable, and the majority of traffic is from tourist and residential narrowboats.

The BCN was built on three main levels: 453 ft, the Birmingham Level; 473 ft, the Wolverhampton Level; and 408 ft, the Walsall Level. These levels were linked by locks at various places on the network; and each level was fed by one or more reservoirs.

The position of Birmingham at the crossroads of a canal and river system extending from Liverpool to London and from Bristol to the Humber estuary meant that it shot to prominence during the Industrial Revolution. This led to the most extensive urban waterway in the world, with almost 160 miles of navigable canal mostly between

Wolverhampton and Birmingham and north into Staffordshire. Most of this system is still present today.

The canal is mostly built on two levels (Wolverhampton and Birmingham levels), with about 20 feet separating the two.

### **Worcester & Birmingham Canal**

The canal links the two cities and was built to connect the River Severn at Worcester to the Birmingham Canal system. Most of canal goes through countryside. There are 58 locks for the climb of 428 feet to Birmingham.

### **Dudley Canal**

The Dudley Canal was built to link the Birmingham Canal Navigations, at Tipton, near Dudley, via the Dudley Tunnel, to the Stourbridge Canal, and thence to the River Severn. The original canal route, which includes the Dudley Tunnel, was built in the 18th century. It later became known as the Dudley Canal Line No 1.

An extension was built, the Dudley Canal Line No 2, to link the Dudley Canal, at Netherton, via Halesowen and Lappal, with a tunnel at Lappal, to the Worcester and Birmingham Canal at Selly Oak. Lappal Tunnel collapsed in 1917 and the section from Selly Oak to Lappal is filled in. Part of the Lappal Tunnel was discovered by accident during the construction of the M5 motorway in the early 1970s and it was filled with concrete. The Dudley Canal Line No 1 was rescued, by the Dudley Canal Trust, and the tunnel reopened in 1973. It is in use today and is now known as the Dudley Canal.

### **Stratford-upon-Avon Canal**

The Stratford canal runs for 25 miles from the Birmingham conurbation to the River Avon at Stratford. Between the two the canal runs through mainly rural areas and small towns and villages.

### **Grand Union Canal**

The Grand Union links London through the Chilterns with Birmingham via the longest single canal in Britain. The 137-mile, 166 lock main line has many branches to towns along the way. The longest of these, the Leicester Line, runs to Leicester, from where the River Soar continues to Nottingham. As the main line from London to the Midlands, the Grand Union Canal was once one of the busiest in the country.

### **Birmingham & Fazeley Canal**

The Birmingham and Fazeley Canal is a canal of the Birmingham Canal Navigations in the West Midlands of England. It runs from the BCN Main Line at Old Turn Junction (near the National Indoor Arena), Birmingham to the Coventry Canal) at Fazeley Junction, just outside Tamworth. This stretch runs 15 miles (24 km) through 38 locks.

Technically the Birmingham and Fazeley Canal extends northwards beyond Fazeley Junction to Whittington, near Lichfield as the Coventry Canal Company had run out of money by the time they reached Fazeley.

### **APPENDIX III:**

*A paper was presented at the 16th International Chironomid Symposium in Madeira from which a paper was published*

## **THE RESPONSE OF CHIRONOMIDS TO WATER AND SEDIMENT CHEMISTRY IN URBAN CANALS (UK)**

By PHIL GREEN\*

With 2 figures and 2 tables

**ABSTRACT.** Forty-six sites on canals in the English Midlands were sampled using the Chironomid Pupal Exuviae Technique (CPET). Species data were associated with water and sediment chemistry at each site. Separate CCA analyses were carried on (i) all species and all chemical variables, (ii) epibenthic species and water chemistry (iii) inbenthic species and sediment chemistry. Dissolved lead, chromium and Total Oxidised Nitrogen, together with sediment lead and fines were the best variables discriminating between sites for (i) Dissolved chromium, lead and Chlorophyll a were best discriminating sites for (ii). Sediment fines, lead and nitrogen were the best discriminating variables for (iii). Biological classifications constrained by each of the significant variables were used to calculate indicator species scores and reveal species assemblages. CPET was effective at determining water and sediment quality within urban canals.

**RESUMO.** Comparamos a eficiência de detecção com o número de géneros exclusivamente recolhidos para exuviae de superfície- pupas flutuantes e métodos de rede de mão, entre dois gradientes de perturbação. O método mais eficiente foi a colheita exuviae, realizada mensalmente. Efectuando a comparação exclusivamente com o mês de Junho, o método tipo rede de mão foi o mais eficiente em todos os locais de amostragem, mas em locais com perturbação não se verificou significância estatística entre os dois métodos. O método de exuviae exclusivamente, colecionou duas vezes mais géneros, tal como o método tipo rede de mão.

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## INTRODUCTION

The recent implementation of the European Commission legislation, the Water Framework Directive (WFD; COUNCIL OF THE EUROPEAN COMMUNITIES, 1999) requires that member states protect and enhance surface waters and groundwater. Good ecological status should be achieved by 2015. The Directive provides additional designations, for Artificial (AWB) and Heavily Modified Water Bodies (HMWB), with canals included as AWB. Unlike all other surface waters, which have to achieve good ecological status, AWB will be required to reach an objective of "Good Ecological Potential" (GEP) for sustainable use of the water body. Canals are difficult for obtaining representative biological samples. Biological assessment of urban canals is further complicated by the variety of impacts from metals, nutrients and organic compounds. An effective WFD-compliant classification tool is required to assess GEP of canals.

In order to develop a tool for the monitoring of urban canals, the aims of this study were therefore to (1) investigate which chemical parameters influence the variation in chironomid taxa composition and (2) determine indicator chironomid taxa for significant chemical variables.

## MATERIAL AND METHODS

Forty-six urban canal sites were chosen from approximately 1000 km of classified canals within the English Midlands and these were predominately urban in nature. The sites were chosen in order to represent as wide a range of water and sediment chemical characteristics as possible, especially metals (Table 1).

Chironomid data were collected over four years (2001-2004). Each site was sampled three times between April and September each year, to cover the different emerging periods of the adults. A 250 mm mesh net on a light extendable pole was used to collect floating debris on the water surface. In the laboratory subsamples from each sample was taken and sorted in a small tray, removing all exuviae. This was done until a random collection of 200 individuals was made (WILSON & MCGILL 1979; RUSE 1993; RUSE 2002). The exuviae were then identified to species level using the keys of LANGTON (1991), WILSON (1996) AND LANGTON & VISSER (2003). Chemical data were obtained from the Environment Agency (Environment Agency 2005) for the twelve month period ending on the month of the final pupal collection. At each site, a one-off sediment sample was obtained.

Species data at each site were combined and abundance recorded as a percentage of the total number of exuviae collected. Separate CCA analyses were carried on (i) all species and all chemical variables, (ii) water chemistry and epibenthic species and (iii) sediment chemistry with inbenthic species. To improve the signal to noise ratio species data were square-root transformed (PRENTICE 1980). All environmental data were tested

for normality by a Ryan-Joiner correlation test and transformations were performed when necessary. Detrended Correspondence Analysis (DCA) was carried out to determine whether there was a unimodal relationship apparent in the species data within the sampling sites (HILL 1980). Environmental variables and biological data were directly related by Canonical Correspondence Analysis (CCA). Biplot-scaling with emphasis on inter-species distances was used. Forward stepwise regression was used to select the minimal number of variables that could significantly explain the species data. Significance was tested by 999 Monte Carlo permutations (probability of random association,  $p < 0.05$ ). To avoid spatial autocorrelation, a covariable file was used to restrict permutations so that sites on the same canal were not in the same block. This restricted permutation was also used to test the significance of the first and second axes. Redundant variables were excluded by reference to Bonferroni-adjustment (MANLY 1991) of probability with  $p = \alpha/n$ , where  $\alpha = 0.05$  and  $n$  is the variable rank.

COINSPAN classification (CARLETON *et al.*, 1996) of sites was constrained by significant environmental variables. COINSPAN classes were analysed using INDVAL (DUFRENE & LEGENDRE 1997) to determine significant indicator assemblages. INDVAL can test for the significance of the association of each species with a particular COINSPAN class and only the indicator with the class at which it reaches its maximum score should be assessed. Indicator values were calculated for each species within each group produced at every level of the classification. Significance of the highest INDVAL score by each species was assessed from 999 random permutations based on there being less than 5% probability of the observed indval score occurring randomly and a one-tailed t-test. Species passing one (\*) or both (\*\*) significance tests were listed. The INDVAL method combines measures of *specificity* (relative abundance) and *fidelity* (frequency of occurrence in a group of sites). Indicators with maximum scores at the top levels of a COINSPAN classification will be more generalist, as opposed to indicators with maximum values at the end groups, which will be regarded as more specialist. The species assemblages have defined mean and variance of the variable under consideration, determined by their associated COINSPAN group.

## RESULTS

A total of 28,772 chironomid pupal exuviae were identified to 88 taxa from 46 sites. The species data set had a gradient length of 2.32 SD units along the primary axis of a DCA. Accordingly, CCA was deemed appropriate as a unimodal model of species distribution (TER BRAAK 1988). After stepwise regression, 5 chemical variables were selected. These were sediment fines, total oxidised nitrogen (TON), lead (dissolved and sediment) and chromium (Fig. 1a). The first two axes explained 17% of the variation seen in the species data and both axes were significant in a restricted permutation test ( $p < 0.001$ ). The eigenvalue for CCA axis 1 was 0.269 and this axis was highly correlated with water

chemistry (TON and lead). Axis 2, with an eigenvalue of 0.196 was highly correlated with sediment characteristics (sediment lead and sediment fines) and was significant ( $p < 0.05$ ). All canonical axes were significant ( $p < 0.05$ ) with an F-ratio of 1.576.

After removing species associated with sediment, this left 52 epibenthic species. Three variables were significantly related to epibenthic species variance, when using water chemistry data: chlorophyll a, lead and chromium (Fig. 1b). There were 36 inbenthic species and three variables were significantly related to species variance: sediment fines, lead and nitrogen (Fig. 1c).

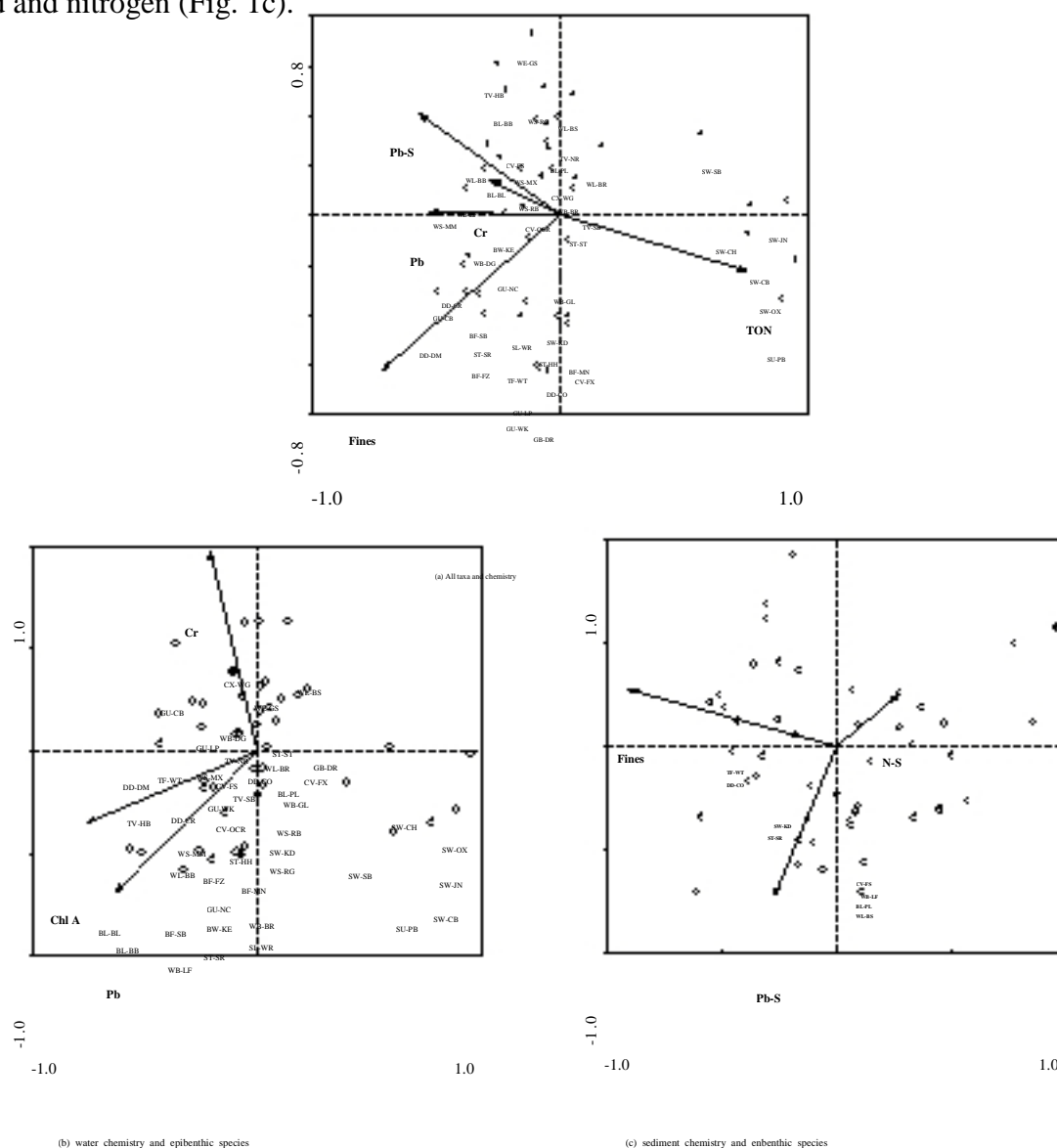


Fig 1: CCA ordination plots (sites and environmental variables): a) water and sediment chemistry and all benthic taxa; b) water chemistry and epibenthic taxa; c) sediment chemistry and inbenthic taxa.

**Figure 1.** CCA ordination plots (sites and environmental variables): a) water and sediment chemistry and all benthic taxa; b) water chemistry and epibenthic taxa; c) sediment chemistry and inbenthic taxa.

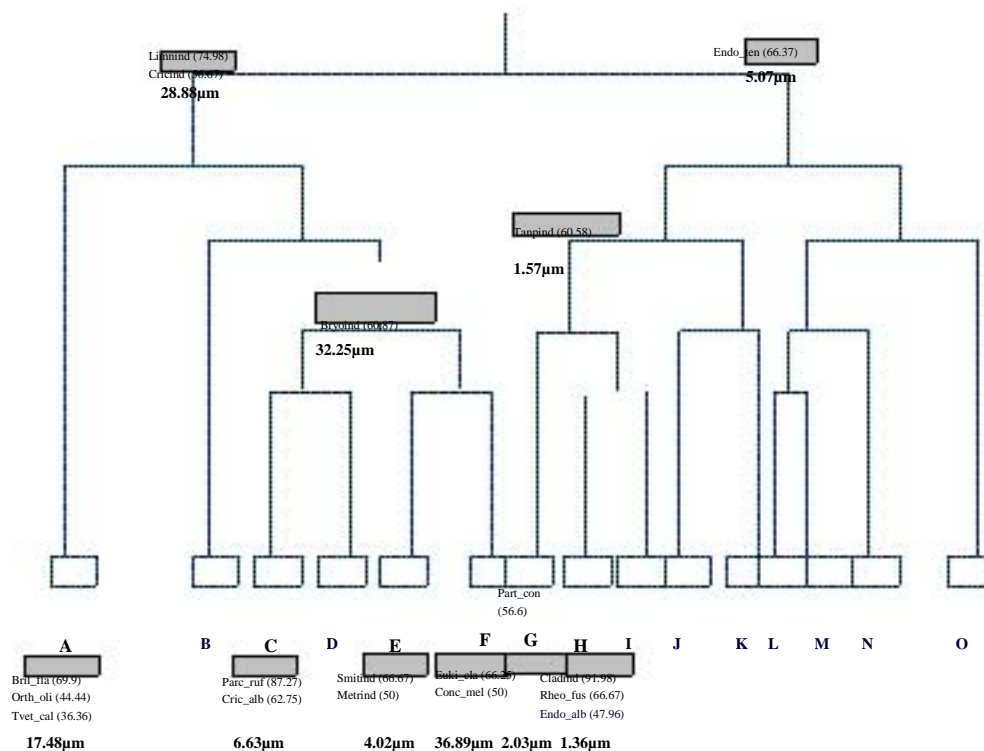


Fig. 2: COINSPAN classification of chironomid data constrained by chromium

**Figure. 2:** COINSPAN classification of chironomid data constrained by chromium.

Each significant variable was used to constrain site classifications. Fig. 2 and table 2 illustrate the COINSPAN classification for chromium and a list of species passing the significance tests for one or more COINSPAN groups. There were *generalist* taxa assemblages, such as *Limnophyes* sp. and *Cricotopus* sp. and *specialist* assemblages such as *Brillia flavifrons* (Johannsen, 1905) to *Tvetenia calvescens* (Edwards, 1929). Each assemblage had a defined mean of chromium, determined by their associated COINSPAN group.

## DISCUSSION AND CONCLUSIONS

This survey has demonstrated that the CPET method is an effective method by which to monitor the biodiversity of canals. A collection of pupal skins integrates both marginal and deep-water habitats from a wide area, which also integrates pupal emergence. Many taxa that were found inhabit the sediment, a habitat normally difficult to sample. Chironomid taxa assemblages were able to differentiate between canal sites of different chemistry. Indicator species assemblages could be identified that could be associated with different COINSPAN groups constrained by each of the significant variables determined

by CCA analysis. The importance of metals in canal systems was also revealed by ordination techniques.

With all chemical variables and species combined it was seen that the first axis of the CCA ordination reflected nutrient enrichment (Fig. 1a). A grouping of sites to the right of the ordination graph (on the Staffordshire & Worcester Canal) shows that there is a nutrient effect from a large sewage works. Nitrogen seems to be the limiting nutrient, with the dominant effect coming via sediment bound nitrogen. This can be seen in Fig. 1c, where sediment nitrogen is the primary variable, whereas chlorophyll a is seen to be dominant when only the water variables are considered.

This study revealed significant chemical variables that influenced the chironomid taxa assemblages across the 46 study sites. The identification of indicator taxa using INDVAL proved to be useful as a tool in the possible future determination of the ecological potential of artificial water bodies. The use of chironomids as bioindicators was shown to be an easy to use and cost effective method by which to ecologically assess canals. The importance of metals revealed by ordination and the identification of indicator taxa could prove to be useful in the ecological assessment of urban canals across the UK. If chironomids are ignored by the WFD, artificial water bodies such as canals will not be adequately monitored.

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Date received: ..... .

**TABLE 1.** Site Locations and chemical characteristics (metals) of canal sites.

Canal	Site	Grid Ref	TV-HB	TV-NR	TV-SB	CV-FX	CV-OCR	CV-FS	WS-RG	WS-MM	WS-MX	WS-RB	WE-GS	CX-WG	SW-KD	SW-SB	SW-CB	SW-JN	SW-OX	SW-CH	SU-PB	WB-BR	WB-GL	WB-DG	WB-LF	ST-SR	ST-HH	ST-ST	BL-BL	BL-PL	BL-BB	WL-BR	WL-BS
Tame Valley	Holloway Bank	SO 990 939																															
	Newton Rd	SP 036 940																															
	Salford Br	SP 096 901																															
Coventry	Foxford	SP 351 839																															
	Old Church Rd	SP 348 821																															
Wyrley & Essington	Foleshill Rd	SP 338 806																															
	Ryders Green Rd	SO 983 917																															
	Moors Mill Lane	SO 977 932																															
Cannock Extn	Moxley	SO 969 955																															
Walsall	Rayboulds Br	SO 985 977																															
	Goscot	SK 016 020																															
	Wyrley Grove Br	SK 019 054																															
Staf fs & Worc	Kidderminster	SO 828 758																															
	Swindon Br	SO 862 906																															
	Compton Br	SO 883 988																															
	Junction	SI 902 011																															
	Oxley	SI 902 017																															
	Coven Heath	SI 914 054																															
Shropshire Union	Pendeford Br	SI 888 034																															
	Bath Row Br	SP 061 860																															
Worc & Bham	Grange Lane	SP 019 712																															
	Worcester	SO 849 539																															
Stratford	Liford	SP 054 803																															
	Stirchley	SP 059 796																															
Bham Level	Hockley Heath	SP 152 725																															
	Stratford	SP 199 555																															
W' ton Level	Bromford Lane	SP 995 903																															
	Park Lane East	SP 966 919																															
Bham & W' ton	Brasshouse Br	SP 019 889																															
	Brades Rd	SO 982 900																															
Bham Level	Baker St, Tipton	SO 954 917																															

**TABLE 2. Part I.** Indicator table of the species indicator power for COINSPAN clustering hierarchy (groups A-F). Species presence is indicated by % abundance. \* Species passing one significance test; \*\* Species passing both significance tests.

	Species	Indval	Mean Cr	A	B	C	D	E	F
F	Euki_cla	66.25**	36.89						<b>10.17</b>
	Conc_mel	50*							
C+D+E+F	Bryoind	60.87*	32.25	2.03		1.34	3.08		0.3
(A+B +C+ D+ E+F)	Limnind	74.98**	28.88	<b>9.63</b>	1.74				
	Cricind	56.67**		<b>10.22</b>	1.36				
A	Bril_fla	69.9**	17.48	0.56	0.09	0.07	0.03		
	Orth_oli	44.44*							
	Tvet_cal	36.36*							
C	Parc_ruf	87.27**	6.63			<b>0.97</b>	0.07		
	Cric_alb	62.75**		12.2		<b>16.59</b>	0.28		
(G+ H+I+J+K + L+M+N+O)	Endo_ten	66.37**	5.07	0.03	<b>1.44</b>				
E	Smitind	66.67**	4.02					<b>0.52</b>	
	Mettrind	50**				0.24	0.07	<b>1.29</b>	0.13
G	Part_con	56.67*	2.03	0.37			0.07		
(G+H+I+J+K)	Tanpind	60.58**	1.57			<b>1.48</b>	0.25		
H	Cladind	91.98**	1.36						
	Rheo_fus	66.67*							
	Endo_alb	47.96*							

**TABLE 2. Part II.** Indicator table showing the species indicator power for COINSPAN clustering hierarchy (groups G - O). Species presence is indicated by % abundance. \* Species passing one significance test; \*\* Species passing both significance tests

	Species	Indval	Mean Cr	G	H	I	J	K	L	M	N	O
F	Euki_cla	66.25**	36,89					0,12				
	Conc_mel	50*										
C+D+E+F	Bryoind	60.87*	32,25	0,08								
(A+B+C +D+E+F)	Limnind	74.98**	28,88									
	Cricind	56.67**										
A	Bril_fla	69.9**	17,48									
	Orth_oli	44.44*										
	Tvet_cal	36.36*										
C	Parc_ruf	87.27**	6,63		0,06							
	Cric_alb	62.75**								2,38		
(G+H+I+J+K												

## **APPENDIX IV:**

*As a result of this Phd, I was assigned to a project in the Science team of the Environment Agency to develop this classification tool for canals in the UK for the requirements of the WFD. This was to support the canal classification tool under development by the SNIFFER research project WFD61. A report has been produced, as has a summary both of which are reproduced here.*

# using science to create a better place



## **Developing a classification tool for UK canals using chironomid pupal exuviae**

**Integrated catchment science programme**  
Science report: SC070062/SR1

The Environment Agency is the leading public body protecting and improving the environment in England and Wales.

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This report is the result of research commissioned and funded by the Environment Agency's Science Programme.

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# Science at the Environment Agency

Science underpins the work of the Environment Agency. It provides an up-to-date understanding of the world about us and helps us to develop monitoring tools and techniques to manage our environment as efficiently and effectively as possible.

The work of the Environment Agency's Science Department is a key ingredient in the partnership between research, policy and operations that enables the Environment Agency to protect and restore our environment.

The science programme focuses on five main areas of activity:

- **Setting the agenda**, by identifying where strategic science can inform our evidence-based policies, advisory and regulatory roles;
- **Funding science**, by supporting programmes, projects and people in response to long-term strategic needs, medium-term policy priorities and shorter-term operational requirements;
- **Managing science**, by ensuring that our programmes and projects are fit for purpose and executed according to international scientific standards;
- **Carrying out science**, by undertaking research – either by contracting it out to research organisations and consultancies or by doing it ourselves;
- **Delivering information, advice, tools and techniques**, by making appropriate products available to our policy and operations staff.



Steve Killeen

Head of Science

# Executive summary

## Background to research

Under the EC Water Framework Directive (WFD) Member States must develop methods to classify the ecological status of their surface water bodies. Under certain circumstances the WFD permits Member States to identify and designate Heavily Modified Water Bodies (HMWB) and Artificial Water Bodies (AWB). Canals are defined as an AWB; therefore, to fulfil UK obligations in the assessment of the ecological potential of canals, the UK needs to introduce a method to classify their ecological status. The WFD develops the concept of ecological potential in AWBs, as opposed to ecological status in natural water bodies to allow their continued beneficial uses such as navigation and recreation. AWBs should attain 'good ecological potential' (GEP) rather than the 'good ecological status (GES) used for natural water bodies.

## Objectives of research

This project aims to support the canal classification tool being developed by SNIFFER (Scotland and Northern Ireland Forum for Environmental Research) project WFD61 by producing CPET (Chironomid Pupal Exuvial Technique) data for an estimated 200 canals and navigable waterways. This tool is based upon the CPET tool already in use with the Environment Agency to classify nutrient impact in lakes.

## Key findings

1. Total oxidised nitrogen (TON) was found to be the best variable for assessing the nutrient status in canals.
2. Boat traffic is a characteristic of the canal system and this was taken into account when developing the tool.
3. Reference sites were defined by the proportion of sensitive and tolerant species, away from significant urban influences and low levels of TON. Using site-specific predictions of expected impact scores enabled EQRs (Ecological Quality Ratios) to be calculated for all samples.
4. Site-specific reference conditions were developed that took into account levels of boat traffic. This avoided splitting the dataset into separate boat traffic classes.
5. EQR boundaries were calculated using the standard deviation of 'fits' for tolerant taxa when the tolerant and sensitive taxa were plotted.
6. Spatio-temporal variability of samples was estimated and used to calculate uncertainty associated with EQR predictions.
7. Converting the measure of temporal variability into estimates of uncertainty suggests that there is no bias to certain seasons and that confidence of canal classification was of high precision.

# Acknowledgements

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# 1 Introduction

## 1.1 Canals and the Water Framework Directive

Under the EC Water Framework Directive (WFD) Member States must develop methods to classify the ecological status of their surface water bodies. Under certain circumstances the WFD permits Member States to identify and designate Heavily Modified Water Bodies (HMWBs) and Artificial Water Bodies (AWBs). AWBs are surface water bodies which have been created in a location where no water body existed before and which have not been created by the direct physical alteration, movement or realignment of an existing water body. Canals are classified as an AWB; therefore the UK needs to introduce a method to classify their ecological status.

The WFD develops the concept of ecological potential in AWBs, as opposed to ecological status in natural water bodies, to allow their continued beneficial uses such as navigation and recreation. AWBs should attain 'good ecological potential' (GEP) rather than 'good ecological status' (GES) for natural water bodies.

Unlike lakes, canal systems are characterised by boat traffic, and by the requirements of the WFD are a source of permitted environmental variation. A system is therefore required that defines maximum ecological potential (MEP) for any specified level of boat traffic.

## 1.2 Canals and the Environment Agency

Historically the Environment Agency has not included canals in biological surveys to any great extent. This is generally because sampling canal habitats is not easy. The paucity of sites available for sampling and the artificial nature of many of the banks make obtaining a representative sample difficult. This sampling issue is exacerbated by the difficulties of interpreting data. Water quality is often influenced by a large number of diffuse and point source inputs, with a wide range of contaminants present. Organic and inorganic pollutants are frequently present together. In addition, the physical characteristics and habitat of urban sites can make sampling and data interpretation difficult.

## 1.3 Chironomids as objects of study

There are now 612 chironomid species recorded from Britain (Langton and Ruse 2005, Wilson and Ruse 2005). There are more species of UK chironomids than all other non-dipteran macroinvertebrate species combined, and they occur in most types of freshwater both within the water column and in the sediment (Coffman 1995). Chironomids comprise all six functional feeding groups exhibited by macroinvertebrates (Berg 1995) and for most species their habitat and water quality preferences are well documented. The aquatic larvae are subjected to the prevalent conditions in the habitat, and thus reflect the quality of both water and sediment.

## 1.4 Objectives

In this project the Chironomid Pupal Exuvial Technique (CPET) was developed as a tool for defining reference conditions which also takes levels of boat traffic into account. More specifically, the project set out to:

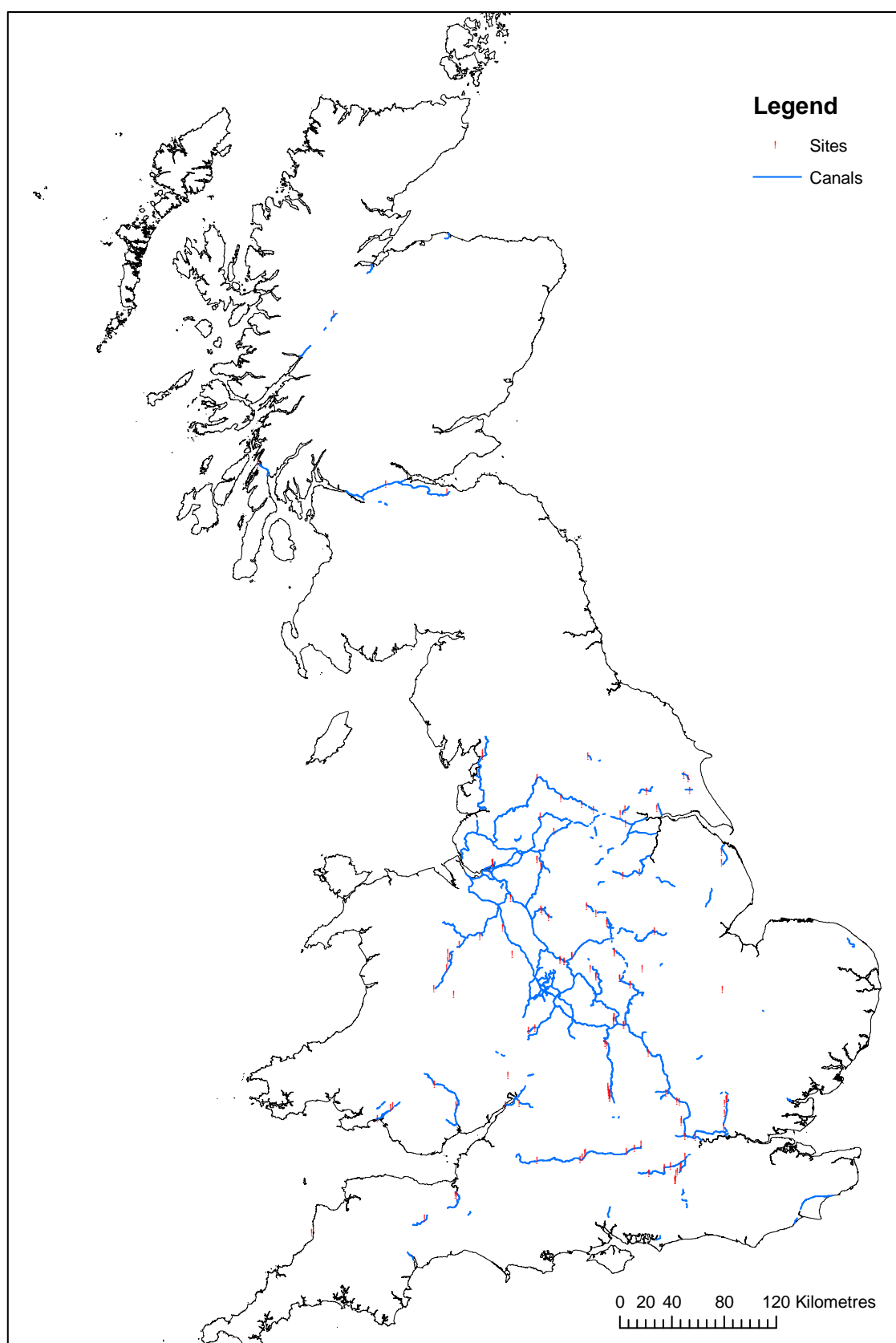
- Gather existing and new data covering chironomid exuviae and associated environmental data.
- Define the expected (reference condition) chironomid community at any canal site.
- Develop a model for assessing ecological potential along environmental gradients.
- Develop a rationale for placing status class boundaries along these gradients.
- Develop estimates of uncertainty associated with status class assessments.

## 2 Methods

### 2.1 Data used in the development of the classification tool

#### 2.1.1 Chironomid pupal exuviae

Data for both chironomid exuviae and environmental data was obtained from several sources. In total, data from 167 canal sites was available for analysis. These sites represented a wide range of canal types from disused to heavily trafficked; urban and rural sites and a full geographic range from South West England to Wales and Scotland (Figure 2.1, Appendix 1). The project also had access to a large volume of chironomid exuviae data from canals collected over the last 15–20 years. Various projects have been carried out by Les Ruse, Environment Agency and previously of the National Rivers Authority (NRA), as well as an initial early attempt to classify canals on a wider scale (Ruse 1998) in which true canal sites were successfully discriminated from more riverine watercourses. A scoring system was proposed and dichotomous key using indicator taxa was developed. Information from 46 sites from a PhD study (Green 2009) were also used – these were mainly urban sites collected from the West Midlands. A further seven sites were used from an ecological assessment of the Montgomery Canal in 2005. The Scottish Environment Protection Agency (SEPA) provided samples from four canals. Additional sampling of canals across the UK and Wales was carried out in 2007–2008.



**Figure 2.1 Location of canal sample sites.**

### **2.1.2 Environmental data**

As far as possible, water chemistry was linked to exuviae sample sites in time and space. Where this was not possible, the nearest site/date was obtained from SlimWIMS, an Environment Agency database that provides water management information. Data were provided by SEPA for sites in Scotland. Meter readings in the field were taken when collecting exuviae samples in 2007–2008 of conductivity, dissolved oxygen (DO) and salinity.

### **2.1.3 Boat traffic data**

Data for boat traffic was provided by British Waterways in 'thousands of boat movements per annum'.

## **2.2 Field and laboratory methods**

### **2.2.1 Chironomid exuviae collection and identification**

#### *Collection*

Pupal exuviae are the skins shed by emerging adults. They can float for two or three days. A collection of these skins represent recently emerged adults within a significant area of the canal. They accumulate at the edge of the water among vegetation and flotsam or behind obstacles such as lock gates. This makes sampling easy, and a collection made represents individuals from all habitats over that period. Water and wind currents aid this accumulation, although on very still days in canals with low boat traffic, skins can accumulate across the entire width of the canal. The larvae will have been subjected to the prevalent conditions over their entire development; therefore the pupae will reflect the quality of the water and sediment during previous months.

Debris which has accumulated on the water surface was collected with a long-handled pond net, with a 250 µm mesh net. The collection was examined on site to determine if enough exuviae had been collected. It is necessary that at least 200 exuviae are present (Wilson and Ruse 2005). The residue was placed in a plastic pot and returned to the laboratory to be preserved in industrial methylated spirit (IMS). Samples were sieved through a coarse and fine (250 µm) mesh to remove most of the debris to leave mostly exuviae to be preserved.

Three samples were collected over the period April to September, to cover as much of the emergence period of adults as possible and the results were amalgamated (Ruse and Wilson 1984, Ruse 1995, Wilson and Ruse 2005). Different species emerge at different times of the day (Wilson and Bright 1973). Collecting accumulated exuviae from the edge of a water body will overcome any diurnal bias in the sample. This offers a two- or three-day rolling average of the exuvial emergence. Meter readings for conductivity, DO and salinity were also taken at the same time as the exuviae.

#### *Laboratory procedure*

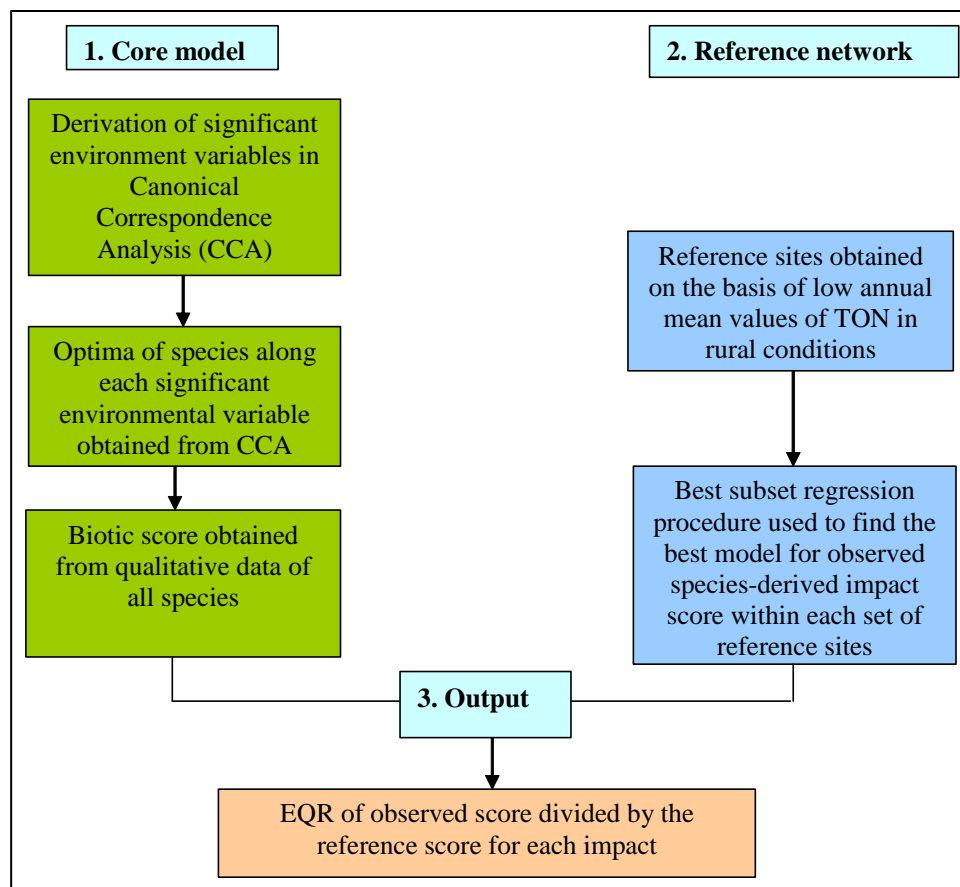
The material was placed in a large white tray with water, and subsamples were taken and placed in small, shallow trays. With the aid of a daylight illuminated magnifier all

exuviae were taken and placed in glass vials until at least 200 were collected. This ensured that a representative subsample of the whole collection is made (Ruse 1993, Wilson and Ruse 2005). Careful examination was required as some of the exuviae are small and transparent (<2 mm).

Skins were examined under both low-powered and high-powered binocular microscopes using the keys of Langton (1991), Langton and Visser (2003), Langton and Visser (2003) and Wilson and Ruse (2005). Samples were generally examined wet under water or 70% alcohol.

## 2.3 Data analysis

The structure of data analysis for this project is shown in Figure 2.2.



**Figure 2.2 Structure of data analysis for this project.**

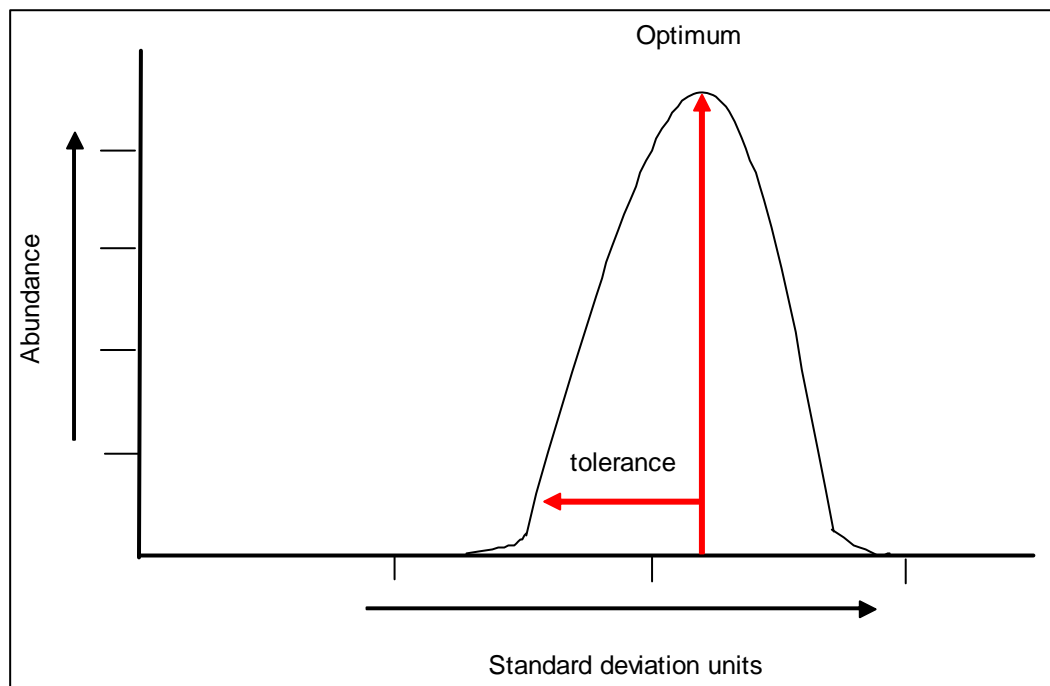
Species data for each survey were amalgamated and abundance recorded as a percentage of the total number of exuviae collected. Percentage abundance was fourth-root transformed to reduce unexplained variance (Prentice 1980). Species were excluded from the analysis if they occurred at only one or two sites, to avoid spurious associations with extreme environmental values. All environmental data were tested for normality (MINITAB™ v14) and transformations were performed where necessary. Reciprocal square roots were used for biological oxygen demand (BOD) and zinc (Zn). Northing, total oxidised nitrogen (TON), ammonia (NH<sub>3</sub>) and copper (Cu) were log transformed. Ortho-phosphate (O-P) and conductivity were square root transformed. All

remaining variables were untransformed. Average width and profile were in the form of categorical data (1–3); emergent, floating and aquatic plants, urban, rural and suburban locations, hard and soft banks or both were all presence/absence data, and average depth was presence/absence data according to whether the depth was greater or less than 1 m.

### 2.3.1 Ordination

Detrended correspondence analysis (DCA) was carried out to determine whether there was a unimodal (bell-shaped) relationship apparent between fourth-root species abundance ( $\sqrt[4]{\%}$ ) data and the primary ordination axis (Lepš and Šmilauer 2003). This was employed to investigate gradient lengths and relationships between surveys based on species data unconstrained by environmental data. The first DCA axis had a gradient length of 2.4 standard deviation (SD) units, the units of measurement along the axis, which is too long for linear ordination but suitable for unimodal analysis.

Relationships between environmental data and chironomid species were investigated by canonical correspondence analysis (CCA). CCA is a direct gradient analysis technique in which the ordination axes are constrained as linear combinations of environmental variables (ter Braak 1986, 1987). Biplot-scaling with emphasis on inter-species distances was used. A species' maximum abundance, the highest point of the distribution curve, gives its optimum value (species score) in relation to the ordination axis, while its niche breadth is measured by the width of the bell-shaped curve and is known as its tolerance (Figure 2.3).



**Figure 2.3 Species optimum and tolerance defined when unimodally related to ordination axes.**

Forward stepwise regression was used to select the minimal number of explanatory variables that could significantly ( $P < 0.05$ ) explain the largest amount of variation in the species data. The significance of the variables was tested by 999 unrestricted Monte Carlo permutations. To protect against inclusion of redundant variables, a Bonferroni adjustment was made (Manly 1991). Probability levels of significance began at  $\alpha = 0.05$

for selection of the first variable and were adjusted with  $P=\alpha/n$ , where  $n$  is the variable rank. Ordination analyses were performed using CANOCO 4.5 (ter Braak 2003).

### 2.3.2 Impact scores

Species optima in relation to the best available variable was obtained from species scores along the first axis of a CCA with only that variable explaining the species–environment relationship.

A weighted-averaging formula was used to calculate a biotic score for each impact measure to take full advantage of the relative abundance and niche breadth data available for all species.

$$\text{Score} = \frac{\sum \text{abundance} * \text{optimum} * (3 - \text{tolerance})}{\sum \text{abundance} * (3 - \text{tolerance})}$$

In this formula the narrower the species niche breadth the greater weight will be attached to its optimum value.

The sum of products of  $\sqrt{\%}$  species abundance multiplied by the species optimum and a measure of niche breadth ( $3 - \text{tolerance}$ ) for all species was divided by the sum of products of abundance and niche breadth. Tolerances were less than 3.0 SD units, therefore the  $3 - \text{tolerance}$  produced the highest value for the narrowest niche breadth.

Biotic scores were also derived using a similar approach but without taking into account niche breadth. This was to test which method was most sensitive to the impact being measured. A third method was also employed by which the mean optimum score for each site was based on qualitative data alone.

$$\text{Score} = \frac{\sum \text{optima}}{\text{No. of taxa}}$$

The resulting scores obtained by the above methods were compared by Pearson product-moment correlation analysis together with the measured variable.

### 2.3.3 Site-specific reference conditions

Reference conditions were derived for negligible impact. Reference sites were chosen on the basis of low annual mean values of the variable being considered. Sites were chosen in rural areas away from any high density urban areas and also irrespective of the level of boat traffic. A best subsets regression procedure was conducted in order to obtain a reference score, using the best model based on the physical and chemical characteristics of the reference sites. First all single predictor models were examined, with the two with the highest  $r^2$  being selected, then all two-predictor models, selecting the best two, and so on until all available predictors were used. The most efficient model was chosen from the adjusted  $r^2$  and Mallows  $C_p$  statistic, where  $C_p$  was small and is close in value to the number of predictors.

### 2.3.4 Ecological Quality Ratio (EQR)

The EQR was derived by dividing the observed score by the reference score for each site. This had to be formulated to take account of negative optima of sensitive species and to produce a high ratio when ecological potential was high. This was achieved by adding 1.2 to both observed and reference scores and then subtracting the score from 0.5:

$$\text{EQR} = 0.5 - (\text{observed score} + 1.2) / 0.5 - (\text{reference score} + 1.2)$$

The resulting EQR was rescaled 0–1 by dividing it by the maximum EQR.

To aid in the setting of class boundaries a classification of sites constrained to TON was carried out using COINSPAN (Carleton et al 1996). COINSPAN is a segmentation of gradients produced by CCA, as opposed to segmentation of gradients produced by correspondence analysis (CA), as with TWINSPAN (Hill 1979). The resulting output aids in the viewing of sensitive and tolerant species.

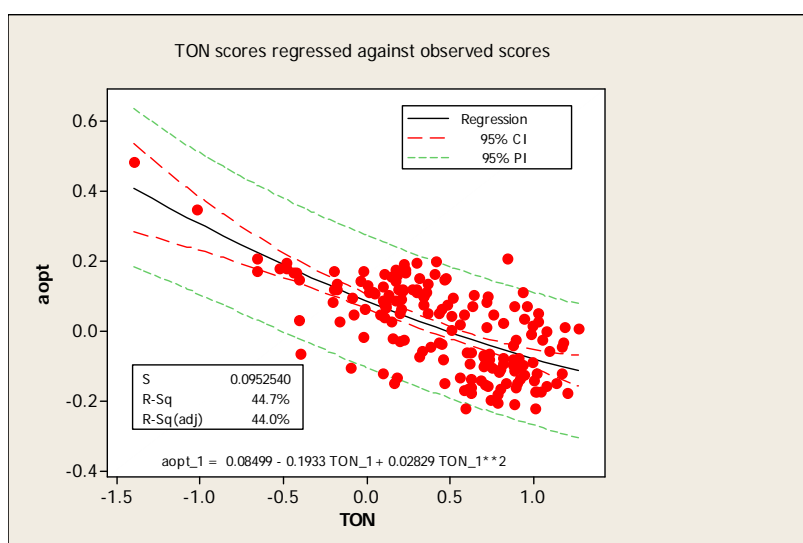
## 3 Results

A total of 97,275 pupal skins comprising 113 taxa from 167 canal sites were available for analysis after removing taxa occurring at less than three sites (Appendix 1). After stepwise forward selection and adjusting for Bonferroni inequality, 13 variables were found to be significantly related to species data:  $\text{NH}_3$ , BOD, easting, boat traffic, Zn, O-P, conductivity, TON, altitude, emergent vegetation, northing, floating macrophytes, urban. Together the significant variables explained 33% of the total variance and 8% of the species data. TON explained 3% of the species data.

### 3.1 Impact scores

#### 3.1.1 TON

Species optima along the first axis for TON were obtained by constraining that variable in a CCA (Appendix 2). Correlations between TON and qualitative data were as good as when using weighted-average data. A quadratic model was found to produce the highest regression coefficient of 44% after adjusting for degrees of freedom (Figure 3.1).



**Figure 3.1** TON impact scores regressed on observed values.

### 3.2 Reference scores and EQR

#### 3.2.1 TON

There were seven sites chosen as reference condition for TON: Caledonian Canal (Fort Augustus), Forth & Clyde Canal (Wyndford), Neath Canal (Resolwen and also

Ynys-Arwed Farm), Peak Forest Canal (Bredbury), Crinan Canal (Crinan) and Llangollen Canal (Bryntysilio). These were selected on the basis of negligible impact, and a rural location. The most efficient model based on best subset regression that took into account boat traffic was:

$$\text{MEP} = 0.667 - 0.000095 \text{ Boat Traffic (movements per year)} - 0.00192 \text{ Alk (mg/l)} - 0.621 \text{ ave width (m)}$$

This had an  $r^2_{\text{adj}}$  of 77% and  $C_p$  of 3.4.

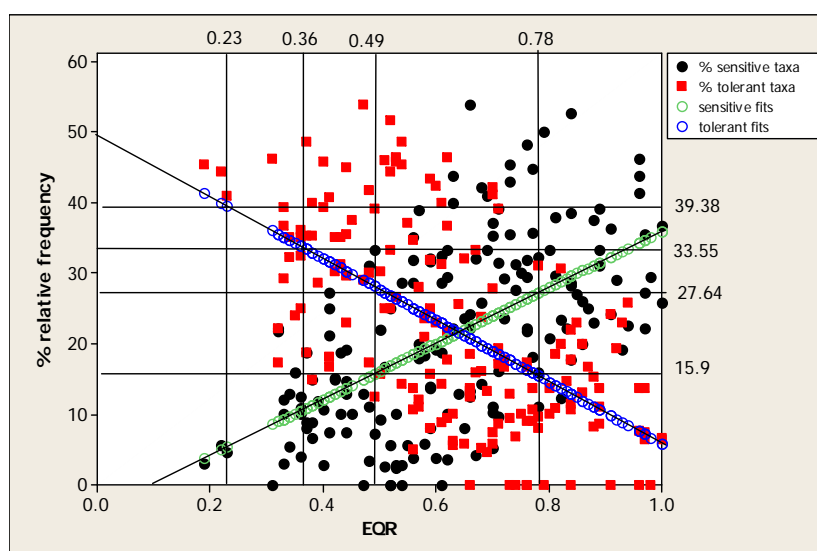
If no alkalinity data is available then this variable can be modelled from conductivity by using the following equation:

$$\text{Alkalinity} = 61 + 0.156 \text{ conductivity}$$

EQR was calculated according to the formula stated in Section 2.3.4. Appendix 4 lists observed, reference scores and EQRs for all sites.

### 3.2.2 Class boundaries

Class boundaries were derived by plotting the relative frequency of sensitive and tolerant species for all 167 sites (Figure 3.2).



**Figure 3.2 Sensitive, tolerant species vs EQR.**

The best fit describing the dataset was a linear equation. Sensitive species exceeded tolerant species at 21.77% at an EQR of 0.64 with a fitted line SD of 5.87. The high/good boundary was placed at the crossover point of 21.77% plus 5.87 giving 27.64% corresponding to an EQR of 0.78. The good/moderate boundary was taken as the crossover minus 5.87. This gave 15.9% equating to an EQR of 0.49. The moderate/poor boundary was placed at a point (2 x SD) from the crossover point, equating to 33.55% and an EQR of 0.36. The poor/bad boundary was fixed at a point (3 x SD) from the crossover point, equating to 39.38% and an EQR of 0.23.

# 4 Uncertainty in ecological potential assessments using chironomid exuviae

## 4.1 Introduction

The WFD requires that there are estimates of confidence and precision in the results of monitoring programmes. In other words, how close are repeated measurements and are they of the same quality?

The previous sections describe the development of a model for assessing ecological potential in canals in the UK. The passive nature by which exuviae are collected generally overcomes any spatial and operator sampling error. This is unlike using diatoms, for example, where they can rapidly respond to environmental change and so will be inherently variable in both space and time (Kelly *et al.* 2008). It is therefore important that the consequences of any variability in chironomid assemblages are properly understood.

In this chapter three questions will be addressed:

- What variation is there in the contingency of which months the samples are collected?
- What variation is there between subsampling and sample size?
- What is the confidence of a canal classification based on its observed EQR?

## 4.2 Seasonal sampling error

Precision of EQR calculated from different months at the same site was measured by sampling Fobney Lock on the Kennet & Avon Canal every month for one year. For the current study three samples were collected between seven months, April and October. There are 35 possible combinations of three months from April to October:

$$[nCr=7!/3!(7-3)!]$$

EQR was calculated for all 35 combinations to measure any variation due to the contingency of which of the three months were sampled. Spatial variation was taken into account as the samples would have been taken at the site point of the canal.

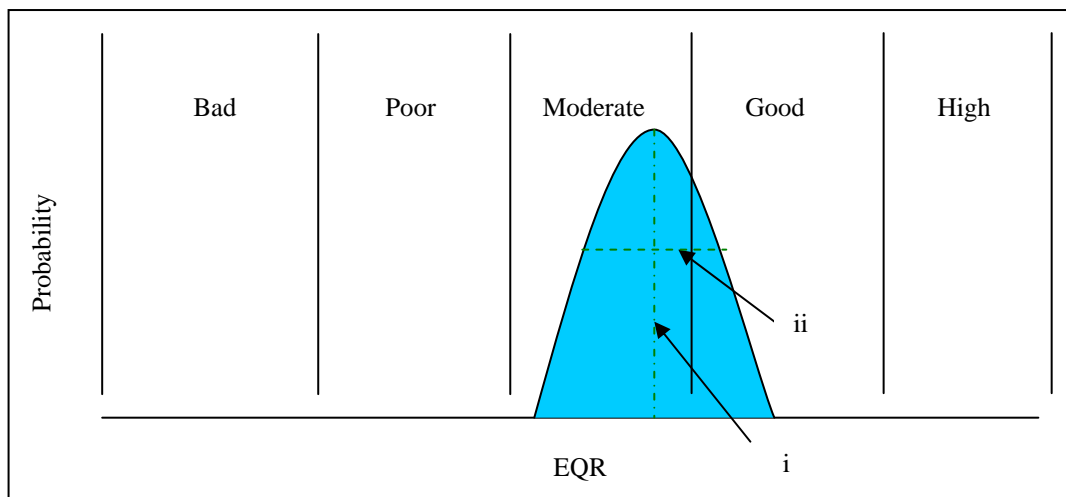
## 4.3 Sample size

Samples used in the analysis for this study consisted of about 200 exuvial skins that were subsampled from the original field sample. Any variation between subsamples taken from the same sample could be important. Variations between subsamples has already been investigated for rivers (Ruse 1993) and lakes (Ruse 2006). These studies showed that obtaining more than 200 skins did not significantly improve quality indices

provided the site was fully surveyed (three times for rivers and canals, four times for lakes), and also that species were randomly distributed between subsamples.

## 4.4 Confidence of classification

A full description of the methodology for estimating confidence of class and risk of misclassification is described in Ellis and Adriaenssens (2006). The seasonal sampling exercise provided a generic EQR that was used to plot per cent confidence of class points based on a symmetrical standard deviation vs mean EQR curve. From this it is possible to determine the confidence of a canal classification based on its observed EQR. This principle is illustrated in Figure 4.1. In this diagram, *i* = observed EQR value; the shaded blue area is a probability distribution associated with this EQR, based on the predicted standard deviation (*ii*). Vertical lines show class boundaries. In this scenario the standard deviation lies across moderate and good ecological potential and while the observed value suggests moderate status, there is also the possibility that the true condition of the site is of 'good potential'.



**Figure 4.1 Schematic diagram showing the basis for calculation of confidence of class and risk of misclassification.**

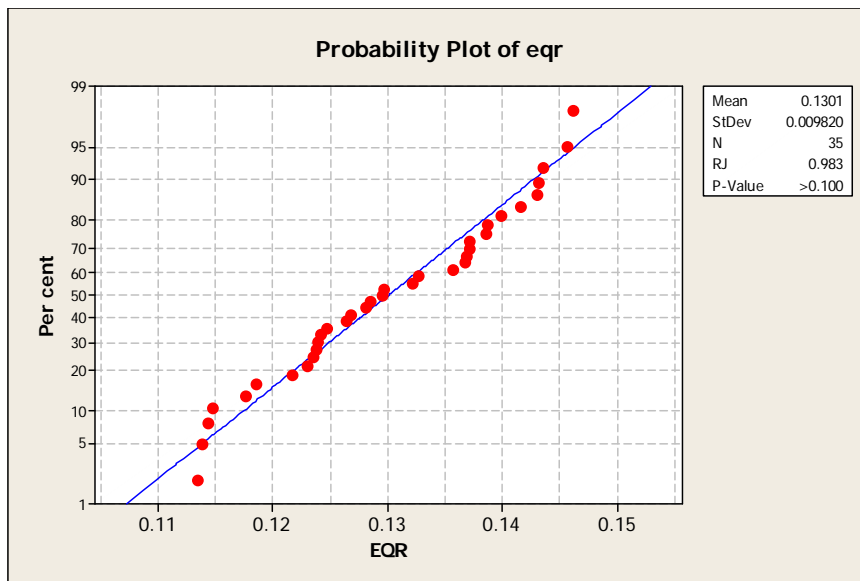
## 4.5 Results

### 4.5.1 Seasonal sampling error

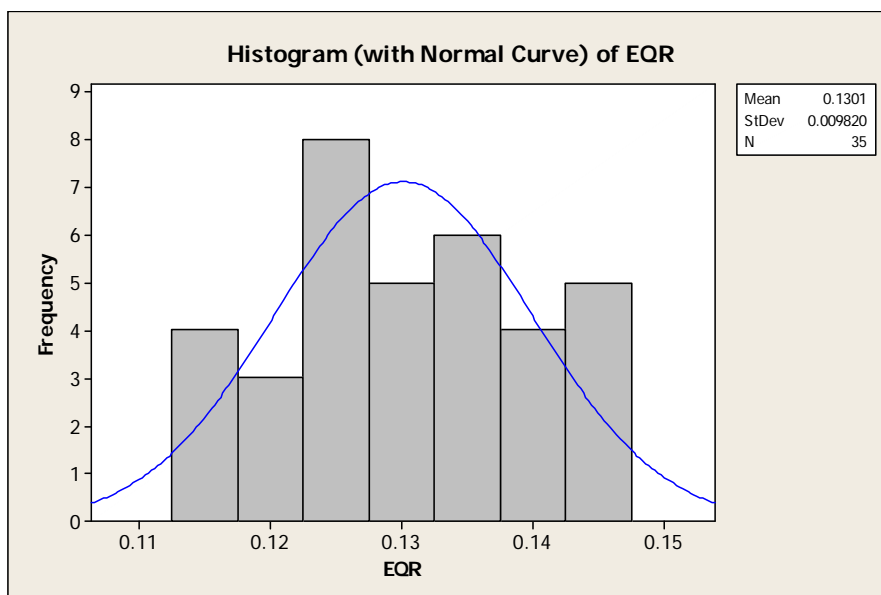
Distribution statistics are summarised in Table 4.1. The distribution was normal ( $p > 0.1$ ,  $r_{\text{corr}} 0.983$ ) about the mean EQR of 0.13 (Figure 4.2). Ratios for all permutations of monthly samples ranged from 0.11 to 0.15. EQR SD was below 0.01 (Figure 4.3).

**Table 4.1 Summary of EQR sampling variation.**

EQR	
Mean	0.13
SE	0.0017
Median	0.1295
<b>SD</b>	<b>0.0098</b>
Variance	0.0001
Range	0.00
Minimum	0.11
Maximum	0.15
Count	35
CL95	0.0032



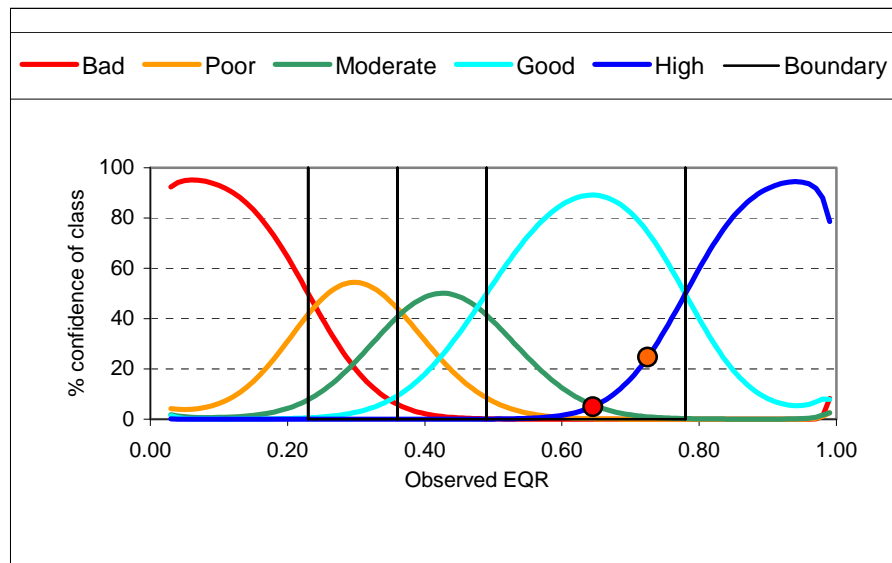
**Figure 4.2 Probability plot of EQR.**



**Figure 4.3 Frequency distribution of EQR variation.**

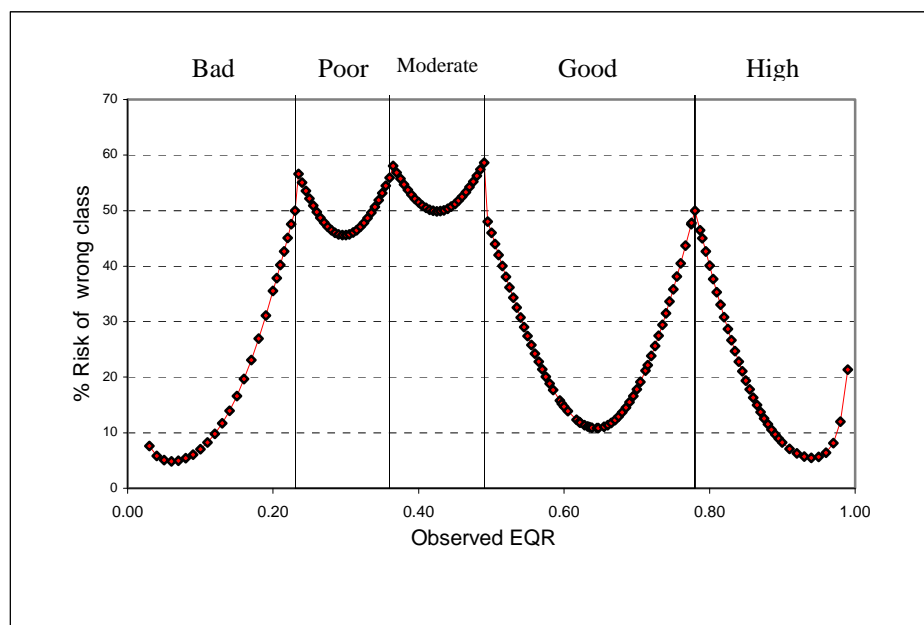
### 4.5.2 Confidence of classification

The seasonal sampling exercise provided an EQR SD of 0.0098 which was taken as the peak in a plot of symmetrical standard deviation vs mean EQR curve (Ellis and Adriaenssens 2006). From this it is possible to determine the confidence of a canal classification based on its observed EQR (Figure 4.4). For example, when the observed EQR is 0.65 (lower red spot), the site would be classified as having good ecological potential with 89% confidence of being correct and 5% confidence of being of high ecological potential. An EQR of 0.73 (higher red spot) would have a 74% confidence of good ecological potential and a 25% confidence of being high ecological potential.



**Figure 4.4 EQR confidence of class curves.**

The information in Figure 4.4 can be described in terms of risk as shown in Figure 4.5. This plots the risk that a face-value interpretation of the observed EQR will put the site into an incorrect class. As the EQR approaches one of the boundaries between classes there will be a loss in the confidence of class. EQR values derived from CPET analysis have a 50% risk at the boundaries; therefore it is theoretically possible for data to have even higher levels of risk at the boundaries.



**Figure 4.5 Risk of misclassifying status from EQR.**

EQR	Confidence of Class (%)				
	Bad	Poor	Moderate	Good	High
88ken5 0.19	100				
88ken6 0.22	89	11			
88ken7 0.23	50	50			
89142 0.31		100			
SWJN 0.32		100			
SWOX 0.32		100			
07TMFJ 0.33		100			
89133(95) 0.33		100			
89089(95) 0.33		100			
07OXCL 0.34		98	2		
89089(91) 0.34		98	2		
89133(91) 0.35		86	14		
89071(91) 0.36		50	50		
07TMKB 0.36		50	50		
89121 0.36		50	50		
34005 0.36		50	50		
08SUAD 0.37		14	86		
89130 0.37		14	86		
33900 0.37		14	86		
89071(95) 0.38		2	98		
89079 0.38		2	98		
town90 0.38		2	98		
07GUWE 0.39			100		
34006 0.40			100		
weystoke 0.40			100		
08GSPT 0.41			100		
08SUNB 0.41			100		
SWCH 0.41			100		
34023 0.41			100		
89135(91) 0.42			100		
35264 0.42			100		
34004 0.43			100		
34027 0.43			100		
08TMEL 0.44			100		
89073 0.44			100		
SWSB 0.44			100		
34014 0.44			100		
stusrm 0.45			100		
89087 0.47			98	2	
362 0.47			98	2	
SWCB 0.48			85	15	
89134(96) 0.48			85	15	
89067 0.48			85	15	
BLPL 0.49			50	50	
89141 0.49			50	50	
08SUPL 0.50			15	85	
07GUCO 0.50			15	85	
07TMBN 0.51			2	98	
462 0.51			2	98	
SUPB 0.52				100	
463 0.52				100	
dsdeep 0.52				100	
89115 0.52				100	
07OXBA 0.53				100	
89070 0.53				100	
89049 0.53				100	
STSR 0.54				100	
89064 0.54				100	
89116 0.54				100	
461 0.55				100	
08KASE 0.56				100	
STST 0.56				100	
GULP 0.56				100	
peas90 0.56				100	
WBDG 0.57				100	
STHH 0.57				100	
07GULE 0.57				100	
07GUGG 0.58				100	
524 0.58				100	
WLBS 0.59				100	
BFFZ 0.59				100	
070XWI 0.59				100	
525 0.59				100	
89054 0.59				100	
89117 0.60				100	
kings88 0.60				100	
CVFX 0.61				100	
WBLF 0.61				100	
89068 0.61				100	
hmar88 0.61				100	
08ERAR 0.62				100	
CVFS 0.62				100	
GUNC 0.62				100	
89055 0.62				100	
enf86 0.62				100	
BLBB 0.63				100	
WBGL 0.63				100	
WLBR 0.63				100	
07GULO 0.64				100	
WBBR 0.65				100	
89083 0.65				100	
BLBL 0.66				100	
08RIRI 0.66				100	
89066 0.66				100	
89082 0.66				100	
SWKD 0.66				100	
08MWNPN 0.67				100	
89113 0.67				100	
WSRB 0.68				100	
TVSB 0.68				100	
89065 0.68				100	
GUCB 0.69				100	
08LOWE 0.69				100	
CXWG 0.70				100	
TVHB 0.70				100	
35240 0.70				100	
89111 0.70				100	
49303 0.70				100	
WSMX 0.71				100	
TVNR 0.71				100	
07ACMB 0.71				100	
CHWK 0.71				100	
DDCR 0.72				100	
GUWK 0.72				100	
08GWFB 0.73				100	
WLBB 0.73				100	
08SHSA 0.73				100	
08DWHW 0.74				100	
WSRG 0.74				100	
DDDM 0.75				100	
08LELL 0.76				99	1
WSMM 0.76				99	1
GBDR 0.76				99	1
BFSB 0.76				99	1
BFMN 0.76				99	1
08GRST 0.77				95	5
UC08GM 0.77				75	25
BWKE 0.77				74	26
07ACSN 0.78				50	50
CVOCR 0.78				50	50
89092 0.78				50	50
CA08FA 0.79				10	90
MTWN 0.80				1	99
MTQH 0.80				1	99
FC08WY 0.81					100
08OHFB 0.81					100
07PFMA 0.82					100
08LOKE 0.82					100
45480 0.83					100
08NERE 0.84					100
DDCO 0.84					100
08NEYA 0.84					100
WEGS 0.84					100
07PFBR 0.84					100
08BRKH 0.85					100
08POWB 0.86					100
08DRWA 0.86					100
08CACD 0.87					100
CR08CR 0.88					100
08NTAW 0.88					100
08LAKB 0.89					100
08DWLW 0.89					100
08CAFG 0.89					100
SLWR 0.91					100
08ROTO 0.91					100
08LBHB 0.92					100
08LLBY 0.93					100
08HUMA 0.94					100
TFWT 0.96					100
08PTJM 0.96					100
08BDFB 0.96					100
08LATF 0.97					100
MTWX 0.97					100
MTTI 0.97					100
MTAB 0.98					100
08PBWA 1.00					100
MTPB 1.00					100

Figure 4.6 Confidence of class for all surveys (reference sites in red).

The calculated confidence of class for all surveys are provided in Figure 4.6. From this figure, it can be seen that there is a 100% confidence of high ecological potential for any site above an EQR of 0.81 . All reference sites were classified as high potential, with six of the seven sites with 99% confidence or higher. One site had 90% confidence of being of high potential.

## 5 Discussion

The use of the most species-rich macroinvertebrate group, with their well known habitat and water quality requirements, as well as their relative ease of collection, has enabled the CPET methodology to be simple and effective. This technique has been shown to define ecological potential of canals by comparing observed to reference conditions. An important criteria for this method was that boat traffic be taken into consideration. This was to allow the canals' continued beneficial sustainable use for navigation and recreation. A system was therefore required that defined Maximum Ecological Potential (MEP) for any specified level of boat traffic. The CPET methodology has already been approved as a standard for ecological assessment (CEN 2006).

Incorporating boat traffic into the best subset regression equations for reference condition has negated the need for MEP to be determined for a specified range of traffic. Boat traffic was one of the best predictors of taxonomic compositional change across the dataset, showing its importance in the distribution of chironomid taxa. The reference equation also included easily obtainable physical parameters and conductivity that can be obtained by field measurement. The importance of geology was also shown by conductivity (although not alkalinity), and latitude and longitude being significant variables. East–west differences were slightly more significant than north–south differences. Altitude was also shown to be significant.

The use of site-specific referencing which included a measure of boat traffic meant it was unnecessary to have separate classifications for ranges of traffic. Putting sites into four or five boat range classes, for example, would have meant having a small number of sites in each class.

### 5.1 Definition of reference sites and 'high potential'

Defining reference conditions for canals is difficult because, even in rural areas away from significant anthropogenic influences, feeder systems can be impacted by diffuse pollution, especially in lowland areas. It was important to allow boat traffic to be considered when selecting reference sites as well as in the final tool. The decision was made to use the best available contemporary data, which took usage into account. As far as possible sites were chosen on the basis of low value of the parameter under consideration. Whether these reference sites do constitute true MEP (Maximum Ecological Potential) or the best available 'point of reference' is debatable. This could be resolved in the future by 'intercalibrating' canal classifications across Europe.

The status of other biological elements in determining reference condition was not considered and so it is possible that a reference site as defined by chironomids is not classified as high ecological potential by macrophytes or macroinvertebrates.

### 5.2 Measuring deviations from reference condition

The use of qualitative taxa presence data proved to be as good to calculate impact score as using weighted-average data and also had the advantage of easy conversion to an EQR.

Only a single pressure gradient has been considered in this study. Although the nutrient gradient assessed here reflects the primary concerns of the regulatory bodies, along with deviations from reference caused by organic pollution, it would be interesting to assess other pressures such as metals pollution. It has been criticised

(Moss 2008) that there is little appreciation of what restoration of ecological status means. Moss quotes Higgs (1997) as having defined restoration: 'as the return of an ecosystem to a close approximation of its condition prior to disturbance. In restoration, ecological damage to the resource is repaired. Both the structure and the functions of the ecosystem are recreated'. This is not achievable in AWBs as there is a requirement for their sustainable use. Also, the use of prescribed lists of species has been criticised in the assessment of ecological quality, especially where reference state is being considered. In this study, taxa have been used with environmental variables to obtain site scores. Ecological potential was then assessed by comparing reference with observed scores. The taxa acquired are representative of the ecological conditions present at each site and not just a random collection to merely provide lists.

### 5.3 Defining 'good ecological potential' and the good/moderate boundary

There are no true boundaries that exist in nature and so to define boundaries between EQR to produce five classes could be problematic. There is also no absolute justification in placing the good/moderate boundary on any point along the ecological potential gradient. A more justified consideration is the crossover point of sensitive and tolerant taxa, which reflect the structural and the ecophysiological changes of the chironomid communities. This is the point where the taxa tolerant to TON become more abundant than the taxa sensitive to TON which tend to be more common in less polluted waters.

From this point it was possible to assign boundaries in accordance with the standard deviations of the 'fits' of tolerant taxa. It is possible that good status could allow the possibility of short-term pollution/disturbance, where some pollution-tolerant taxa will be found at the lower end of the 'good' boundary.

It is possible that there will be overlaps between the 'good' and 'moderate' boundaries due to the definitions assigned to these terms. In other words, 'good' status or potential allows *slight* changes to the community under consideration, whereas 'moderate' status or potential allows *moderate* changes. This is the critical point where a water body will require remedial measures to achieve good ecological potential.

### 5.4 Site-specific predictions

Allowing boat traffic to be included in the regression equation has enabled site-specific reference conditions to be applied. Any classifications based on the need to divide boat traffic into classes would have meant a small number of sites being assessed in each class. The system under consideration here, and other classification systems being assessed (Kelly *et al.* 2008, Ruse 2008) for rivers and lakes, all show continuous variation in the response of biota to environmental conditions. Although the WFD requires that type-specific reference conditions are applied to generate EQRs, this approach requires larger datasets than were available for this study. For this reason, the site-specific approach was applied here.

## 5.5 Uncertainty in chironomid-based estimates of ecological potential

Monthly sampling of the Kennet & Avon Canal at Fobney over a period of a year provided the means by which the effects of sampling in different months could be assessed. It was found that there was no bias to certain seasons, with EQR normally distributed about the mean. Sampling variability did not have a significant effect on observed/reference values with an EQR SD of 0.0098.

## 5.6 Recommendations

External validation of this tool across Europe and intercalibration of class boundaries will be required.

## 6 Conclusions

This work defines the chironomid fauna of canals that is expected in the absence of any significant anthropogenic influences. It provides a means to assess the ecological potential of canals by taking into account boat traffic, a necessary proviso in allowing for the sustainable use of these water bodies.

This CPET tool will enable statutory agencies to commence monitoring to assess the ecological potential of canals with a statistically sound basis for determining the need or otherwise of any 'programme of measures'.

This work also provides a framework by which metrics to assess other pressures may be developed in the future.

Confidence of canal classification was of high precision. This, together with the significant relationship between TON impact score and observed TON values, supports the adoption of the CPET tool to implement the objectives of the WFD for canals.

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# List of abbreviations

AWB	Artificial Water Bodies
BOD	biological oxygen demand
CCA	canonical correspondence analysis
CPET	Chironomid Pupal Exuvial Technique
DCA	detrended correspondence analysis
DO	dissolved oxygen
EQR	Ecological Quality Ratio
EU	European Union
GEP	good ecological potential
GES	good ecological status
HMWB	Heavily Modified Water Bodies
IMS	industrial methylated spirit
MEP	maximum ecological potential
SD	standard deviation
SE	standard error of the mean
SEPA	Scottish Environment Protection Agency
TON	total oxidised nitrogen
WFD	Water Framework Directive

# Glossary

Ecological potential	Developed by the WFD to distinguish from ecological status by allowing the continued beneficial use of canals such as by navigation.
Intercalibration	An EU-wide exercise to ensure the status classification systems developed by different Member States produce comparable results.
Programme of measures	Actions taken by a Member State to improve a water body which does not achieve at least good ecological potential.
Uncertainty	Parameter associated with the result of a measurement which characterises the dispersion of values that could reasonably be attributed to the quantity subject to measurement.

# Appendices

# Appendix 1 List of CPET canal sample locations

CODE	CANAL	SITE	NGR	EASTING	NORTHING
89142	Aire & Calder	Woodlesford Lock	SE 3670 2948	436700	429480
362	Aire & Calder	Pollington	SE 6120 1940	461200	419400
07ACMB	Ashby	Market Bosworth	SK 387 024	438700	302390
07ACSN	Ashby	Snarestone	SK 343 085	434300	308500
89067	Basingstoke (Wey)	Crookham	SU 7918 5171	479180	151710
89055	Basingstoke (Wey)	Brookwood Lock 12	SU 9582 5719	495820	157190
89082	Basingstoke (Wey)	Frimley Lock 28	SU 9115 5655	491150	156550
BFFZ	B'ham & Fazeley	Fazeley	SK 203 019	420300	301900
BFSB	B'ham & Fazeley	Salford Br	SP 097 901	409700	290100
BFMN	B'ham & Fazeley	Minworth Lock	SP 152 923	415200	292300
GBDR	B'ham & W'ton	Dudley Rd, Tipton	SO 978 903	397800	290300
BWKE	B'ham & W'ton	King Edwards Rd	SP 060 869	406000	286900
BLPL	B'ham Level	Bromford Lane	SO 995 903	499500	290300
BLBB	B'ham Level	Brasshouse Br	SP 019 889	401900	288900
BLBL	B'ham Level	Bromford Lane	SO 995 903	499500	290300
08BRKH	Bridgewater	Keckwith Hill Br	SJ 57209 82615	357209	382615
08BDFB	Bude	Falcon Br	SS 207 061	220728	106153
08CACD	Caldon	Cheddleton	SJ 9738 5251	397384	352507
08CAFG	Caldon	Frogghall	SK 0238 4749	402383	347497
08LBHB	Caldon (Leek Branch)	Horse Br	SJ 9624 5374	396243	353737
CA08FA	Caledonian	Fort Augustus	NH3758509146	237585	809146
CXWG	Cannock Extn	Wyrley Grove Br	SK 019 054	401900	305400
49303	Chesterfield (Ryton)	Retford	SK 7210 8200	472100	382000
CHWK	Chesterfield (Ryton)	Worksop	SK 595 791	459500	379100
CVFX	Coventry	Foxford	SP 351 839	435100	283900
CVFS	Coventry	Foleshill Rd	SP 338 806	433800	280600
CVOCR	Coventry	Old Church Rd	SP 348 821	434800	282100
CR08CR	Crinan	Crinan	NR 79303 93762	179303	693762
89117	Cromford	Ripley	SK 3880 5070	438800	350700
89066	Cromford	Cromford	SK 3140 5590	431400	355900
08DRWA	Driffield	Wansford	TA 06251 56172	506251	456172
08DWHW	Droitwich	Hanbury Warf	SO 9202 6304	392017	263039
08DWLW	Droitwich	Ladywood	SO 8681 6106	386814	261056
DDCR	Dudley	Cradley Rd	SO 948 874	394800	287400
DDDM	Dudley	Dudley Mixed	SO 934 888	393400	288800
DDCO	Dudley	Cherry Orchard	SO 963 861	396300	286100
08ERAR	Erewash	Ilkeston	SK 46928 43097	446928	343097
FC08WY	Forth & Clyde	Wyndford	NS7752378778	277523	678778
08GSPT	Gloucester/Sharpness	Purton	SO 692 042	369238	204199
07GUWE	Grand Union	Welton	SP 598 654	459750	265370
07GUCO	Grand Union	Cosgrove	SP 795 424	478950	243550
GULP	Grand Union	Lapworth	SP 194 723	419400	272300
07GULE	Grand Union	Leicester	SK 569 010	456900	301050
07GUGG	Grand Union	Great Glen/Leicest	SP 648 960	464800	296000
GUNC	Grand Union	Nechells	SP 095 877	409500	287700
07GULO	Grand Union	Loughborough	SK 528 209	452850	320900
GUCB	Grand Union	Catherine-de-Barne	SP 180 803	418000	280300
GUWK	Grand Union	Emscote Rd, Warwick	SP 297 655	429700	265500
33900	Grand Union (Batchworth Reach)	Above Springwell Lock	TQ 0434 9296	504340	192960
34023	Grand Union (Colne)	Coppermill Lane, Harefield	TQ 0407 9111	504070	191110
34005	Grand Union (Cowley Reach)	Horton Rd Br	TQ 0664 8007	506640	180070
34006	Grand Union (Cowley Reach)	Above Lock 97	TQ 1498 7963	514980	179630
34004	Grand Union (Pix Farm Reach)	1500m d/s Berkhamstead STW	TL 0270 0630	502700	206300
34027	Grand Union (Pix Farm Reach)	Above Berkhamstead STW	TL 0070 0710	500700	207100
35264	Grand Union (Wendover Arm)	Tring	SP 924 132	492400	213200
08GWFB	Grand Western	Fenacre Br	ST 071 177	307083	117707
08GRST	Grantham	Stenwith	SK 83475 36693	483475	336693
08HUMA	Huddersfield	Marsden	SE 06350 13105	406350	413105
88ken5	Kennet & Avon	Ufton	SU 618 686	461800	168600
88ken6	Kennet & Avon	Burghfield Br	SU 682 708	468200	170800
88ken7	Kennet & Avon	u/s R. Thames	SU 731 738	473100	173800
08KASE	Kennet & Avon	Seend	ST 93536 61417	393536	161417
89083	Kennet & Avon	Great Bedwyn	SU 279 642	427900	164200
89113	Kennet & Avon	Oakhill	SU 299 672	429900	167200
89065	Kennet & Avon	Crofton	SU 264 623	426400	162300
35240	Kennet & Avon	Froxfield Br	SU 304 677	430400	167700
08LAKB	Lancaster	Kellet Br	SD 51853 71149	351853	471149
08LATF	Lancaster	Tewitfield	SD 51922 73786	351922	473786
34014	Lee (Navigation)	Above Dobbs Weir/D/s Rye Meads STW	TL 3850 0830	538500	208300
89134(96)	Lee (Navigation)	u/s Rye Meads STW	TL 391 091	539100	209100
kings88	Lee (Navigation)	King's Weir	TL 373 052	537300	205200
hmar88	Lee (Navigation)	Hackney Marsh	TQ 366 865	536600	186500
enf88	Lee (Navigation)	Enfield Lock	TQ 373983	537300	198300
462	Leeds & Liverpool	Gargrave	SD 9350 5450	393500	454500
463	Leeds & Liverpool	Bingley	SE 1180 3840	411800	438400
461	Leeds & Liverpool	Canal Rd, Leeds	SE 2770 3400	427700	434000
08LELL	Levan	Little Levan	TA 10395 44894	510395	444894
08LLBY	Llangollen	Bryntysilio	SJ 20143 43448	320143	343448
08LOWE	Louth	West End	TF 35048 97532	535048	397532
08LOKE	Louth	Kedington	TF 35073 89023	535073	389023
08MWNP	Market Weighton	Newport	SE 8540 3096	485401	430958
89111	Monmouth-Brecon	Malpas Lock Newport	ST 2958 8891	329580	188910
89092	Monmouth-Brecon	Llangynidr Locks	SO 1473 1999	314730	219990

CODE	CANAL	SITE	NGR	EASTING	NORTHING
45480	Monmouth-Brecon	Croes y Pant	SO 3131 0399	331310	203990
MTWN	Montgomery	Wern	SJ 2518 1413	325180	314130
MTQH	Montgomery	Queens Head	SJ 3390 2680	333900	326800
MTWX	Montgomery	Welshpool Cross	SJ 2410 0890	324100	308900
MTTI	Montgomery	Tannat Intake	SJ 2511 2040	325110	320400
MTAB	Montgomery	Aberbechan	SO 1430 9330	314300	293300
MTPB	Montgomery	Parson Bridge	SJ 2640 1730	326400	317300
08NERE	Neath	Resolwen	SN 82727 03137	282727	203137
08NEYA	Neath	Ynys-Arwed Farm	SN 81145 01921	281145	201921
08NTAW	Nottingham	Awsworth	SK 4760 4342	447606	343396
08OHFB	Old Howe	Frodingham Br	TA 09130 53836	509130	453836
89133(95)	Oxford	u/s King's Br	SP 492 121	449200	212100
89089(95)	Oxford	Kidlington Lock	SP 492 128	449200	212800
07OXCL	Oxford	Claydon Locks	SP 457 512	445750	251240
89089(91)	Oxford	Kidlington Lock	SP 492 128	449200	212800
89133(91)	Oxford	u/s King's Br	SP 492 121	449200	212100
89071(91)	Oxford	D/S King's Br	SP 488 113	448800	211300
89121	Oxford	Roundham Lock	SP 483 140	448300	214000
89130	Oxford	Thrupp	SP 482 159	448200	215900
89071(95)	Oxford	D/S King's Br	SP 488 113	448800	211300
89079	Oxford	Enslow	SP 479 178	447900	217800
89135(91)	Oxford	u/s King's Br	SP 492 121	449200	212100
89073	Oxford	u/s King's Br	SP 492 121	449200	212100
89141	Oxford	Wolvercote Lock	SP 494 099	449400	209900
07OXBA	Oxford	Barby	SP 525 712	452530	271160
07OXWI	Oxford	Willoughby	SP 523 682	452300	268200
07PFMA	Peak Forest	Marple	SJ 967 866	396700	386600
07PFBR	Peak Forest	Bredbury	SJ 935 916	393500	391600
08POWB	Pocklington	Walbut Br	SE 77152 44163	477152	444163
08PTJM	Port Tennant	Jersey Marine	SS 71207 93892	271207	193892
08PBWA	Prees Branch	Waterloo	SJ 49738 33238	349738	333238
stusrm	R. Stort	u/s Rye Meads STW	TL 392 093	539200	209300
08RIRI	Ripon	Ripon	SE 32387 70355	432387	470355
08ROTO	Rochdale	Todmorden	SD 95927 24677	395927	424677
dsdeep	Salmon Bk	D/S Deephams	TQ 356 927	535600	192700
524	Selby	West Haddlesey	SE 5720 2650	457200	426500
525	Selby	Brayton	SE 6100 3030	461000	430300
08SUAD	Shropshire Union	Adderley	SJ 67043 39218	367043	339217
08SUNB	Shropshire Union	Nanneys Br	SJ 65463 58525	359172	389483
08SUPL	Shropshire Union	Platt Lane	SJ 51088 36512	351088	336512
SUPB	Shropshire Union	Pendeford Br	SJ 888 034	388800	303400
SLWR	Soho Loop	Western Rd Br	SP 051 880	405100	288000
08SHSA	St Helens	Sankey	SJ 59171 89483	359172	389483
SWJN	Staffs & Worc	Junction	SJ 902 011	390200	301100
SWOX	Staffs & Worc	Oxley	SJ 902 017	390200	301700
SWCH	Staffs & Worc	Coven Heath	SJ 914 054	391400	305400
SWSB	Staffs & Worc	Swindon Br	SO 862 906	386200	290600
SWCB	Staffs & Worc	Compton Br	SO 883 988	388300	298800
SWKD	Staffs & Worc	Kidderminster	SO 828 758	382800	275800
STSR	Stratford	Stirchley	SP 059 796	405900	279600
STST	Stratford	Stratford	SP 199 555	419900	255500
STHH	Stratford	Hockley Heath	SP 152 725	415200	272500
TVHB	Tame Valley	Holloway Bank	SO 990 939	399000	293900
TVNR	Tame Valley	Newton Rd	SP 036 940	403600	294000
TVSB	Tame Valley	Salford Br	SP 096 901	409600	290100
89087	Taunton & Bridgewater	Huntsworth	ST 318 344	331800	134400
89068	Taunton & Bridgewater	Crossways	ST 309 353	330900	135300
TFWT	Titford	W'ton Rd	SO 988 878	398800	287800
08TMEL	Trent & Mersey	Elworth	SJ 7304 6209	373038	362090
07TMFJ	Trent and Mersey	D/S Fradley Junction	SK 142 140	414110	314060
07TMKB	Trent and Mersey	Kings Bromley	SK 111 152	411110	315210
07TMBN	Trent and Mersey	Barton-under-Needwood	SK 203 184	420300	318430
UC08GM	Union	Gilmore	NT2440472692	324404	672692
WSRB	Walsall	Rayboulds Br	SO 985 977	398500	297700
WSMX	Walsall	Moxley	SO 969 955	396900	295500
WSRG	Walsall	Ryders Green Rd	SO 983 917	398300	291700
WSMM	Walsall	Moors Mill Lane	SO 977 932	397700	293200
town90	Wey (Navigation)	Town (Weybridge) Lock	TQ 068 647	506800	164700
wey Stoke	Wey (Navigation)	Stoke Lock	TQ 002 516	500200	151600
89115	Wey (Navigation)	Papercourt Lock	TQ 034 568	503400	156800
89070	Wey (Navigation)	D/S Godalming	SU 996 465	499600	146500
89049	Wey (Navigation)	Artington Lock (St Catherine)	SU 996 477	499600	147700
89064	Wey (Navigation)	(Craft) Unstead Lock	SU 992 460	499200	146000
89116	Wey (Navigation)	Pyrford	TQ 039 574	503900	157400
peas90	Wey (Navigation)	Peasmarsh	SU 993 463	499300	146300
89054	Wey (Navigation)	Bowers Lock	TQ 012 529	501200	152900
WBDG	Worc & B'ham	Diglis Basin, Worcester	SO 849 539	384900	253900
WBLF	Worc & B'ham	Pershore Rd, Lifford	SP 054 803	405400	280300
WBG	Worc & B'ham	Grange Lane	SP 019 712	401900	271200
WBBR	Worc & B'ham	Bath Row Br	SP 061 860	406100	286000
WLBS	W'ton Level	Baker St, Tipton	SO 954 917	395400	291700
WLBR	W'ton Level	Brades Rd	SO 982 900	398200	290000
WLBB	W'ton Level	Brasshouse Br	SP 019 889	401900	288900
WEGS	Wyrley & Essington	Goscot	SK 016 020	401600	302000

## Appendix 2 Taxa in analysis

Code	Name and author	Code	Name and author
ABLA_LON	Ablabesmyia longistyla Fitt	ORTH_EUO	Orthocladius (Euorthocladius) spp
ABLA_MON	Ablabesmyia monilis (Linnaeus)	ORTHss	Orthocladius spp
ABLA_YIA	Ablabesmyia spp	PARC_ARC	Parachironomus arcuatus (Goetghebuer)
ACRI_LUC	Acricotopus lucens (Zetterstedt)	PARC_BIA	Parachironomus biannulatus (Staeger)
APSE_TRI	Apsectrotanypus trifascipennis (Zetterstedt)	PARC	Parachironomus spp
ARCT	Arctopelopia spp	PARC_LMA	Paracladopelma spp
BRIL_BIF	Brilia bifida Kieffer	PARC_NGA	Paracladopelma nigritula (Goetghebuer)
BRIL_FLA	Brilia flavifrons Johannsen	PARDCAMg	Paracladopelma camptolabis (Kieffer) gp
BRYO	Bryophaenocladus spp	PARH	Paraphaenocladus spp
CARD_FUS	Cardiocladius fuscus Kieffer	PARK_FEN	Parakiefferiella fennica Tuiskunen
CHIR_PLU	Chironomus plumosus (Linnaeus)	PARK	Parakiefferiella spp
CHIR	Chironomus spp	PARL_CON	Paracladius conversus (Walker)
CLAD	Cladopelma spp	PARM	Parameria spp
CLAT	Cladotanytarsus spp	PART_TLL	Paratanytarsus tenellulus (Goetghebuer)
CLIN_NER	Clinotanytarsus nervosus (Meigen)	PART	Paratanytarsus spp
CONC_MEL	Conchapelopia melanops (Meigen)	PATD	Paratendipes spp
CONC	Conchapelopia spp	PATR_RUV	Paratrachocladius rufiventris (Meigen)
CORY_URA	Corynoneura spp	PATR	Paratrachocladius spp
CRIC(C)	Cricotopus s.g Cricotopus	PHAE	Phaenoscpectra spp
CRIC(I)	Cricotopus s.g Isocladius	POLY_NUB	Polypedium nubens (Edwards)
CRIC_BIC	Cricotopus (C.) bicinctus (Meigen)	POLY	Polypedium spp
CRIC_INT	Cricotopus (I.) intersectus (Staeger)	POLYSORg	Polypedium (Pent) sordens (Wulp) gp
CRIC_SYL	Cricotopus (I.) sylvestris Fabricius	POTT_TIA	Potthastia spp
CRYP_MUS	Cryptochironomus spp	PROC_HOL	Procladius (Holo) spp
CRYPOBRg	Cryptochironomus obreptans gp	PROC_SUS	Procladius spp
CRYT	Cryptotendipes	PROD_OLI	Prodiamesa olivaea (Meigen)
DEME_RUF	Demeijerea rufipes (Linnaeus)	PSEC_BPS	Psectrocladius (ss) barbatipes Kieffer
DEMI	Demicryptochironomus	PSEC_OBV	Psectrocladius (Allo) obivus (Walker)
DICR_NER	Dicrotendipes (Lim) nervosus (Staeger)	PSEC_OCT	Psectrocladius (ss) octomaculatus Wulker
DICR_NOT	Dicrotendipes (Lim) notatus (Meigen)	PSEC_ss	Psectrocladius (ss) spp
DICR_TRI	Dicrotendipes (Lim) tritonus (Kieffer)	PSET_VAR	Psectrotanytarsus varius (Fabricius)
DICR	Dicrotendipes spp	PSEU_PRA	Pseudochironomus prasinatus (Staeger)
EINF_PAG	Einfeldia pagana (Meigen)	RHEO_ss	Rheocricotopus spp
ENDO	Endochironomus spp	RHET	Rhetantanytarsus spp
EPOI_FLA	Epoicocladius flavens (Malloch)	SMIT	Smittia spp
EUKI_CLA	Eukiefferiella claripennis (Lund)	STEM_BAU	Stempellina bausei (Kieffer)
EUKI	Eukiefferiella spp	STEM	Stempellina spp
GLYP_cau	Glyptotendipes (Caulochironomus)	STEN	Stenochironomus spp
GLYP_ss	Glyptotendipes spp	STIC	Stictochironomus spp
HARN_CUR	Harnischia curtilamellata (Malloch)	SYNE	Synendotendipes spp
HARN_HIA	Harnischia spp	SYNO_SEM	Synorthocladius spp
HETE	Heterotrissocladius spp	TANP_PUN	Tanytarsus punctipennis Meigen
HETT_API	Heterotanytarsus apicalis (Kieffer)	TANP	Tanytarsus spp
KIEF_TEN	Kiefferulus tendipediformis (Goetghebuer)	TANY_BRU	Tanytarsus brundini Lindeberg
LAUT_AGR	Lauterborniella agrayloides (Kieffer)	TANY_PCS	Tanytarsus pallidicornis (Walker)
LIMN	Limnophyes spp	TANY_PT2	Tanytarsus (part 2)
MACR_NEB	Macropelopia nebulosa Meigen	TANY_PT3	Tanytarsus (part 3)
METR	Metriocnemus spp	TANY_SUS	Tanytarsus spp
MICC_TEN	Microchironomus tener (Kieffer)	TANY_SYL	Tanytarsus sylvaticus (Wulp)
MICR_ATR	Micropsectra atrofasciata (Kieffer)	TANYMEDg	Tanytarsus medius gp Reiss & Fittkau
MICR	Micropsectra spp	THIE	Thienemanniella spp
MICT	Microtendipes spp	THIY	Thienemannimyia spp
NANO_BAL	Nanocladius balticus Palmen	TVET_DIS	Tvetenia discoloripes (Goetghebuer)
NANO	Nanocladius spp	TVET	Tvetenia spp
NEOZ	Neozavrelia spp	XENO_XEN	Xenochironomus xenolabis (Kieffer)
ODON_FUL	Odontomesa fulva (Kieffer)	XENP	Xenopelopia spp
ORTH_EUD	Orthocladius (Eudactyloccladius) spp	ZAVR	Zavrelimyia spp

## Appendix 3 Species optima (TON) in tolerance order. Codes from Appendix 2

Taxa	Optima	Taxa	Optima
ODON_FUL	-1.13	CORY_URA	-0.03
ORTH_EUO	-0.69	MICC_TEN	-0.02
CRIC_BIC	-0.66	MICT	-0.02
TANY_BRU	-0.64	LIMN	-0.01
CLAT	-0.59	PHAE	0.01
CONC	-0.59	NANO	0.01
SYNO_SEM	-0.59	ARCT	0.01
CRYT	-0.51	PSEC_OBV	0.02
PATR_RUV	-0.48	POLY	0.03
THIY	-0.47	GLYP_ss	0.06
EUKI_CLA	-0.47	PART_TLL	0.09
CRYP_MUS	-0.46	KIEF_TEN	0.09
EPOI_FLA	-0.45	MICR	0.10
STEM	-0.43	TANP_PUN	0.11
HARN_HIA	-0.38	EINF_PAG	0.13
THIE	-0.37	PARK_FEN	0.13
PARC	-0.37	PARL_CON	0.13
ABLA_YIA	-0.37	CHIR	0.15
POTT_TIA	-0.34	ENDO	0.20
PROC_SUS	-0.34	BRIL_BIF	0.21
CONC_MEL	-0.33	CRYP_OBRg	0.22
TANY_MEDg	-0.32	PARH	0.23
NANO_BAL	-0.31	PARD_NGA	0.25
APSE_TRI	-0.31	BRYO	0.25
HARN_CUR	-0.31	STEN	0.26
CARD_FUS	-0.31	PSEC_ss	0.28
METR	-0.27	DICR	0.30
PSEU_PRA	-0.25	CLIN_NER	0.30
PARD_LMA	-0.24	STIC	0.31
ORTH_ss	-0.23	ABLA_MON	0.32
TANY	-0.23	GLYP_cau	0.33
PARM	-0.19	DICR_NOT	0.33
MICR_ATR	-0.19	DEMI	0.34
TVET	-0.18	SYNE	0.34
PATD	-0.17	DEME_RUF	0.34
XENO_XEN	-0.16	PROC_HOL	0.35
ABLA_LON	-0.16	ACRI_LUC	0.35
POLY_NUB	-0.16	PARC_ARC	0.39
NEOZ	-0.16	PARC_BIA	0.40
RHEO_ss	-0.14	TANY_PT2	0.43
ORTH_EUD	-0.14	POLY_SORg	0.45
EUKI	-0.13	TANY_SYL	0.47
CRIC_INT	-0.13	PSET_VAR	0.49
RHET	-0.13	PARK	0.55
PART	-0.12	DICR_TRI	0.60
DICR_NER	-0.09	CHIR_PLU	0.63
CLAD	-0.09	MACR_NEB	0.69
BRIL_FLA	-0.09	ZAVR	0.70
PSEC_OCT	-0.08	TANY_PT3	0.72
PATR	-0.08	TANP	0.75
CRIC (C)	-0.07	SMIT	0.85
PROD_OLI	-0.07	PARD_CAMg	0.87
TVET_DIS	-0.07	HETE	1.01
PSEC_BPS	-0.07	STEM_BAU	1.02
CRIC (I)	-0.06	XENP	1.24
TANY_PCS	-0.06	HETT_API	3.19
CRIC_SYL	-0.03		

## Appendix 4 Reference data, scores and EQR (TON). Reference site names in blue

Site	Observed Score	Reference Score	EQR	Site	Observed Score	Reference Score	EQR
88ken5	-0.22	-0.64	0.19	enf88	-0.20	-2.11	0.62
88ken6	-0.17	-0.74	0.22	WB-GL	0.10	-2.11	0.63
88ken7	-0.14	-0.78	0.23	BL-BB	0.16	-2.12	0.63
89142	-0.16	-1.06	0.31	WL-BR	0.11	-2.13	0.63
SW-JN	-0.04	-1.07	0.32	07GU-LO	-0.09	-2.17	0.64
SW-OX	-0.03	-1.10	0.32	89083	-0.15	-2.18	0.65
07TM-FJ	-0.10	-1.10	0.33	WB-BR	0.12	-2.19	0.65
89089(95)	-0.15	-1.11	0.33	SW-KD	-0.01	-2.22	0.66
89133 (95)	-0.11	-1.12	0.33	89066	0.06	-2.23	0.66
07OX-CL	-0.06	-1.14	0.34	08RI-RI	0.10	-2.24	0.66
89089(91)	-0.10	-1.15	0.34	89082	0.03	-2.24	0.66
89133 (91)	-0.07	-1.20	0.35	BL-BL	0.20	-2.25	0.66
07TM-KB	-0.08	-1.21	0.36	08MW-NP	0.07	-2.27	0.67
89121	-0.10	-1.21	0.36	89113	-0.14	-2.27	0.67
34005	-0.16	-1.22	0.36	WS-RB	0.14	-2.30	0.68
89071(91)	-0.06	-1.22	0.36	TV-SB	0.05	-2.30	0.68
08SU-AD	0.00	-1.26	0.37	89065	-0.09	-2.30	0.68
33900	-0.13	-1.26	0.37	GU-CB	0.14	-2.34	0.69
89130	-0.08	-1.26	0.37	08LO-WE	0.11	-2.34	0.69
89071(95)	-0.03	-1.30	0.38	49303	-0.14	-2.35	0.70
town90	-0.16	-1.30	0.38	35240	-0.09	-2.36	0.70
89079	-0.09	-1.30	0.38	CX-WG	0.10	-2.36	0.70
07GU-WE	-0.08	-1.31	0.39	89111	-0.11	-2.38	0.70
34006	-0.11	-1.34	0.40	TV-HB	0.09	-2.38	0.70
wey-stoke	-0.17	-1.35	0.40	CH-WK	-0.17	-2.39	0.71
08GS-PT	0.10	-1.38	0.41	TV-NR	0.11	-2.40	0.71
34023	-0.12	-1.38	0.41	WS-MX	0.16	-2.40	0.71
08SU-NB	0.02	-1.38	0.41	07AC-MB	0.04	-2.41	0.71
SW-CH	0.03	-1.40	0.41	DD-CR	0.08	-2.44	0.72
35264	-0.08	-1.41	0.42	GU-WK	0.07	-2.44	0.72
89135(91)	-0.10	-1.42	0.42	08SH-SA	0.12	-2.46	0.73
34027	-0.07	-1.45	0.43	WL-BB	0.17	-2.47	0.73
34004	-0.04	-1.45	0.43	08GW-FB	0.21	-2.47	0.73
89073	-0.07	-1.48	0.44	08DW-HW	0.15	-2.50	0.74
34014	-0.17	-1.49	0.44	WS-RG	0.17	-2.51	0.74
SW-SB	-0.06	-1.50	0.44	DD-DM	0.12	-2.53	0.75
08TM-EL	0.03	-1.50	0.44	BF-MN	0.01	-2.55	0.76
stusrm	-0.16	-1.52	0.45	08LE-LL	0.20	-2.56	0.76
89087	-0.09	-1.59	0.47	BF-SB	0.02	-2.56	0.76
362	-0.21	-1.59	0.47	WS-MM	0.18	-2.57	0.76
89134(96)	-0.14	-1.61	0.48	GB-DR	0.09	-2.57	0.76
SW-CB	0.01	-1.63	0.48	08GR-ST	0.17	-2.59	0.77
89067	-0.15	-1.64	0.48	BW-KE	0.06	-2.59	0.77
BL-PL	0.12	-1.65	0.49	UC08-GM	0.18	-2.61	0.77
89141	-0.12	-1.66	0.49	07AC-SN	0.07	-2.62	0.78
07GU-CO	-0.07	-1.68	0.50	CV-OCR	0.08	-2.63	0.78
08SU-PL	0.11	-1.71	0.50	89092	-0.03	-2.64	0.78
462	-0.15	-1.71	0.51	CA08-FA	0.49	-2.67	0.79
07TM-BN	-0.08	-1.72	0.51	MT-QH	0.08	-2.70	0.80
dsdeep	-0.18	-1.74	0.52	MT-WN	0.18	-2.72	0.80
463	-0.14	-1.75	0.52	FC08-WY	0.18	-2.73	0.81
89115	-0.21	-1.77	0.52	08OH-FB	0.01	-2.75	0.81
SU-PB	0.01	-1.77	0.52	08LO-KE	-0.03	-2.78	0.82
89049	-0.18	-1.78	0.53	07PF-MA	-0.03	-2.78	0.82
07OX-BA	-0.04	-1.78	0.53	45480	0.07	-2.79	0.83
89070	-0.18	-1.79	0.53	WE-GS	0.13	-2.83	0.84
89064	-0.16	-1.81	0.54	08NE-YA	0.17	-2.83	0.84
89116	-0.18	-1.81	0.54	07PF-BR	-0.02	-2.84	0.84
ST-SR	0.05	-1.81	0.54	DD-CO	0.19	-2.84	0.84
461	-0.12	-1.86	0.55	08NE-RE	0.21	-2.85	0.84
peas90	-0.12	-1.89	0.56	08BR-KH	0.05	-2.87	0.85
ST-ST	0.09	-1.90	0.56	08PO-WB	0.05	-2.90	0.86
08KA-SE	0.17	-1.90	0.56	08DR-WA	0.03	-2.91	0.86
GU-LP	0.05	-1.91	0.56	08CA-CD	0.11	-2.93	0.87
WB-DG	0.04	-1.91	0.57	08NT-AW	0.07	-2.97	0.88
07GU-LE	-0.02	-1.92	0.57	CR08-CR	0.35	-2.99	0.88
ST-HH	0.09	-1.93	0.57	08CA-FG	0.15	-2.99	0.89
524	-0.12	-1.95	0.58	08DW-LW	0.15	-3.01	0.89
07GU-GG	0.00	-1.97	0.58	08LA-KB	0.16	-3.01	0.89
WL-BS	0.12	-1.99	0.59	08RO-TO	0.06	-3.07	0.91
525	-0.10	-2.00	0.59	SL-WR	0.12	-3.08	0.91
07OX-WI	-0.03	-2.00	0.59	08LB-HB	0.13	-3.11	0.92
89054	-0.15	-2.00	0.59	08LL-BY	0.03	-3.15	0.93
BF-FZ	0.05	-2.01	0.59	08HU-MA	0.07	-3.16	0.94
kings88	-0.15	-2.01	0.60	08PT-JM	0.15	-3.23	0.96
89117	-0.04	-2.03	0.60	TF-WT	0.20	-3.25	0.96
89068	-0.06	-2.05	0.61	08BD-FB	0.14	-3.25	0.96
WB-LF	0.05	-2.06	0.61	MT-WX	0.12	-3.26	0.97
CV-FX	0.09	-2.07	0.61	08LA-TF	0.14	-3.28	0.97
hmar88	-0.22	-2.07	0.61	MT-TI	0.10	-3.28	0.97
CV-FS	0.09	-2.09	0.62	MT-AB	0.11	-3.33	0.98
GU-NC	0.05	-2.09	0.62	08PB-WA	0.17	-3.36	1.00
89055	-0.07	-2.10	0.62	MT-PB	0.15	-3.38	1.00
08ER-AR	0.08	-2.10	0.62				

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## Developing a classification tool for UK canals using chironomid pupal exuviae

Science summary SC070062/SS

The Environment Agency has developed a new technique to assess water quality of canals by looking at the different species of the Chironomid family of non-biting midges that are present in the water. The Chironomid Pupal Exuvial Technique (CPET) involves identifying the type and abundance of chironomid from pupal exuviae (skins) in a sample of water and relating this to the levels of nutrients in the water. This allows the canal to be classified according to its ecological potential as part of the European Union Water Framework Directive (WFD).

A dataset of chironomid pupal exuviae samples were taken across the UK (England, Wales and Scotland) and a classification of nutrient impact using total oxidised nitrogen (TON) was developed. The dataset allowed us to define what chironomids we would expect in canals if there was no human activity, but also to see the effect of boat traffic. This is important to enable the canals to be used sustainably and is specifically allowed within the context of the WFD.

CPET has many advantages over other techniques. Floating pupal exuviae are easy to collect and give a more comprehensive snapshot of the species present than direct sampling of chironomid larvae or other macroinvertebrates. Also, their ecological requirements are generally well documented or can be worked out. This study has allowed us to understand the uncertainties in CPET and develop a framework for the assessment, which may allow us to develop the technique to assess other pressures on the waterbody in the future. We found that CPET can classify the status of the canal with high precision.



This summary relates to information from Science Project SC070062, reported in detail in the following output:

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