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THE EFFECTS OF COAL MINE CLOSURE ON MACROINVERTEBRATE COMMUNITIES.

By

Christopher David Reynolds

Thesis submitted in partial fulfilment of the requirements of The Nottingham Trent University for the degree of Doctor of Philosophy.

This research programme was carried out in co-operation with the Creswell Heritage Trust and the Environment Agency.

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DECLARATIONS

1. The observations presented in this study are, except where otherwise stated, entirely the work of the author.

2. The author has not submitted any part of the thesis in partial fulfilment of any other higher degree.

3. The author has attended conferences and programmes of study relevant to the present research.

4. Due acknowledgements have been made for the assistance given during the course of this work and in the presentation of the thesis on which it is based.

Signed.....

(Candidate)

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(Director of Studies)

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ABSTRACT

Creswell colliery (north-east Derbyshire) pumped saline effluents into Millwood Brook for almost 100 years until spring 1993, a year after it closed. Since the brook had been sampled prior to mine closure (Bird, 1989), it was possible to examine the subsequent changes in species distributions here, and in the River Idle catchment, into which 16 mines, 12 now closed, once discharged their effluents.

From correlation analysis, and using the Millwood Brook as a case study, the major changes in species-dependent site groupings are caused by the loss of a chloride gradient from the polluting mine discharges. Species previously existing up and downstream of the polluted areas have now extended their range. It is now physical river parameters such as width, discharge rate and substratum type which generally control species distribution in the catchment. The saline tolerant amphipod *Gammarus tigrinus* has ceased migration upstream (towards the discharge), and is now retreating. *G. pulex* is re-colonising previously saline sites occupied exclusively by *G. tigrinus*.

To investigate the effects of mine closure on a wider area, data from 165 river sites sampled by the Environment Agency around 59 abandoned coal mine discharges in Yorkshire, Northumbria and Wales were examined. Macrofauna assemblages have been ordinated by DECORANA, classified by TWINSPAN and related to physico-chemical factors using correlation and Multiple Discriminant Analysis (MDA).

In Yorkshire, there is a change in significant correlations of variables with water chemistry in low impact sites, to include water turbidity and substratum quality in high impact sites, including coverage by 'ochre'. The DECORANA ordination for Northumbria, with low impact sites in more rural areas, illustrates changes in macroinvertebrate assemblages in relation to spatial changes in substratum and position along the length of the river.

Application of novel modelling techniques to the regional data to predict BMWP and ASPT scores and Number of Taxa shows that Group Method of Data Handling (GMDH) performs better than multiple regression (MR) as determined by R^2 comparison (multiple coefficient of determination). The use of these techniques in pollution forecasting is discussed, and limitations of the study considered.

'Caddis'

Struggle - drudge - with the ideas of a crocodile
And the physique of a foetus
Absurd mudlark - living your hovels' life -
Yourself your own worst obstacle.
Lugging Castle Paranoia
Through that moonland, like a train off its track,
Under the rivers' hurricane.
You should have been a crab. Its no good.
Trout in March are crammed
With the debris of your hopeful redoubts.
Wasp - face, orphaned and a waif too early,
Improvising - with inaudible war-cries -
This Herculean makeshift, hoisting your house,
You can nip my finger but
You are still a baby.
Your alfresco Samurai suit of straws,
Your Nibelungen mail of agates, affirms
only fantasies of fear and famine.
Hurry up. Join the love-orgy,
Up here among the leaves, in the light rain,
Under a flimsy tent of dusky wings.

Ted Hughes, 1983.

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Chapter 1. General Introduction.

1.1 Historical Aspects of Coal Mine Pollution

Freshwater pollution in Britain from mines of coal, iron ore and other minerals has been documented back to the 16th century (Hynes, 1970). In 1874 the first of several Acts was passed by Parliament to limit such pollution, which had escalated as a result of the power and raw material needs of the Industrial Revolution. In England and Wales the regional Water Authorities were formed in 1974, with the statutory duty to control the inputs into water bodies. This duty was passed to the National Rivers Authority (NRA) in 1989, and to the Environment Agency (EA) in 1996.

For many years abandoned mines have been a source of poor water quality in England and Wales, but recently the consequences of coal mine closure have been highlighted (NRA, 1994). In October 1992, the Department of Trade and Industry announced proposals for the 'streamlining' of coal production by the state owned corporation, British Coal. This included the closure of 31 out of 50 state owned coal mines, with the loss of an estimated 30,000 jobs. These pit closure plans came during a transition in the coal market, from its nationalisation in 1947 to private ownership in 1994. Robb (1994) explained that cheaper and often cleaner coal now comes from Poland and Brazil, markets are now dominated by light industry and service industry and technological advances have led to the adoption of much larger coal workings employing fewer miners. There has also been the move to cleaner energy sources, such as gas and nuclear.

The outcry from coal mining communities, due to the social consequences, provoked the Government to review plans, with a 'stay of execution' for the pits due for closure. During this time, environmental concerns were highlighted; namely the decay of mine buildings, abandonment of spoil heaps, and particularly the leakage of mine waters from disused pits.

1.2 The Nature of Effluents from Abandoned Coal Mines

The mining industry creates freshwater pollution from two major sources: run off from spoil heaps and the water continuously pumped out of the mines to allow them to be worked (Down and Stocks, 1978). The industry's heritage consists of thousands of abandoned mine shafts and large areas of land made derelict by mineral spoil from closed underground mines (Glover, 1983).

Mine waters are defined as 'waters which can be traced as having had contact with mine workings' (Henton, 1974). They are produced from two sources, water which infiltrates vertically from the land surface (leakage) and water entering mine workings from adjacent geological rock strata; dependent on the nature of those strata. The latter is called 'connate' water (Robb, 1994). Mine waters may run from an old mine shaft, seep out as a spring from the surface or emit directly into a river.

Water can leave a mine by gravitational discharge through a variety of exits or by pumping (Henton, 1981). In 1994, the NRA had some 100 polluting discharge cases from abandoned mines, mostly from underground workings concentrated in the principal coal fields of Britain (NRA, 1994; see Table 1.1). It must be stated that not all mine water is necessarily of poor quality; some of it is very good indeed and may be used to offset the effects of poor quality surface waters by providing additional dilution. Thus, the closure of the mines providing the dilution may indirectly reduce the quality of the receiving watercourse, and reduce the quality and quantity of water available for extraction for agriculture if pumping ceases. Indeed some pumps in the East Midlands area are maintained purely to provide this dilution (R. Harris, EA, personal communication). In the East Midlands, mine water discharges also help to support base flows in the Rivers Poulter, Maun and Meden. A loss of flow may lead to the loss of dilution for sewage works discharges, which may in turn lead to more restrictive discharge consents to preserve the river water quality. Mines are often interconnected and water is pumped from abandoned mines to keep the water table low. This prevents flooding of the pit, which may flood other pits in the system.

Table 1.1. Discharges from coal mines which cause significant pollution problems (NRA, 1994).

Region	Discharges by number [1]	Length of Watercourse Affected (km) [2]	British Coal	Other Coal	Working	Abandoned
Northumbria	15 (all)	18 (12)	11	4	4	11
North West	24 (14)	57 (25)	19	5	Nil	24
Severn-Trent	4 (all)	19 (4)	Nil	4	Nil	4
Welsh	21 (all)	54 (22)	6	15	Nil	21
Yorkshire	36 (most)	50 (11)	Most	Few	Nil	36
Total	100	198	/	/	/	/

Notes.

[1] Main occurrences from coal mines (originating from underground mines in brackets)

[2] Estimated total for main occurrences (numbers of waterways listed in brackets)

The process of dewatering of the coal fields is best envisaged as a series of adjacent underground 'ponds', which are areas of self-contained mining activity, each of which must be pumped to prevent over spilling into the next if flooding of the final pond (perhaps a mine still operating) is to be prevented (S. Dumpleton, British Geological Survey, pers. comm.; Harrison *et al.*, 1989). When mining stops and the pumps are turned off, the mine water will rise to the normal level of the water table ('ground water rebound') until the water escapes into another pond with which there is a connection. This is illustrated in Figure 1.1. A 'leaky bucket' conceptual model of mine water movement suggests that there is an overflow of contaminated waters into the final operating pit.

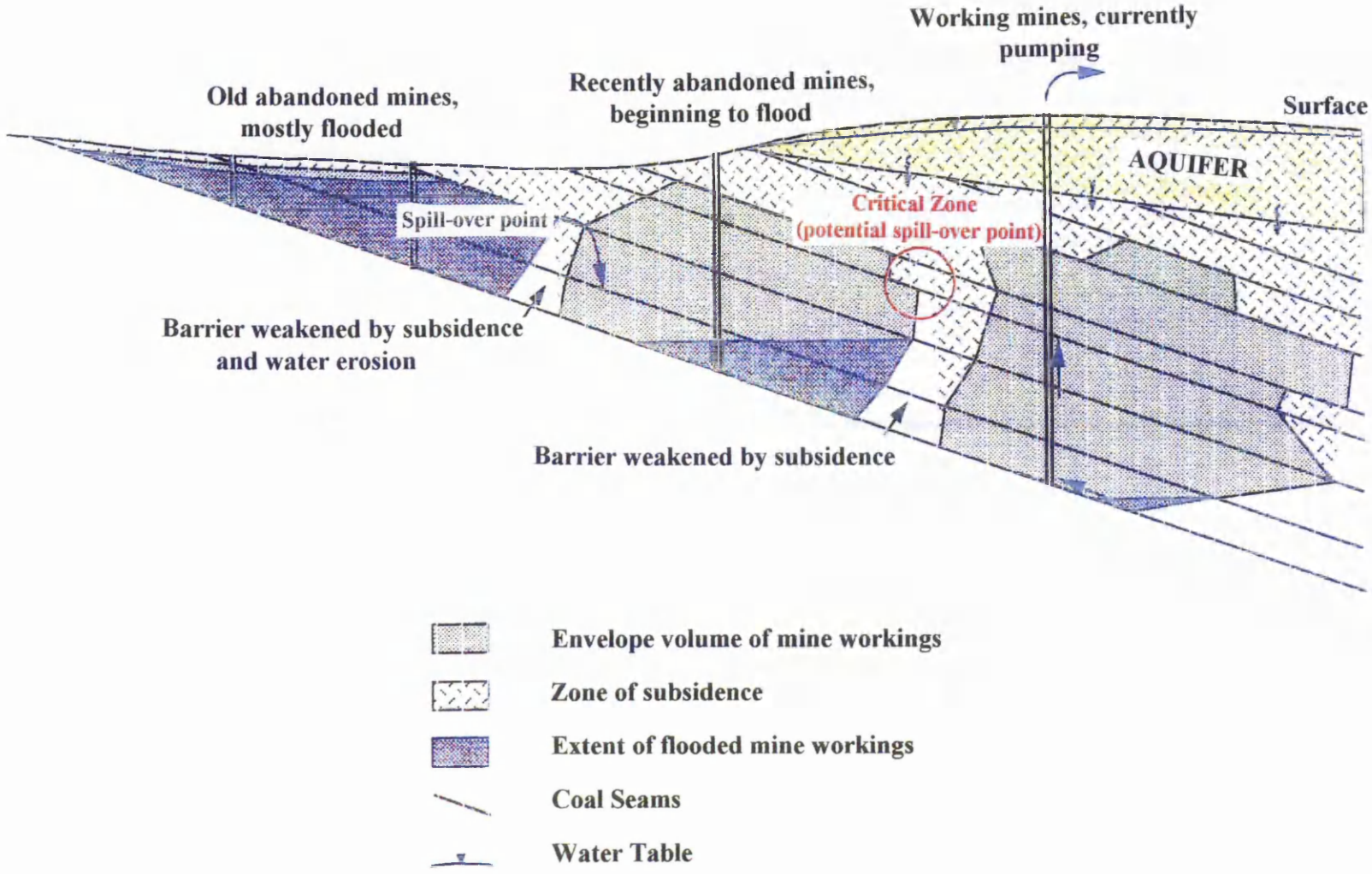


Figure 1.1. The 'Leaky Bucket' conceptual model of mine water movement.

(S. Dumpleton, British Geological Survey).

Eventually all the voids in the mined area become full and the water level stabilises at a position dictated by the point at which it can escape to the surface, often marked by orange 'ochreous' discharges of ferric hydroxide (Younger, 1993), deposited and stabilised by a combination of biological and physical processes. In the East Midlands, Creswell and Langwith collieries (abandoned in 1992 and 1989 respectively) pumped to keep the 'ponds' of the remaining operating mines at Welbeck, Thoresby, Clipstone and Bilsthorpe free from flooding (S. Dumpleton, British Geological Survey, pers. comm). Similarly, Morton, 'A' Winning and Woodside mines are pumped to protect the adjacent Annesley-Bentinck and Calverton collieries. If all collieries closed and pumping ceased completely then mine water would eventually build up and emerge in the surface water system. The River Erewash and possibly the River Leen are potential recipient watercourses. The nature of mine waters varies considerably and the structure of the mine itself affects the nature of the pollution as mines have inter-linked pumping arrangements and water chemistry varies with depth. Glover (1983) states the approximate percentage of mine drainage waters in each class: hard (55%), alkaline (25%), moderately saline (10%), highly saline (2%), alkaline and ferruginous (7%), and acid and ferruginous (1%). 80% of the mine drainage waters are of reasonable quality, but a discussion of two problematic discharges (acidic ferruginous and saline waters) follows.

1.2.1 Acidic, Ferruginous Waters

Acid coal mine drainage is commonly found in South Wales, Northwest England and North America. Numerous studies tackle its effects on macroinvertebrates (eg. Dills and Rogers, 1974; Greenfield and Ireland, 1978; Scullion and Edwards, 1980; Armitage and Blackburn, 1985). When a mine is abandoned, the pumps removing water from the workings are switched off. This allows flooding of the shaft, creating acidic ferruginous waters after oxidation of pyrites in the ideal humid oxidising environment. These waters can in turn dissolve heavy metals e.g. copper and aluminium. Refer to Box 1.1 for equations.

Box 1.1. Chemical reactions involved in the production of ferruginous discharges from coal mines. (Koryak *et al.*, 1972)

Pyrite (FeS_2), a natural component of coal bearing rock, (amongst other metal sulphides) is exposed to dry atmospheric conditions prior to coming into contact with the rising ground waters. Iron pyrites, by the action of sulphur and iron bacteria eg. *Thiobacillus* spp, is oxidised to produce acidic waters with a high iron content:

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4$$

The oxidation of ferrous iron by oxygen in acid solution occurs in the stream:

$$2Fe^{2+} + \tfrac{1}{2}O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$$

The pH rises as the solution is diluted and neutralised in the receiving stream, and the ferric iron hydrolyses, with the precipitation of ferric hydroxide:

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+$$

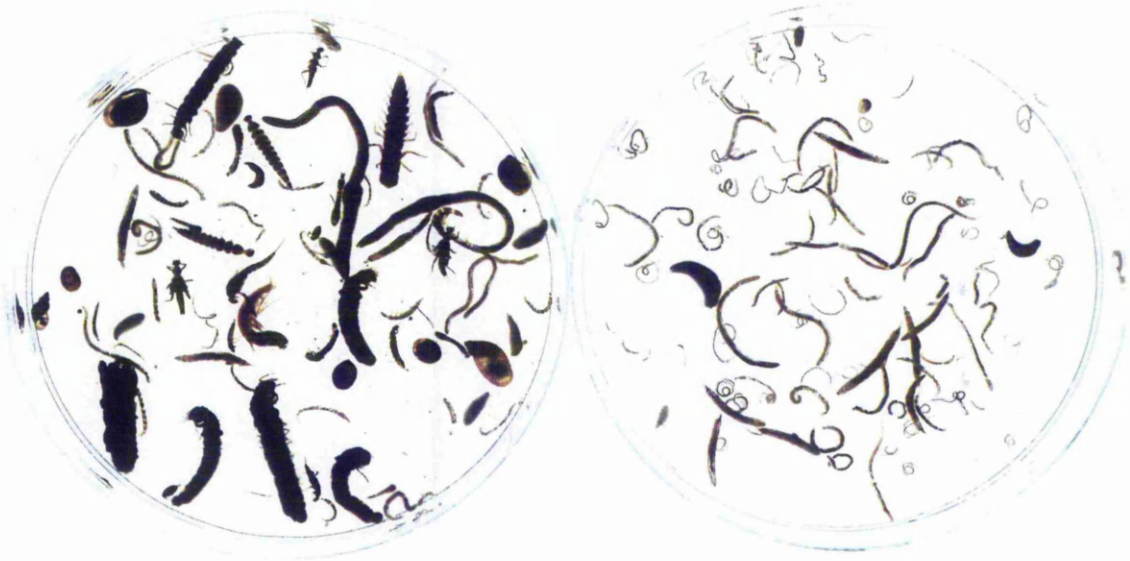
The resulting brownish orange ferric hydroxide may remain suspended in the stream or coat the stream bed as 'ochre'.

The effects of this discharge on the receiving watercourse are well documented, and include the following:

(i) Acidification of the water with subsequent reductions in floral and faunal diversity. Dills and Rogers (1974) used community structure and diversity indices to measure stressed and non-stressed ecosystems in Cane Creek, Alabama. Maltby and Booth (1991) found reduction in aquatic *Hyphomycetes* spp. fungi by mine effluent. These fungi break down leaves for shredders such as the amphipod *Gammarus*, so this has possible knock on effects higher in the food chain. Detailed simulation studies of acid episodes have been carried out (McCahon and Poulton, 1991; Ormerod *et al.*, 1987), and they discuss the immediate drift of members of the families Leuctridae, Baetidae and Ephemerellidae away from pollution inputs in such systems. The gammarid genus is one of the first to disappear from waters undergoing acidification (McCahon and Poulton, 1991).

(ii) Precipitation of iron.

This produces an orange plume, blanketing substrata so killing natural fauna and



A B

Plate 1.1. A sample of bottom fauna from an iron polluted stream, Stagshawbank Burn, upstream (A) and downstream (B) of Halton mine, Northumbria. Notice the low species diversity as a result of pollution downstream of the mine discharge, with a high number of different species upstream.

reducing fish spawning grounds and nurseries (eg. Greenfield and Ireland, 1978). Plate 1.1 illustrates a sample of bottom fauna from an iron polluted stream area, to compare impacted and non-impacted areas. Hynes (1970) considers the presence of an iron hydroxide precipitate in acid streams is as important in the elimination of aquatic species as a reduction in pH. Indeed, many streams affected by the deposition of iron are also acidic, therefore this makes the cause of faunal change sometimes difficult to establish (Scullion and Edwards, 1980). A reduction in ephemeropterans, certain plecopterans, trichopterans, molluscs and crustacea, but an increase in chironomids and oligochaetes, is observed within several studies (Dills and Rogers, 1974; Greenfield and Ireland, 1978; Hildrew *et al.*, 1985; Koryak *et al.*, 1972; Scullion and Edwards, 1980). The ferric hydroxide may disrupt ingestion and interfere with respiratory, feeding and attachment mechanisms, or have indirect effects on plant growth by smothering and reducing light penetration for photosynthesis (Scullion and Edwards, 1980). A detailed review of these effects on specific macroinvertebrate families is presented in section 4.4.3.

(iii) Increasing turbidity of the water.

This may reduce light penetration with a consequent decline in phytoplankton, and deleterious effects on saprobic life (Maltby and Booth, 1991). By inhibiting the growth of bacteria and other organisms which contribute to the self-purification process, organic matter is preserved, so creating serious oxygen depletions (Koryak *et al.*, 1972). Coal dust particles from washeries produce similar effects, particularly influencing substrate microhabitats (Learner *et al.*, 1971).

(iv) A reduction in fish community diversity.

This particularly affects Salmonid species, due to a reduction in food sources, abrasion to gills by precipitated iron, and loss of spawning gravel (Robb, 1994; Dalzell, 1996).

(v) Reduction in potability and amenity of water.

Natural conditions may take a long time to return, with consequent economic effects on sports and industrial extraction activities.

Following mine closure and cessation of pumping, it is not always possible to predict the level to which the water table will rebound and a variety of problems can result. Previously dry areas can become prone to flooding and waterlogging, affecting livestock; land may become unstable so putting roads and buildings at risk from landslides. Enhanced surface emissions of carbon dioxide and methane may occur;

increasing flows in sewers could overload treatment plants, and leaching of metals from landfills previously below the water table may take place (Younger, 1993). Foundations can also be attacked by the sulphate rich mine water (Coalfield Communities Campaign (CCC), 1995). This could have high treatment costs (S. Dumpleton, BGS, pers. comm.). It is considered that the risk to the water bearing Sherwood sandstone aquifer in the East Midlands is low because of the low permeability of the intervening strata (NRA, 1996). Younger (1993) reports more severe problems for the basal Permian sands aquifer in County Durham.

Shallow mines containing pyritic deposits are susceptible to ground water changes, so a cycle of flushing and drying is created, producing a major pollution threat. The Dalquharran mine in Ayrshire is typical in that the concentration of iron in the discharge and the pH fell over time as the pyrite was exhausted (Robb, 1994). As a deep mine is flooded, the oxidising environment is lost, but oxygen in the water may maintain oxidation. Seepage into adjacent ground waters may occur. Rising of mine water to the surface tends not to be a problem in the Nottinghamshire area, as the shafts are plugged after abandonment, and the coal is deep below the Sherwood sandstone (R. Harris, EA, pers. comm.)

1.2.2 Saline Waters

The effluents from the collieries of the English East Midlands are typically saline (with a high chloride content) and neutral in pH. It is thought that rather than originating from solution of the rock salt (Schoeller, 1962; Anderson, 1967), the saline groundwaters are essentially marine in origin, originating from the pores of the limestone during periods of uplift, and diluted by rainfall. The ionic ratios of the groundwater differ from that of seawater, due to reactions between the sea water and sediments over time. Sulphates are reduced in the groundwater (Emery and Rittenburg, 1952) and magnesium and potassium concentrations decrease (Chave, 1960). After mixing with colliery process water, pumping water is discharged into watercourses, so salinity in these watercourses can be unnaturally high. The effluents are neutral as the solution is buffered by large quantities of limestone and magnesium carbonated in the strata. This results in the oxidation of any metal species, such as iron, found in the effluent, which rapidly precipitates on the substratum of the receiving watercourse. The

area includes the counties of Nottinghamshire and Derbyshire, also parts of South Yorkshire, North Leicestershire and Lincolnshire. Bird (1989) and Terrell-Nield (1995) document the effects of coal mine saline discharges, although studies of ponds and streams (Short *et al.*, 1991; Savage 1981) and the saline River Weaver on the Cheshire salt flats from salt mining (Holland, 1976) have also examined the effects of raised salinity on macroinvertebrate communities. In other countries, brine laden wastewater creates saline streams from oil production (or oil extraction) eg. in the USA. Short *et al.* (1991) highlighted how organisms inhabiting brine contaminated benthic systems are confronted by a wide range of salinities along the stream, so community structure conforms to any imposed salinity gradient with a redistribution of fauna according to species-specific differences in ionic and regulatory ability. Glover (1983) indicates that saline discharges cannot be controlled other than by not working in strata liable to yield such waters.

1.2.3 Metallic Waters

Metallic waters have been produced from the dissolution of heavy metals from deposits in metal mines, often resulting in spectacular pollution problems such as that in the Carnon River by the Wheal Jane tin mine when closed in 1991. However, Wheal Jane only discharged contaminated waters following the switching off of the pumps. In general, there is recovery of macroinvertebrate communities from reduced metal pollution discharges as highlighted by North American studies of improved mine wastewater treatment and mine closures (Hoiland *et al.*, 1994; Ellis and Hoover, 1990; Deniseger *et al.*, 1990 and Chadwick *et al.*, 1986). There may be, however, some delay due to poor habitat structure, a lack of an undisturbed headwater source of colonisers, or leaching from tailings.

1.3 Responsibility for Effluents from Abandoned Mines

The most difficult issue surrounding the current climate of mine abandonment is that current legislation fails to place responsibility for the prevention of environmental impacts in the hands of any one organisation. Indeed, this 'vacuum of responsibility' (Younger, 1993) needs to be addressed urgently before a practical methodology for

environmental protection can be pursued. Under the Environment Act, 1995, mine owners are liable for pollution they cause this year (2000). All mines closed before 2000, including the thousands already disused, will have exemption. No responsibility is assigned to abandoned mines that do not revert to the Coal Authority, including all non-coal mines. Where there is significant pollution occurring the Environment Agency will be able to take preventative action using powers under S.161 of the Water Resources Act 1991, but at its own expense. There is also the option of civil action whereby pumping is prevented by seeking an injunction against the mine operator (NRA, 1996). Fortunately, there is much legislation provided by the Town and Country Planning Act (1971) to prevent an abandoned mine becoming an eyesore. However, there is no obvious provision made in legislation to ensure that mine owners are accountable for discharges from abandoned mines.

Discharges into water courses are covered by Section 85 of the 1991 Water Resources Act which, in the traditional 'polluter pays' manner, confers liability to the person who 'causes or knowingly permits any poisonous, noxious or polluting matter into any controlled waters.' However, the clarity of who is responsible for abandoned mines is lost by Section 89 reading 'no pollution offence is committed by reason of permitting water from an abandoned mine to enter controlled water'. Therefore a prosecution can only be made and responsibility delegated if it can be established that the owner of an abandoned mine has 'caused' rather than 'permitted' pollution. It needs to be established when pollution of a watercourse from an abandoned mine is 'permitted' or 'caused' and what exactly an abandoned mine is. Because mines can date back more than three hundred years, land/mine owners have changed, and owners often pump shafts dug by others. Often those with rights to the land now cannot be expected to take positive action to prevent mine water discharge. Ground water rebound can take from months to several years to occur and the ownership of the mine could change during that period (NRA, 1994). Younger (1993), stated lucidly that British Coal has merely 'inherited the unconscious sins of our forefathers'. Perhaps the most useful legal changes would be those which relate to the opening/reopening of mines so that full provision for any pollution is made at the start. This area is controlled by the planning system (NRA, 1994).

In order to allocate responsibility for polluting discharges from abandoned mines, avenues to follow could include prosecutions under certain sections of the

Environmental Protection Act (1990) and Salmon Fisheries and Freshwater Fisheries Act (1975), although it is unclear how they may be applied (NRA, 1994). The newly formed Coal Authority (or where appropriate the Scottish Executive and Welsh Office) has already been identified as the responsible body to co-ordinate the environmentally sound decommissioning of coal fields; for other kinds of mining the Department of the Environment could assume this role (CCC, 1995). This would involve a full research and design programme, using the skills of all bodies connected with the industry, with academic and consultancy input. Mines should also not be closed until satisfactory monitoring and control procedures are established, with easy access to the information. The Environment Act also indicates that mine operators are required to give 6 months notice of an abandonment. The Welsh Development Agency has funded 12 projects across the U.K. (NRA, 1994), such as a Severn-Trent EA project whereby metal rich waters are purified in wetlands (Welsh Affairs Committee, 1992). A strong emphasis on such projects is their potential use elsewhere and that research and monitoring will ensure the spread of information throughout the international community. European Community support is available through the LIFE fund for environmental initiatives (Water Guardian, February/March 1994).

1.4 The Use of Macroinvertebrates in Biomonitoring

For the purpose of this study, benthic macroinvertebrates are taken as those organisms that inhabit the bottom substrates (sediments, debris, logs, macrophytes, filamentous algae etc.) of freshwater habitats, for at least part of their life cycle, and are retained by mesh sizes between 200 to 500 μm (Rosenberg and Resh, 1992). Such benthic macroinvertebrates provide an invaluable tool for the long term monitoring of water quality, and are used by the EA to detect and assess any pollution and measure the effects of river management. As they are sedentary, their populations will respond to sporadic polluting discharges, which may not be collected in a spot sample for a chemical test. Theoretically, a small but permanent change in water quality will alter community structure if it results in a survival advantage of one species over another.
The use of macroinvertebrates is based on three principles (Hellawell, 1986):

(i) Certain species are tolerant of pollution, others are sensitive.

(ii) Species richness is high in clean sites although the number of individuals

may be low.

(iii) Species richness is low in polluted sites, but numbers of individuals may be high.

Minor differences in susceptibilities translate into a competitive disadvantage, decreased resistance to predation and lowered reproductive success for the most sensitive organisms (Metcalfe, 1989).

Macroinvertebrates are widely distributed in freshwater, estuarine and marine environments and respond to a wide range of pollutants, and are relatively easily and cheaply sampled. There is also much general information on macrobenthic invertebrate studies from the marine field (for example, see McManus and Pauly, 1990 and Elliott, 1994). Many macroinvertebrates have life histories longer than six months, so providing an overview of prevailing, not just immediate conditions. 'r' and 'K' strategists exist, which describes lifestyles and can be used to evaluate the success of a species in polluted conditions. 'r' strategists have a short life history, early maturity, many small young, and large reproductive effort. They experience population explosions in suitable conditions (eg. for *Gammarus tigrinus* in saline pollution – Section 2.2.2). 'K' strategist species have a longer life, late maturity, few large young, a large size, and smaller reproductive effort. This is characteristic of eg. Stoneflies in clean freshwater conditions.

A good range of keys is available for help with identification of macrofauna, and there are numerous scoring systems to assign a comparable score to each site, such as the widely used Biological Monitoring Working Party (BMWP) system (Chesters, 1980). With the latter, a numerical value is assigned to each invertebrate taxon, based on its tolerance to organic pollution; the higher the BMWP score, the greater the water quality. Average Score per Taxon (ASPT) can be calculated from the BMWP score divided by the number of taxa which the sample contains. This average score indicates the sensitivity of each family to pollution. Armitage *et al.* (1983) indicated the usefulness of ASPT in that it is less sensitive than BMWP to seasonal differences and sampling effort. Caution must be applied however in the interpretation of such biological surveys as seasonal (ie. emergence of insects), substratum and physiogeographic parameters (including altitude and flow rate) can override the effects of pollution on species diversity and community composition. Indeed, low diversity is not necessarily a sign of pollution. Drift behaviour may carry individuals into a

sampling area in which they do not normally occur (Hellawell, 1977). Rosenberg and Resh (1992) indicated that the presence of many keys may indicate a dissatisfaction amongst authors in the identification to species level, particularly for chironomids, oligochaetes and some trichoptera. There is the additional problem of the use of families in calculating diversity scores. For example, the family gammaridae contains species which are tolerant to saline pollution (eg. *Gammurus tigrinus*, *G. duebeni*, *G. zaddachi*), but also species that are intolerant (eg. *G. pulex*). This presents a problem in the identification of taxa to family level for use in calculating diversity scores.

The Shannon-Wiener Diversity Index (Zand, 1976) additionally takes into account the numbers of organisms of each species present in a given sample, to calculate an index of species diversity. The index relates to the uncertainty that exists regarding the species of an individual selected at random from a population. The greater the number of species present and/or the more evenly the individuals are distributed among the species, the greater the uncertainty in selection and hence the greater the diversity value (Dills and Rogers, 1974). Calculated from this, the value for equitability (evenness) indicates how closely the community in question approaches the maximum possible diversity, hence the effects of pollution are assumed to reduce this value. Wilhm (1970) reported, after sampling various habitats, that clean water streams generally have a Shannon-Wiener diversity score (calculated with log to base 2) of 3-4, whilst polluted streams will tend to score less than 1. Community structure provides a useful tool by identifying where ecosystem condition is poor and where investigative efforts should be focused (Metcalfe, 1989). Chemical data provide a cost effective back up to biological data, to provide details of the nature of any pollutant present. The principles of both biological and chemical sampling are the basis of the methods used in this thesis.

1.5 The Use of Models in Ecology

A model is a representation of the structure or function of a real system, and may be either conceptual (words or diagrams) or mathematical (equation based). Such models can never totally represent the real system, as the complexities of living systems mean that regardless of the information we have about them, we always remain outside, looking in, and the system remains a 'black box'.

1.5.1 Conceptual Models

Ecological systems inherently involve multiple, non-linear interactions. The multi-component nature of freshwater systems is illustrated in a conceptual model (Figure 1.2).

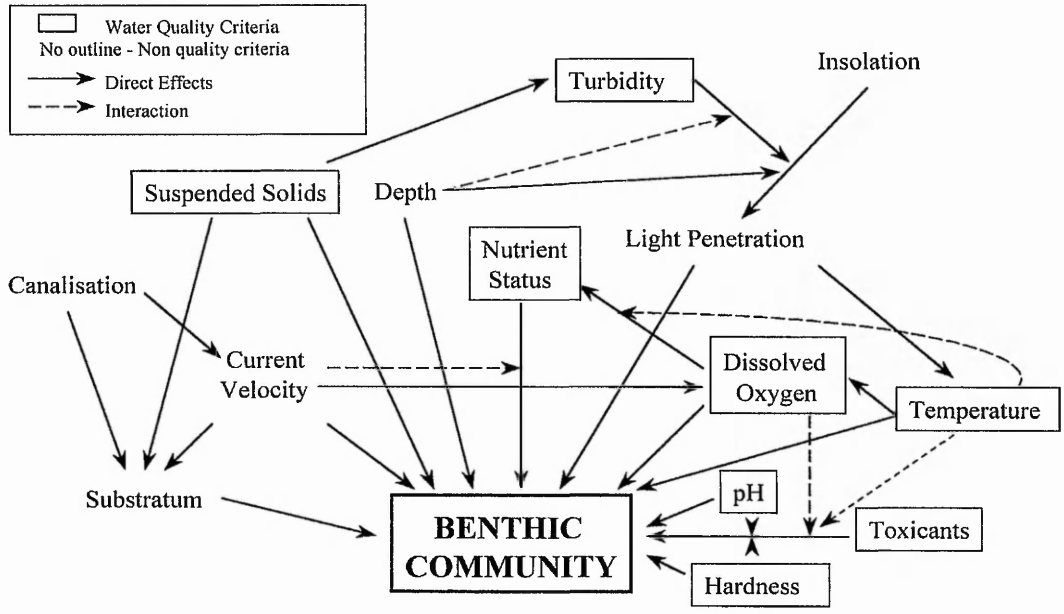


Figure 1.2. Water Quality and Non-Water Quality Determinants of Benthic Communities in Rivers.

The interactions between chemical, physical and biological components in such simple models allow basic decisions to be made by authorities who wish to manipulate water environments to mitigate polluting discharges, such as using wetlands/lagoons to treat mine drainage.

1.5.2 Mathematical Models

Conceptual models, however, often lack rigour, and can be interpreted in different ways by different people. Mathematical models can be evaluated and validated by using data from the real system.

However, such models are only as accurate as the data used to establish the

model, and are only as good as the underlying knowledge of the system. A mathematical model of a biological system may be as simple as a single equation relating one variable to another, or it may be a multi-component model involving the interactions of many equations having several mutually dependent variables (Keen and Spain, 1992). Such mathematical models of freshwater systems are ideal tools for the water industry, as they can be used to simulate systems which would be impossible to investigate experimentally because of the amount of time and cost involved (Keen and Spain, 1992). For example, the effects on the biological diversity of a receiving watercourse by using wetland systems to 'clean up' ferruginous pollutants can be examined in minutes using a computer model, instead of taking time and resources in the field.

1.5.3 Univariate and Multivariate Analysis

The simplest mathematical model is a linear regression equation. This type of analysis fits a linear equation to the variable to be predicted (dependent variable) from the predicting variables (the independents). However, ecological systems involve many interacting variables, and the processes are often non-linear. A multivariate approach is therefore needed. One method, if one independent variable (such as a biological diversity index score) is to be predicted, is multiple regression analysis. This method produces an equation relating the dependent variable to all of the independents. Studies have used multiple regression techniques to produce predictive equations for biological diversity scores, in unpolluted British rivers. Wright *et al.* (1983) predicted species lists, the Average score per Taxon (ASPT) and BMWP diversity scores from physical and chemical data, and this methodology was used by Wright *et al.* (1989) to model ASPT scores for hypothetical rivers, in lowland and highland environments. Another method used to analyse complex multivariate data, particularly in ecology where species and environmental variables are to be correlated, is Two-way Indicator Species Analysis (TWINSPAN) followed by Detrended Correspondence Analysis (DECORANA). These techniques are discussed further in Chapter 3 and 4.

1.5.4 Artificial Neural Networks and the Group Method of Data Handling.

Artificial neural networks (ANNs) and Group Method of Data Handling (GMDH) present a new computing tool for model generation, and are now receiving much interest in many scientific, industrial and technological disciplines. They are very useful for the interpretation of non-linear trends in data, as they can 'learn' the patterns present in the data, and then predict upon new 'unseen' data. The non-linear nature of the factors controlling the distributions of freshwater macrofauna prompted an examination of the possible uses of these novel techniques to the field of freshwater ecological management. The study is presented in chapter 5.

1.5.5 The Role of Modelling in Freshwater Environments

For a number of years, the increase in concern about the quality of aquatic ecosystems has forced legislating bodies to address important ecological problems (Lek *et al.*, 1996). Models which can predict changes in biological diversity with changes in pollution levels, have beneficial uses within those industries safeguarding running water environments. The RIVPAC System (River Invertebrate Prediction and Classification System) has been developed by the Institute of Freshwater Ecology to predict biotic scores and species lists for unpolluted rivers throughout the UK. RIVPACs was developed in 11 years over 3 phases, incorporating 268, 370 then 438 sites from 41, 60 and 80 unpolluted river systems respectively (Wright *et al.*, 1984; Furse *et al.*, 1984; Moss *et al.*, 1987; Wright *et al.*, 1989). The models used in RIVPACs have a high accuracy of prediction, more than adequate for freshwater management. Moss *et al.* (1987) successfully predicted 75% of sites to the correct species group.
The RIVPACs system was developed with anticipation of the following uses:

(i) To aid in predicting biological responses to environmental change, whereby existing and projected values of environmental parameters could be used to predict the change in species expected (including BMWP/ASPT), also providing a list of those species most at risk. Any loss of biological quality due to environmental stress can be measured by the 'observed/expected' (Environmental Quality Index) ratio, and the nature of the perturbation at a site may also be indicated by the taxa that are absent (Moss *et al.*,

1987). Target macroinvertebrate community lists for individual sites could be provided (Furse *et al.*, 1984). The accuracy of prediction in RIVPACS is high (Armitage *et al.*, 1987), making the system extremely valuable for management purposes.

(ii) To address the problem of biotic scores such as BMWP reflecting regional differences due to local geological, geographic and physiographic features (Chave, 1990).

(iii) To identify key sites as example ecosystems for conservation (Furse *et al.*, 1984).

(iv) To provide water authorities with an inventory of site types, along with their relative abundance, for use when attempting to fulfil their statutory obligations with regard to conservation (Furse *et al.*, 1984).

(v) Useful applications exist in interpreting pollution surveillance programmes (Furse *et al.*, 1984).

(vi) Basic questions about the structure and functioning of benthic communities can be answered.

1.6 Aims

The objectives of this research are as follows:

1. Biological, chemical and physical data collected from the River Idle catchment, including intensive sampling of Millwood Brook, are used to investigate the changes that have taken place in species distribution since the study of Bird (1989), during which time 12 mines have been abandoned in the catchment. This study includes qualitative and quantitative analysis of macrofauna.

2. The status and distribution of *G. pulex* and *G. tigrinus* populations in the River Idle catchment are examined, the aim being to predict the future of *G. tigrinus* by establishing the factors underlying the success of the species in colonising Millwood Brook.

Furse *et al.* (1984) encouraged future studies of chemically or physically perturbed sites to test the usefulness of modelling techniques used in the design of RIVPACS, which was developed using only unperturbed sites nationwide. To date, no such studies have taken place. Wright *et al.* (1984) also highlight that in order to be of

practical value, the RIVPACs scheme cannot be restricted to sites thought to be uninfluenced by man, particularly relevant to lowland Britain. In light of this, this study also aims:

1. To create mathematical models to compare and contrast the effects of coal mine pollution on macroinvertebrate distributions in Yorkshire, Northumbria and Wales. This will provide valuable information towards the management of abandoned coal mines in these areas and will allow the assessment of the effects of ferruginous discharges on the receiving watercourses.
2. To use TWINSPAN groupings and indicator families to suggest a national index of family sensitivity to coal mine pollution, to replace the organic pollution-based BMWP scoring system.
3. To compare traditional modelling techniques (namely multiple regression) with the novel approach of the Group Method of Data Handling and artificial neural networks, to assess potential uses to the management of freshwater communities.

Chapter 2. Invertebrate, Chemical and Physical Surveys of Millwood Brook and the River Idle Catchment.

2.1 Introduction. Coal Mining in the East Midlands

2.1.1 Geological and Groundwater Features

The East Midlands coalfield covers an area of 6216 square kilometres, the coal measures reaching a maximum depth of 1667 metres. The coal measures are exposed (shallow) in the west, and concealed (deep) in the east, buried under increasingly thicker layers of Permo-Triassic and Jurassic rock (Ministry of Fuel and Power, 1945). Salient features of the geology of the area include the layer of Permian limestone, with a composition of 44% magnesium carbonate, and beds of gypsum, which overlay the carboniferous coal measures. Gypsum is commercially quarried in Nottinghamshire. Triassic sandstones, covering two thirds of the coalfield overlay this and although beds of rock salt up to 6 metres thick can be found in the limestones (Downing and Howitt, 1969).

2.1.2 Historical Aspects

Coal mining in the North Nottinghamshire and South Derbyshire areas dates from the Middle Ages, with shallow mining of the exposed coalfields. There was a move from the exhausted exposed coalfields from the mid nineteenth century onwards to the concealed coalfields of the East in the Leen Valley. The current status of collieries in the Idle catchment can be found in Table 2.1.

Table 2.1. Colliery Status in the River Idle Catchments (September, 1995)

Number	Colliery	Status	Discharge Details [2] (Number of Discharges) [3]
1	Bilsthorpe	Operating	Rainworth Water (2)
2	Clipstone	Operating	Vicar Water (R. Maun) (3)
3	Thoresby	Operating	R. Maun (1)
4	Welbeck	Operating	R. Meden (2)
5	Pleasley	Abandoned 1985	Pleasley Brook (R. Meden) (2)
6	Whitwell	Abandoned 1986	Whitwell Brook (Millwood Brook) (3)
7	Mansfield	Abandoned 1988	Vicar Water (R. Maun) (2)
8	Sutton	Abandoned 1989	Skegby Brook (R.Meden) (2)
9	Warsop	Abandoned 1989	R. Meden (1)
10	Langwith [1]	Abandoned 1989	R. Poulter (2) 0.35 Ml D^{-1}
11	Sherwood	Abandoned 1992	R. Maun (1)
12	Creswell [1]	Abandoned 1992	Millwood Brook (R.Poulter) (2) 1.88 Ml D^{-1}
13	Silverhill	Abandoned 1992	Teversal Brook (R. Meden) (2)
14	Shirebrook	Abandoned 1993	Sookholme Bath (R. Meden) (4)
15	Rufford	Abandoned 1993	Rainworth Water (4)
16	Bevercotes	Abandoned 1993	R. Meden/Maun (6)
17	Ollerton	Abandoned 1994	Bevercotes Beck (R. Idle) (3)

Notes. [1] Pumping stations to protect operating pits 1 - 4. [2] Figures for discharge are given as mega litres per day.[3] Number indicates discharges with consent from EA. Data supplied by EA / Steve Dumpleton at the British Geological Survey / Nottingham County Council (1992a,b).

By 1945, there were 92 collieries in Nottinghamshire and Derbyshire, 26 in the older exposed area and 66 in the newer, concealed coalfield (Griffin, 1971). As pits were sunk deeper into the water table, so the removal of water became more of a problem. Saline waters were reached which were removed by pumping to the surface and then piped into lagoons and drainage channels, often into small receiving streams.

2.1.3 Creswell Colliery

The Millwood Brook at Creswell Crags is typical of this system (See Figures 2.1 and 2.2). The Crags is a Site of Special Scientific Interest (SSSI) partly due to a history of limestone cave dwellers. Opened in 1895 by the Bolsover Colliery Company, Creswell colliery pumped saline effluents into Millwood Brook for 98 years (Plate 2.1A). The effluent had a pH above 6.5, a salinity approximately equal to that of seawater and a temperature of 20°C due to the depth from which the groundwaters originated (see Bird, 1989; Terrell-Nield, 1995). The mine closed in 1992 and the pumping water ceased in spring 1993 (Plate 2.1B). Creswell has provided the perfect case study for coal mine pollution in the River Idle catchment, due to the large output of saline water in relation to total flow, so the effects of fluctuating discharge and cessation of output on the benthic macroinvertebrates can be studied. A run off stream from the spoil heaps also enters Millwood Brook (Left Hand Stream).

2.1.3.1 Site Descriptions - Millwood Brook/River Poulter.

The 24 sample sites on Millwood Brook were numbered 1 to 19 on Millwood Brook and A to E for sites on other streams and rivers, as for Bird (1989). These sites are illustrated in Figures 2.1 and 2.2, with the main physical characteristics of each site in Table 2.2.

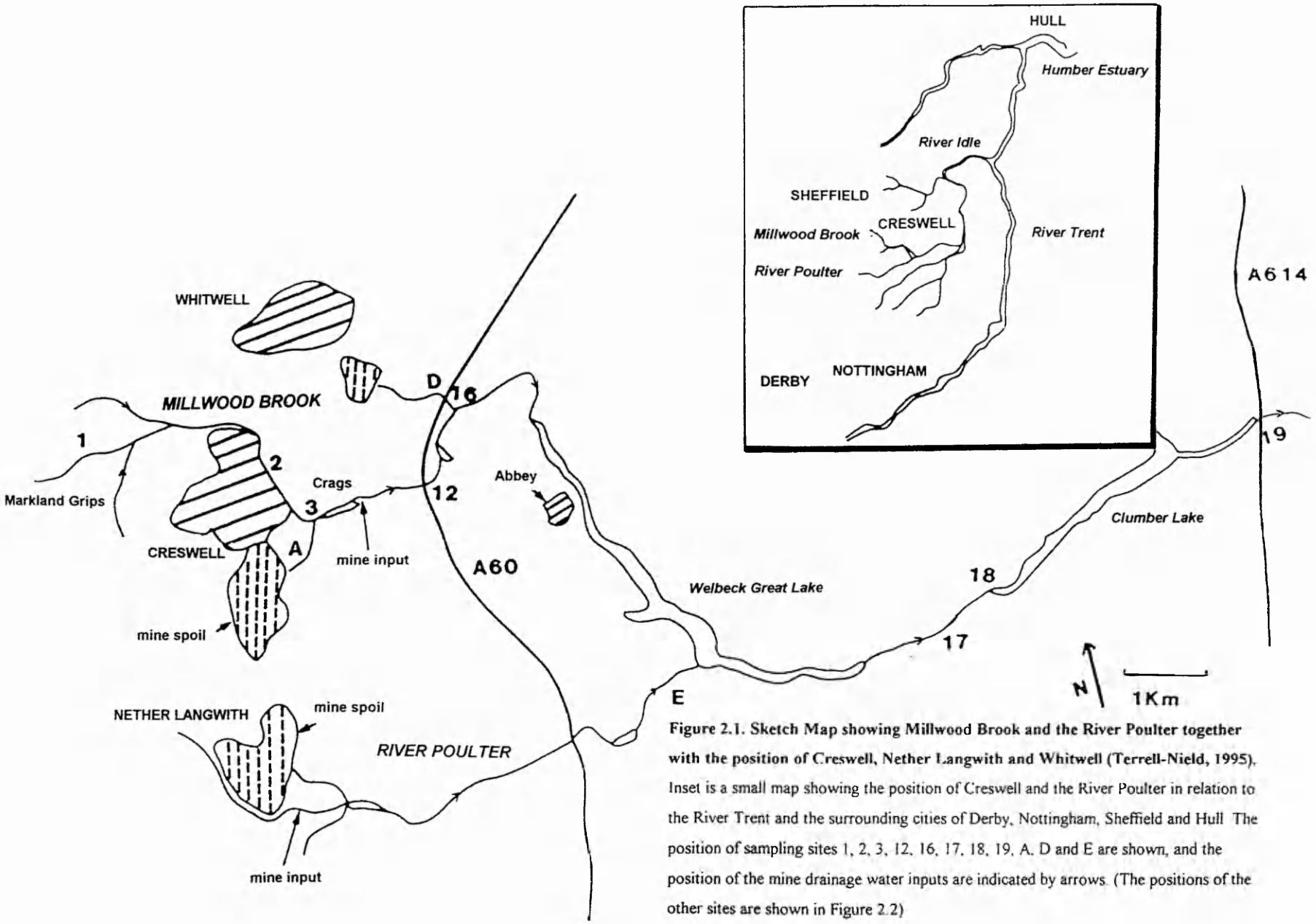


Figure 2.1. Sketch Map showing Millwood Brook and the River Poulter together with the position of Creswell, Nether Langwith and Whitwell (Terrell-Nield, 1995). Inset is a small map showing the position of Creswell and the River Poulter in relation to the River Trent and the surrounding cities of Derby, Nottingham, Sheffield and Hull. The position of sampling sites 1, 2, 3, 12, 16, 17, 18, 19, A, D and E are shown, and the position of the mine drainage water inputs are indicated by arrows. (The positions of the other sites are shown in Figure 2.2)

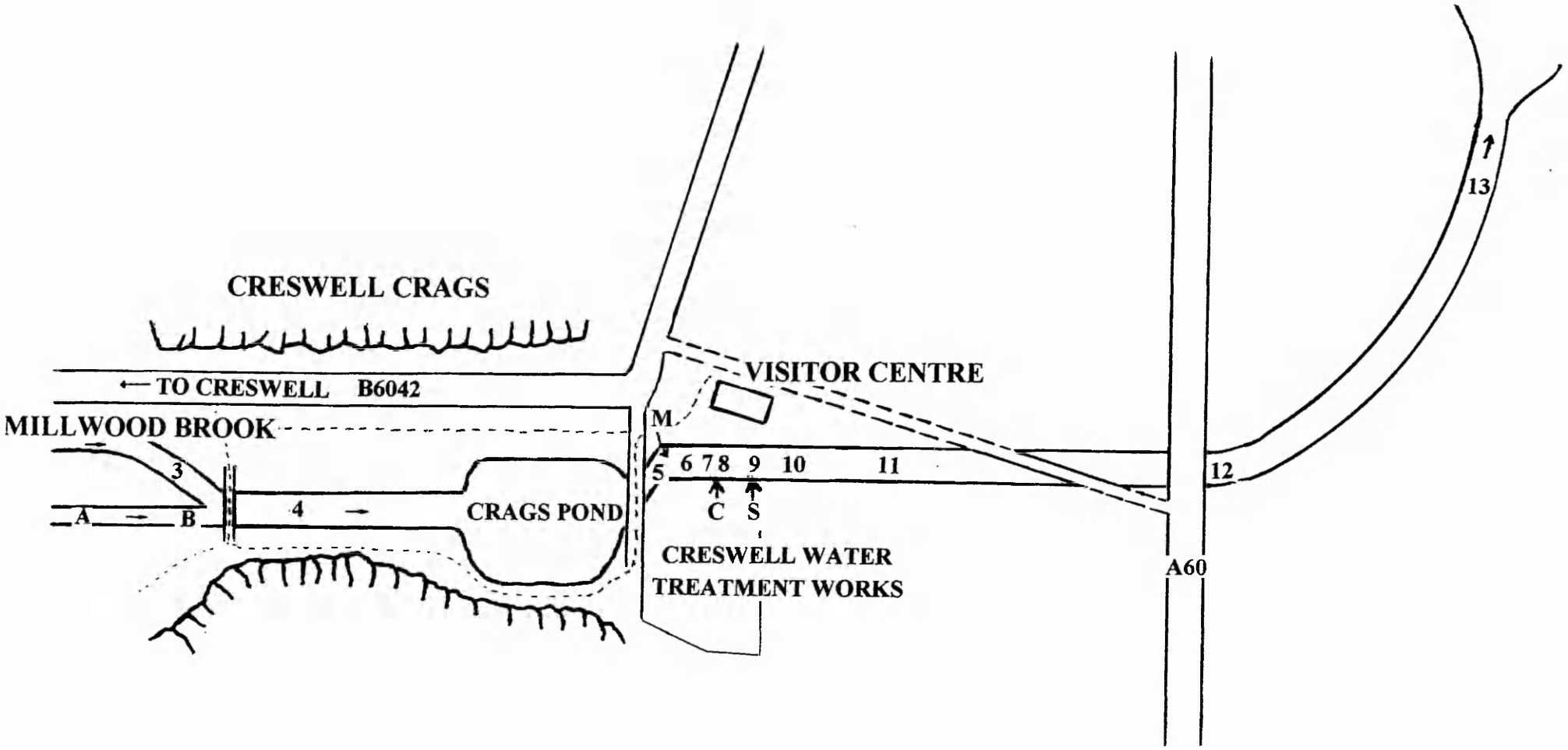


Figure 2.2. Map showing Millwood Brook in the area of Creswell Crags (Terrell-Nield, 1995). Positions of sampling sites are shown. Arrows show direction of flow. Position of inputs of Mine Pumping water (M), water from Crags Pond (C), and sewage effluent (S) are indicated by letter.

A.

B.

Plate 2.1. The Discharge Site of Creswell Colliery into Millwood Brook before (A) and after (B) mine closure in 1992. This is between sites 5 and 6.

Table 2.2. Grid reference, mean width, depth, flow rate and substratum type for the Millwood Brook / River Poulter sites, as measured during winter 1994 to summer 1995. Refer to Figures 2.1 and 2.2 for site positions. 3 samples per site. Results for Bird (1989) shown in brackets. Codes for substrates: 1=Silt, 2=Sand/Clay, 3=Gravel, 4=Pebbles/Cobbles, 5=Large Stones/Boulders.

Site Code	Site	Grid Reference	Mean Width (m)	Mean Depth (cm)	Mean Flow ($m\ s^{-1}$)	Substrate Type
1	Markland Grips	SK503747	0.95 (1.65)	44 (13)	0.29 (0.142)	Sand/Clay (2)
2	Lower Mill Farm	SK526746	1.87 (2.4)	18 (15)	0.35 (0.895)	Large Stones (5)
3	Right Hand Stream	SK534742	2.12 (2.2)	13 (18)	0.71 (0.895)	Large Stones (5)
A	Cattle Bridge	SK532738	1.73 (1.8)	3 (37)	0.07 (0.407)	Silt (1)
B	Left Hand Stream	SK533740	2.02 (3.2)	21 (44)	0.27 (0.407)	Silt (1)
4	Boulder Site	SK534742	2.89 (3.3)	31 (20)	0.31 (0.552)	Sand (5)
5	-10m	SK537743	2.32 (4)	13 (7)	0.3 (1.57)	Large Stone/Rock (5)
6	+5m	SK537743	3.73 (3)	13 (7)	0.13 (0.71)	Large Stone/Rock (5)
7	+50m	SK537743	2.88 (4)	10 (25)	0.22 (0.506)	Gravel (3)
C	+75m	SK537743	3.43 (3.8)	13 (24)	0.44 (0.646)	Gravel (3)
8	+80m	SK537743	3.28 (3.95)	15 (18)	0.25 (0.728)	Gravel (3)
9	+130m	SK538743	3.75 (3.5)	27 (17)	0.08 (0.515)	Silt (1)
10	+250m	SK538743	3.75 (3.2)	37 (25)	0.08 (0.515)	Silt (1)
11	+450m	SK543745	3.23 (2.8)	26 (29)	0.23 (1.127)	Silt (3.5)
12	A60	SK546745	2.78 (3)	19 (27)	0.3 (1.143)	Sand/Pebbles (3)
13	Below A60	SK548749	3.93 (3.5)	18 (22)	0.33 (0.562)	Sand/Pebbles (1.5)
14	After Lake	SK547751	5.25 (4)	34 (115)	0.08 (0.514)	Silt (1)
15	After Bridge	SK549755	4.77 (5)	11 (45)	0.47 (0.681)	Sand/Pebbles (1.5)
D	Whitwell Stream	SK548755	1.53 (1.1)	10 (11)	0.83 (0.334)	Gravel (3)
16	Farm	SK549756	3.76 (3.5)	14 (24)	0.41 (0.567)	Sand/Pebbles (3.5)
E	Norton	SK574719	3.78 (6.9)	32 (66)	0.33 (1.08)	Silt (1)
17	Site B6034	SK606727	6.41 (6)	62 (50)	0.18 (1.63)	Sand/Pebbles (2.5)
18	Carburton	SK616734	11.25 (10.4)	40 (57)	0.22 (0.81)	Pebbles/Silt (2.5)
19	A614 Poulter	SK649757	8.65 (10.8)	24 (28)	0.4 (3.38)	Pebble/Gravel (3)

Rising in a limestone gorge (Markland Grips, site 1), the brook forms a rocky bed (site 2) supporting growths of the moss *Fontinalis* spp. and eventually flows into the limestone valley (site 3) of Creswell Crags. Just downstream of this site the brook is joined by a stream of mine spoil tip run-off from Creswell mine. Sites A and B were situated on this stream. Millwood Brook flows in a silty channel through Creswell Crags (site 4) and into Crags Pond. The water from this pond flows over a concrete spillway into a rocky channel; site 5 is situated 10 metres above the site of previous mine discharge, sites 6, 7 and 8 are situated 5, 50 and 80m respectively below this discharge point. 75m below the mine water input is an outflow of water from Crags Pond which has been ducted through the water treatment works (site C). The brook also receives intermittent untreated sewage after sudden heavy rain, directly opposite the mine discharge pipe. 130m downstream from the mine discharge point, the brook receives effluent from the water treatment works (1-5 Ml day $^{-1}$ of treated sewage, with a Biochemical Oxygen Demand of 10-15 mgl $^{-1}$ 0_2, Severn-Trent Water, 1988a). Here, at sites 9 and 10, the sediment becomes black, glutinous and anaerobic. The brook recovers somewhat from this discharge by site 11.

The watercourse then passes under the A60 road and enters Welbeck Estate (site 12) where it enters a small lake (site 13 is situated above this). Downstream the sediment becomes silty and deep (site 14), joined by a swift stream after site 15, from the coal tips at Whitwell (input site D), which also receives discharges from Steetly Minerals and a small sewage works. Site 16 is situated below this. The brook flows across Welbeck Estate, joining the River Poulter (input site E at Norton), flowing into a series of managed lakes. Site 17 was situated just outside Welbeck Estate, and site 18 at the entrance to Clumber estate. Site 19 is located after Clumber Park, and has a broad, shallow, pebbly bed.

Millwood Brook, along its 14km length from its source at Markland Grips to its exit from Welbeck Great Lake where it joins the Poulter (see Figure 2.1), has consent for an additional 27 discharges. Of these, 16 are from treatment works, 10 from industry (eg. effluents from limestone quarries, Creswell and Whitwell collieries), and one from farm drainage.

2.2 Studies of Gammarids (including the River Idle Catchment)

2.2.1 *Gammarus pulex* (L.) (Crustacea: Amphipoda)

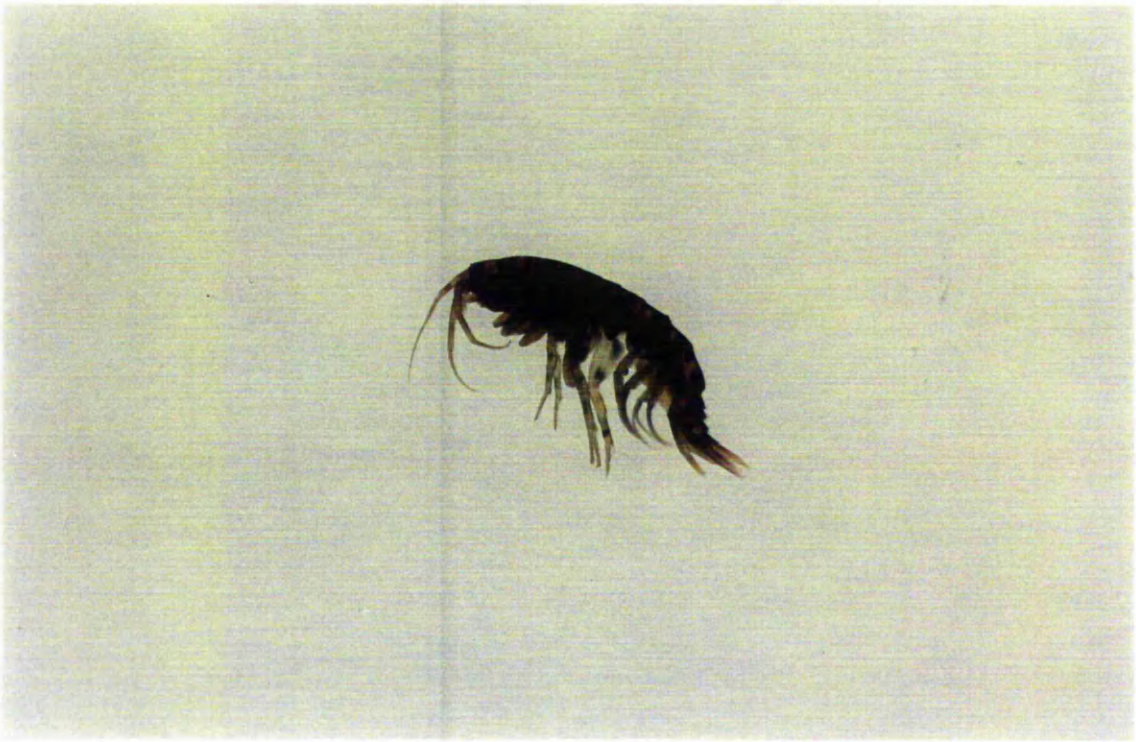
Gammarus pulex (L.) (Crustacea: Amphipoda) is a freshwater amphipod crustacean (Plate 2.2A). It lives under stones in shallow water, affording protection from the current. It is regarded as one of the most important invertebrate species in chalk streams in terms of biomass and food for fish and one of 5 species of *Gammarus* found in Britain. The other species are *G. lacustris* Sars, the brackish water/saline species *G. duebeni*, *G. tigrinus* Sexton and *G. zaddachi* (Hynes, 1955). Larger individuals of *Gammarus pulex* associate predominantly with large sized substrata, juveniles with weed (Graca *et al.*, 1994). It is found normally in highly oxygenated waters in the headwaters of streams (Welton, 1979). The life history of *G. pulex* in Britain is well documented (eg. Welton, 1979; Sutcliffe *et al.*, 1981; Graca *et al.*, 1994). *G. pulex* is often found parasitised by the acanthocephalan parasite *Pomphorynchus laevis* (L.) which has been shown to increase its drift (McCahon *et al.*, 1991). As a detrital shredder, the leaves of deciduous trees form a major part of its diet (Sutcliffe, 1981). Fungi are one important factor in the modification of this leaf material (Graca *et al.*, 1993). *G. pulex* feeds on this fungi-leaf complex by secretion of enzymes active on cellulose and fungal cell walls (Chamier and Willoughby, 1986). The effluents from a disused mine in the River Don, Sheffield, have been implicated in reducing the diversity of fungal assemblage below the discharge, with subsequent deleterious effects on Crustacea populations (Maltby and Booth, 1991).

2.2.2 Invasions of *Gammarus tigrinus* (Sexton, 1939)

The different species of Gammaridae have restricted distributions in relation to salinity gradients. Salinity tolerance is of primary importance in determining the distribution and success of each species, but other factors may be important (Savage, 1982), such as interspecific competition (Hynes, 1955). The work of Dorgelo (1974) and Ruoff (1968), has demonstrated that whilst *G. pulex* can tolerate little salt, *G. tigrinus* (Sexton, 1939), illustrated in Plate 2.2B, can extend its range from freshwater to seawater, so is a true euryhaline species, although it prefers oligohaline waters (Dorgelo, 1974) and therefore demands a less specialised habitat (Nijssen and Stock, 1965).

Plate 2.2. Gammarids Examined in the Present Study.

A. *Gammarus pulex* (L.) Length (mm)



B. *Gammarus tigrinus* (Sexton, 1939) Length (mm)

Morphological differences are apparent between *G. pulex* and *G. tigrinus* and these are used in identification. Stripes are apparent on *G. tigrinus*, absent in *G. pulex*. Whereas *G. pulex* has oval shaped eyes, *G. tigrinus* eyes are more kidney shaped. Peduncle segments four and five of male *Gammarus pulex* normally have groups of short setae, whose lengths are less than the distances between groups, implanted in three longitudinal rows (Pinkster, 1972). The second antennule and peraeopods in the male *G tigrinus* have numerous fine curled setae and the second peduncle segment on the antennule has 2-4 equally strong median ventral groups of setae (Bousfield, 1958).

Few studies have been conducted into the British *G. tigrinus* populations, first documented in Droitwich in 1931 (Sexton, 1939). Further investigations took place in the saline Cheshire meres (Savage, 1982) and in drainage dykes below high tide level (Hynes, 1954). Hynes (1955) noted that all *G. tigrinus* sites appeared to be saline, muddy/weedy and more pond like than most Gammarid habitats. Nijssen and Stock (1965) report its ability to colonise organically polluted waters, withstanding pollution better than other Gammarid species, and the species has the ability to colonise empty habitats. The species invasion of Europe is well documented, a discussion of which follows.

2.2.2.1 Britain

G. tigrinus was introduced into Britain from the Liverpool docks, from where it has spread into the Trent and Mersey Canal, possibly in the rope fenders of boats or on bird's feet, around the Cheshire salt fields. It is now common in certain coastal brackish waters and in freshwater where pollution has raised the ion content, in the English East Midlands and Northern Ireland. *G. tigrinus* is native to the North American Atlantic coast where it lives in the upper portions of the estuaries. Hynes (1955) supposes that this English population was transported in bilge water or ballast tanks; it is significant that it occurs in the great ports of Bristol and Liverpool. It would be informative to identify if it is present in the saline marshes by the Humber and Thames estuaries. It has now been present in the River Idle catchment for nearly 25 years (Severn -Trent Water Authority, 1988b). The high conductivity of the mine drainage waters in the catchment is the greatest influence in allowing *G. tigrinus* to outcompete *G. pulex* to which such salinity is deleterious. Bird (1989) demonstrated the restriction of *G. pulex* to the headwaters of streams above coal mine inputs where the conductivity was low (<1 mS cm^{-1}). Below mine inputs with higher conductivity

(>2 mS cm^{-1}), *G. tigrinus* predominated. No marked changes in *G. tigrinus* distribution were found by Everett (1991). Strange and Glass (1979) documented the spread of *G. tigrinus* from Lough Neagh, and now dominates L. Erne in Northern Ireland. L. Erne was stocked with fish in 1968, and *G. tigrinus* could have been introduced from the stock ponds. The presence of canals enables new catchments to be rapidly colonised, emphasised by the location of the majority of *G. pulex* sites in Northern Ireland being in river systems associated with a canal (Strange and Glass, 1979).

2.2.2.2 Germany

In 1959, *G. tigrinus* was deliberately introduced, as a food source for fish, into the Rivers Weser and Werra which have a high ion content due to heavy pollution (Schmitz, 1960). It has been spreading there since introduction.

2.2.2.3 The Netherlands

Similar spreading of *G. tigrinus* has occurred since 1964 in the Netherlands, with Dutch fishermen of Ysellake ('Ijsselmeer') noticing damage to nets by the massive levels of the shrimp only a year later (Nijssen and Stock, 1965). Despite of competition with local species, including *G. pulex* and *G. duebeni*, *G. tigrinus* pushed its way forward in the Dutch inland waters over a distance of 30-40 km in a year (Pinkster and Stock, 1967). Since then, the range extension of this species into nearly all oligohaline waters in the Netherlands has been documented (Dennert *et al.*, 1968; Gras, 1971; Smit, 1974). Smit (1974) reported the replacement of all other species of Gammarid by *G. tigrinus*, where the species colonised. In 1973 a more or less stable situation seemed to have been established, in which the range of *G. tigrinus* was bounded by running freshwaters in the eastern and southern parts of the country, and by the meso to polyhaline waters in coastal areas (Pinkster *et al.*, 1977). *G. tigrinus* coexists with *G. pulex* where salinity is lowered by the inflow of several streams (Nijssen and Stock, 1965). A second invasion of *G. tigrinus*, from Germany, was shown by electrophoretic tests (Pinkster *et al.*, 1992).

2.2.2.4 Natural habitats of *G. tigrinus* in the North American Atlantic Coast

The genus *Gammarus* is one of the most abundant macroinvertebrates in estuaries of the east coast of North America (Bousfield, 1969). It is pertinent for this

study to examine in detail the natural habitat of *G. tigrinus* on the North American Atlantic coast. This was conducted on a visit during July 1996 to the South Carolina Natural Resource Institute, Charleston, South Carolina and site visits to the River Cooper and River Edisto, where the organism is present.

According to Hynes (1955), *G. tigrinus* seems adapted to a climate with warm summers and springs characterised by a rapid rise in temperature. Such marine climatic conditions prevail along the coastal plain of South Carolina, on the Cooper River estuary a rapid rise in temperature takes place in early spring, while the water temperature attains rather high values during summer. It is common to waters of larger streams with sandy substratum, as found in the Cooper River and is normally found in the more upstream parts of estuaries, with a salinity range of 0-22 parts per thousand (Van Maren, 1978). It is replaced downstream in higher salinities by *Gammarus palustris* (Bousfield, 1969); at very low salinities by *Gammarus fasciatus* (Say).

Niche segregation operates between species, and Ristich *et al.* (1977) conclude that salinity is the most important factor controlling species range and community boundaries, whilst body size is the main adaptation allowing *Gammarus* species to occupy their extensive geographic range from the North pole to the Gulf of Mexico. Temperature also determines the geographical distribution of species as water temperature dictates the spawning times of species. Northern larger species (eg. *G. wilkitzkii* and *G. setsus*) have a single, large well timed brood release within a short period of optimum conditions (Steele and Steele, 1972a). Medium sized species (eg. *G. duebeni* and *G. oceanicus*) have several medium sized broods spread over a longer optimum season. *G. tigrinus* and *G. lawrencianus* females have a large number of small broods and mature second generations in the much warmer and longer optimum season characteristic of the southern environment. Steele and Steele (1972b) also identify that *G. tigrinus* is more successful in the Southern parts of the USA, with more generations per year due to higher temperatures than the northern states. The young can reproduce in the same season because of the higher environmental temperatures and their smaller size at maturity as compared to other Gammarids. Van Maren (1978) also details the colonisation of *G. tigrinus* in widely different salinities in North Carolina, and its tolerance to high water temperatures (up to 34°C). It has been confused with *G. daiberi* (D. Knott, South Carolina Department of Natural Resources, pers. comm.) and has apparently been introduced during the last 15 years

to San Francisco Bay on the West Coast of North America, as a 'bilge water tramp' species, perhaps in a similar fashion to *G. tigrinus* in Europe (E. L. Bousfield, pers. comm.).

2.2.3 Competitive features of *G. tigrinus*

Bird (1989) found that the chloride tolerance of *G. tigrinus* is 20-100 mM l^{-1} as compared to 0.8-1.1 for *G. pulex* in the saline polluted Millwood Brook. However, to maintain itself in a certain habitat, a species must not only survive but also has to reproduce (Pinkster *et al.*, 1977). The rapidity in reaching sexual maturity together with the short incubation period can possibly explain the success of *G. tigrinus* in its competition with indigineous species such as *G. pulex* (Pinkster *et al.*, 1977) (see Table 2.3). Since *G. tigrinus* is such a fast growing and breeding species, it is able to produce up to 16 generations in one season (Pinkster *et al.*, 1977), so out-competing *G. pulex* for food and habitat resources. Indeed, Bird (1989) found *Gammarus tigrinus* to be more r-selected than *Gammarus pulex*, having a life history characterised by many early maturing young, with a large reproductive effort and short life.

Table 2.3. Comparison of the reproductive capacities of *G. pulex* and *G. tigrinus*.

	G. pulex	*G. tigrinus*	Source
Size at sexual maturity (mm)	6mm	4mm	Chambers (1977)
Eggs per brood	up to 34	up to 95	Bird (1989)
Mean egg development time (days, at [1]15°C and [2]14°C)	23.9[1]	15.3[2]	[1]Welton (1979) [2]Chambers (1977)
Juvenile development time to maturity (days, at 10°C)	135	32	Bird (1989)

Thus, if *Gammarus tigrinus* tends to occupy the same habitats as the original *Gammarus* species and it is subjected to the same density controlling factors, then its greater reproductive capacity will enable the species to outnumber and gradually replace the existing species (Pinkster *et al.*, 1977).

2.2.4 Mechanisms of coexistence of *G. tigrinus* with other Gammarids

2.2.4.1 Habitat Segregation and Salinity Gradients

It is clear that upon invasion, *Gammarus tigrinus* may coexist with other Gammarid species. Such coexistence with *G. pulex* was first documented in the Newry Canal, in Northern Ireland (Strange and Glass, 1979). In the Netherlands, whilst Pinkster and Stock (1967) found that *G. tigrinus* can only compete with *G. pulex* in stagnant (not running) waters in the Netherlands, Bird (1989) and Everett (1991) demonstrated full coexistence of the two species in fast flowing streams and rivers of the River Idle catchment. Strange and Glass (1979) highlight how in Northern Ireland, *G. tigrinus* does not penetrate from the Lough to the river occupied by *G. pulex*. Similar segregation of *G.tigrinus* in lakes and *G. pulex* in streams occurs in the Netherlands (Nijssen and Stock, 1965). The authors conclude that *G. tigrinus* seems to prefer larger water bodies.

In Britain, *G. tigrinus* also coexists with *G. d. duebeni*, and *G. zaddachi* in the Bridgewater and Trent and Mersey canals (Holland, 1976). During the latter study, the salinity of the sampling points remained fairly constant, and could not be used to explain the changes in species dominance that took place. Similar observations were made in the central Baltic Sea (Fenchel and Kolding, 1979). This does not agree with the findings of niche separation of 5 species of *Gammarus* along the salinity gradient in Danish waters (Kolding and Fenchel, 1979). This segregation appeared to optimise food availability, and minimising predation by relaxing competition for hiding places. Male gammarids actively touch females with their antennae to test the females and assess their suitability for mating, and if so, forming a precopula pair (Dick and Elwood, 1989). Kolding and Fenchel (1979) go on to suggest that preferences for different salinities, hence niche segregation, could maximise reproductive output by preventing precopulation pairing and subsequent sterile matings between dissimilar gammarid species.

The exact nature of the competition which is responsible for the habitat

limitation of regionally coexisting Gammarids is therefore not clear. Fenchel and Kolding (1979) dismiss competition for food resources as the sole factor responsible for the narrow zones of overlap, as *Gammarus* species are essentially omnivorous, feeding on a wide variety of food items, abundant in the field. Competition for shelter from predators also seems to be a negligible component as although *G. tigrinus* buries in the substratum, *G. pulex* is usually found in crevices beneath stones, so segregating the two species (Bird, 1989). Differences in the life cycles and reproductive rates mean that the two species are represented by different size classes at any one time, hence competition could be relieved by microhabitat selection (Fenchel and Kolding, 1979). It is interesting to note that *G. tigrinus* is generally found in stagnant, very slow running waters in its natural habitat in southern USA (Van Maren, 1978) such conditions exist in its principal habitat along the River Poulter (Section 2.5.1.2).

Lough Neagh, Northern Ireland, provided Dick (1996) with an ideal case study for the habitat segregation of three non-indigenous invading amphipod species; *G. tigrinus*, *Crangonyx pseudogracilis* (both North American in origin) and the European *G.pulex.* In the study, *G. tigrinus* was identified as having a preference for deep, mid-Lough areas, and, to a limited extent, it colonised sheltered, vegetated shores. Both *G. tigrinus* and *C. pseudogracilis* preferred larger water bodies and avoided fast flowing streams and lake shores where the wave action is strong (Nijssen and Stock, 1966; Pinkster *et al.*, 1977 and Strange and Glass, 1979). Despite the prolific output of *G. tigrinus,* a rather erratic population structure (formed *via* invasions from deeper water areas) was identified on the shores of Lough Neagh, dominated by the other species. The survey suggested that although habitat selection may partly account for differences in the distributions of the species, it is the interactions between species which are probably major determinants of community structure. However, it is anticipated that the time taken for populations to switch from one species to another, during which Dick and Platvoet (1996) argue coxistence is observed, could be dictated by the availability of suitable habitats for species segregation. The interactions of predation that will dictate the time to switch populations thus may be delayed.

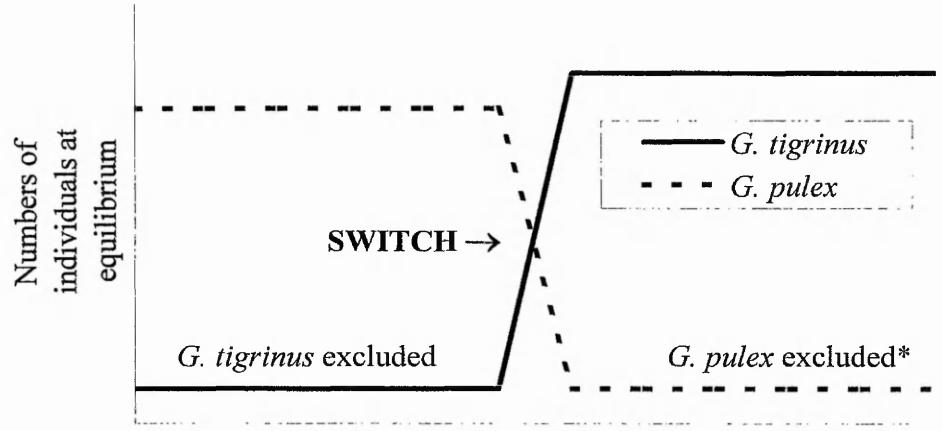
2.2.4.2. Intraguild Predation

There have been several studies investigating the interspecific interactions between *Gammarus* species, which go some way in explaining the success of *G.*

tigrinus in colonising Millwood Brook (Bird, 1989) during the discharge of saline effluents. Dick and Platvoet (1996) recently identified the importance of differential predation as a strong selection pressure in the coexistence between *G. pulex* and *G. tigrinus*.

Classical competition theory states that when resources are limiting, competing species can only coexist by some form of niche differentiation. Complete competitors cannot exist; one species should drive the other to extinction. Intra-Guild Predation (IGP) is the killing and eating of species that use similar, often limiting, resources and are thus potential competitors (Polis *et al.*, 1989). Symmetrical competition may occur, with a differential strength of interaction between species A (e.g. *G. tigrinus*) and species B (e.g. *G. pulex*), exacerbated by the lower output of the weaker species (e.g. *G. pulex*). The recent mathematical modelling of Dick and Platvoet (1996), focusing on coexistence of *G. pulex* and *G. tigrinus* in Northern Ireland, indicates that the greater reproductive output of *G. tigrinus* will exclude *G. pulex* when mutual predation is balanced. However, predation frequencies were differentially in favour of *G. pulex* under the ionic conditions to which the species is physiologically adapted (freshwater), but no advantage is gained in ionic conditions to which *G. tigrinus* is physiologically adapted (oligohaline). Such conditions were found in the 'Beemster' region studied in the Netherlands, which receive salt drainage water from German coal mining in the Rhine (Admiraal *et al.*, 1993).

Dick and Platvoet (1996) argue that *G. tigrinus* is excluded by *G. pulex* by differential mutual predation under freshwater conditions; however, there is a sudden switch at higher conductivities; due to its adaptation to a wide range of salinities, *G. tigrinus* then appears less vulnerable to predation and cannibalization than *G. pulex*, especially around the particularly sensitive physiological period around moulting. Predation by *G. pulex* on *G. tigrinus* is consequently less severe. In addition, *G. tigrinus* kills and eats more *G. pulex* at high compared to low conductivities, probably due to 'pressure by numbers' rather than an increased rate of predation. Hence *G. tigrinus* will dominate when its higher reproductive rate is combined with reduced predation pressure. The model devised by Dick and Platvoet (1996) is illustrated in Figure 2.3.



*The predation rate of *G. tigrinus* on *G. pulex* is slightly greater than that of *G. pulex* on *G. tigrinus.*

Figure 2.3. The effect of biasing mutual intraguild predation in favour of *G. pulex* **whilst simultaneously increasing the bias in reproductive rate in favour of** *G. tigrinus* **(conditions of high conductivity).** *The predation rate of *G. tigrinus* on *G. pulex* is slightly greater than that of *G. pulex* on *G. tigrinus.* Adapted from Dick and Platvoet (1996).

The authors discuss the temporary periods of apparent unstable coexistence of the two species. The computer simulations show that the time taken to switch from one species to another, whereby populations stabilise, is manifested as periods of apparent coexistence.

Thus so far, only raised ionic concentration, such as that found below the discharges of saline effluents, has been investigated, and appears to act as a 'trigger' for 'switching' the balance of predation in favour of *G. tigrinus*, resulting in the species out-competing and colonising areas in which *G. pulex* exists.

2.3 Aims

The aim of this chapter is to establish the changes in macroinvertebrate communities since the sudden cessation of saline discharges from mines in the River Idle catchment, using Creswell colliery as a case study. A focus will be made on the

distributions of *Gammarus pulex* and *Gammarus tigrinus* in light of their differing tolerances to saline pollution.

2.4 Methods

2.4.1 Sampling Sites

46 sites were selected for sampling, 19 on Millwood Brook (Figures 2.1 and 2.2, and Table 2.2), 5 on inputs and tributaries, and a further 22 at sites within the River Idle catchment (Table 2.4, illustrated later in Figure 2.19). These sites were originally selected by Bird (1989) on the basis of their distance from a source of pollution and their ease of access with regards to depth. 3 new sites were chosen due to their proximity to pollution from Thoresby (site MA) and Bilsthorpe (sites BL and ST), mines still operating in the catchment. A control site in a clean Derbyshire river (site LA) and a site downstream of a salt mine on the Cheshire salt flats (site CM) were also included for comparative purposes.

2.4.2 Chemical Analysis of the Watercourses

Each site was sampled in October 1994, May and July 1995. On each occasion of sampling, 250ml of water was collected in polypropylene bottles from below the water surface, across the width of the river at each site to obtain a representative water sample. These samples were analysed in the laboratory using:

(i) A Corning model 240 pH meter to determine the pH. Analar buffers of pH 4 and pH 7 were used to calibrate the meter.

(ii) A Corning 925 Chloride analyser to measure total halide ion concentration.

(iii) A Dionex DX100 unit to measure chloride/fluoride/nitrite/phosphate/sulphate anion levels (ppm) by means of suppressed ion chromatography.

(iv) For selected samples, a Cambridge stereoscan 600 scanning electron microscope was used to give the relative proportions of ions with an atomic number greater than 16. 100ml samples of water and also samples of sewage/mine discharge sediment and effluent were evaporated to dryness. Small samples of these were placed on carbon stubs and analysed by the electron-probe analyser calculating peaks of activity corresponding to each element present in the sample. This method simply shows the relative percentage composition of a restricted number

Table 2.4. Grid reference, mean width, depth, flow rate and substrate type for the River Idle sites, as measured during winter 1994 to summer 1995. 3 samples per site. Results for Bird (1989) shown in brackets. Codes for substrates: 1=Silt, 2=Sand/Clay, 3=Gravel, 4=Pebbles/Cobbles, 5=Large Stones/Boulders.

Site Code	Site	Grid Reference	Mean Width (m)	Mean Depth (cm)	Mean Flow ($m\ s^{-1}$)	Substrate Type
ME1	River Meden (Mansfield)	SK558679	6.35 (10)	36 (25)	0.26 (3)	Pebble/Stones (4)
ME2	River Meden (Mansfield)	SK568686	7.4	19	0.82	Pebbles/Cobbles
ME3	River Meden (Mansfield)	SK594700	5.69 (5)	30 (100)	0.33 (0.5)	Silt (1)
ME4	River Meden (Mansfield)	SK618702	6.7 (5)	27 (22)	0.34 (1.1)	Pebbles/Stones (4)
ME5	River Meden (Mansfield)	SK652718	10.16	21	0.47	Pebbles/Stones
S1	Sookholme Bath (Mansfield)	SK541665	1.47	13	0.02	Clay
S2	Sookholme Bath (Mansfield)	SK543667	1.47 (2)	7 (13)	0.31 (0.9)	Pebbles/Gravel (2)
S3	Sookholme Bath (Mansfield)	SK544668	1.31 (1)	9 (16)	0.56 (0.5)	Pebbles/Gravel (1)
S4	Sookholme Bath (Mansfield)	SK544669	2.71 (1)	23 (8)	0.57 (0.6)	Pebbles/Stones (3)
S5	Sookholme Bath (Mansfield)	SK545669	2.69 (1)	17 (10)	0.5 (0.6)	Pebbles/Stones (3)
P1	Poolsbrook (Staveley)	SK438738	4.13	24	0.36	Pebbles/Cobbles
VW1	Vicar Water (New Clipstone)	SK600632	1.46	26	0.16	Silt
VW2	Vicar Water (New Clipstone)	SK606649	n/a (7)	n/a (9)	n/a (0.4)	Silt (4)
P3	Poolsbrook (Staveley)	SK438738	4 (10)	41 (12)	<0.02 (1)	Silt (1)
LA	River Lathkill (Lathkill Dale)	SK203662	13.5	27	0.032	Stones (4)
CM	Crabmill Lane Flash (Cheshire)	SJ628994	6.08 (40)	32 (50)	0.25 (0)	Stones/Boulders (1)
RW1	Rainworth Water (Rainworth)	SK636604	1.42 (5)	11 (20)	0.27 (0.9)	Pebbles (3)
RW2	Rainworth Water (Rainworth)	SK591586	0.9 (3)	10 (12)	0.41 (0.9)	Sand/Pebbles (4)
L1	River Poulter at Nether Langwith	SK526702	5.42 (10)	25 (20)	0.21 (0.4)	Stones (2)
MA	River Maun at Edwinstowe	SK642670	6.6	34	0.26	Sand/Pebbles
BL	Stream from Bilsthorpe Spoil Heaps	SK649646	2.58	7	<0.02	Gravel
ST	Bilsthorpe Stream at Stonish Hill	SK662622	0.89	10	0.17	Silt

of elements in each sample, and does not allow comparison of concentrations.

(v) At each site, a conductivity measurement (of total ions) was made using a Hanna HI 9033 portable conductivity meter.

(vi) A filter photometer to measure phosphorous, after treating with reagents according to the method of Olsen *et al.* (1954) and Schofield (1955).

(vii) A Nitrachek 404 meter (QuoMed Ltd, England) to measure the nitrate concentration semi-quantitatively by means of test strips.

2.4.3 Physical Analysis of the Watercourses

At each site:

(i) The current flow rate at a depth of 20cm below the water's surface was determined using a 'Braystroke' miniature current meter.

(ii) The water temperature was measured at a depth of 10cm below the water's surface using an ink filled glass thermometer.

(iii) The width of the river was measured using a 30m tape, and the depth was measured using a metre rule.

(iv) A site description was made (substrate/vegetation cover/grid reference).

(v) The discharge rate of the watercourse was calculated by multiplying the flow rate by the cross sectional area (as calculated by multiplying the mean depth of 5 points by the width)

(vi) The percentage of emergent and pre-emergent macrophyte cover was estimated by a random half-metre square quadrat.

(vii) The substrate phi value, as used by Wright *et al.* (1984), is an index used to condense substrate data into a single score. The percentage cover by boulders and cobbles (having a phi value of -7.75), pebbles and gravel (phi -3.25), sand (phi 2.0), and silt and clay (phi 8.0) was estimated by means of a random $1m^2$ quadrat. This quadrat was thrown within the immediate area used for the 3 minute kick sample. The four percentages were each multiplied by their respective phi values and the results then totalled and divided by four to give an average phi value.

(viii) Suspended solids were estimated by evaporating to dryness a disc of pre-weighed filter paper through which had been filtered 500ml of river water, and washed with de-ionised water to remove salts.

(ix) Oxygenation measurements of the watercourses were attempted; however due to

problems with the oxygen probe during sampling sessions and lack of the ability to recalibrate in the field, measurements were not used in subsequent analysis. Millwood Brook is fast flowing and shallow, so oxygen was not considered a limiting variable for macrofauna distributions.

2.4.4 Qualitative and Quantitative Invertebrate Sampling

The invertebrate fauna at each site was sampled using a standard 3 minute kick and search technique. This consists of placing a fine mesh net (900 μm) firmly into the river bed downstream of the operator, then disturbing the sediment by kicking, allowing the cloud of debris containing the displaced animals to be washed into the net. Sampling was timed for a 3 minute period per site, moving across the river bed, with an additional manual search under rocks. Three samples were taken in October 1994, May and July 1995 from each site to minimise sampling errors due to seasonal variations etc. Hynes (1961) reported that the minimum number of times a British stream needs to be sampled in order to collect most of the major species is twice. 3 samplings each of a 3 minute duration will not give a comprehensive family species list, but is satisfactory for a preliminary classification; and a 3 minute sample was found to collect approximately 62% of families and 50% of species that can be collected by 18 minutes of netting (Furse *et al.,* 1981). All samples were preserved in 5% formaldehyde at the site, then sorted and identified in the laboratory, using a variety of keys (e.g. see Quigley, 1977). BMWP and Shannon-Wiener diversity index values were calculated, the latter with the aid of a simple BBC computer program. Note that Chironomidae, Simuliidae and Oligochaetes were not scored as individual species for ease of calculation of this index. It must also be noted that the Environment Agency calculate BMWP scores by scoring for each representative of a family. In this study, as for Bird (1989), family presence or absence from a sample is used to calculate the score, regardless of how many species are collected from that family.

To assess the quantitative changes in sediment fauna present in different stretches of Millwood Brook, core samples were taken during June 1995 at 10 sites. The core consisted of an 11cm diameter plastic pipe pushed into the sediment to a fixed depth of 5cm. 3 core samples were taken at each site, the macrofauna preserved in 5% formaldehyde, then sorted and identified in the laboratory.

2.4.4.1 Distribution of *G. pulex* and *G. tigrinus* in the River Idle Catchment.

Between October to December 1994, an additional 31 sites on the Rivers Idle, Meden, Maun, Rainworth Water and Vicar Water were selected for kick sampling to determine the presence of *G. pulex* and *G. tigrinus* (site details in Table 2.5).

These sample sites were generally several kilometres apart on each river, and conductivity was measured with a Hanna HI 9033 portable conductivity meter to give an indication of salinity.

2.4.5 Further statistical analysis of Millwood Brook species data

To test the significance of species changes at Millwood Brook up and downstream of the mine discharge before (Bird, 1989) and after the cessation of discharges, a chi-squared analysis was performed on the species frequency data derived from species lists in Appendix IV and Bird (1989). This analysis used the species numbers found by Bird (1989) up and downstream of the Creswell mine discharge as expected values, and numbers of species for the same sites from the current study as observed values, to establish if there has been a significant change in numbers since the cessation of pollution discharge in 1993. Mean values for species per site were calculated to allow direct comparison between upstream and downstream.

2.5. RESULTS

2.5.1 Millwood Brook/River Poulter.

2.5.1.1 Chemical and Physical Analysis.

A comparison of the mean values for the four chemical parameters measured seasonally in 1994/5 and monthly in 1986/87 (Bird, 1989) are shown in Figures 2.4 - 2.8 (The mean values for each site are held in Appendix I). Note that due to the lack of replicate data from the study of Bird (1989), no standard error calculations were possible, and an analysis of variance could not be performed.

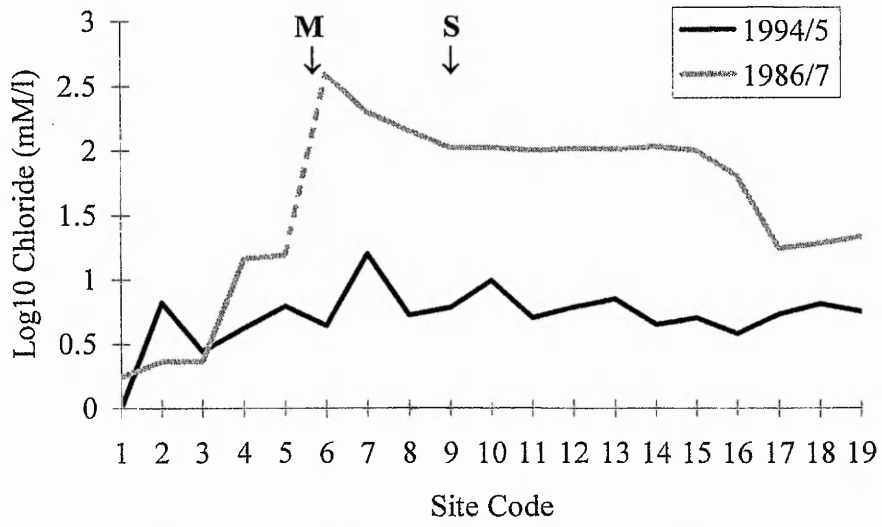


Figure 2.4. Log10 Chloride concentrations (mM/l) for Millwood Brook / River Poulter (1994/5 vs 1986/7). Data for 1986/87 from Bird (1989). Sites of inputs from (M)ine (up to 1993) and (S)ewage works indicated by letter.

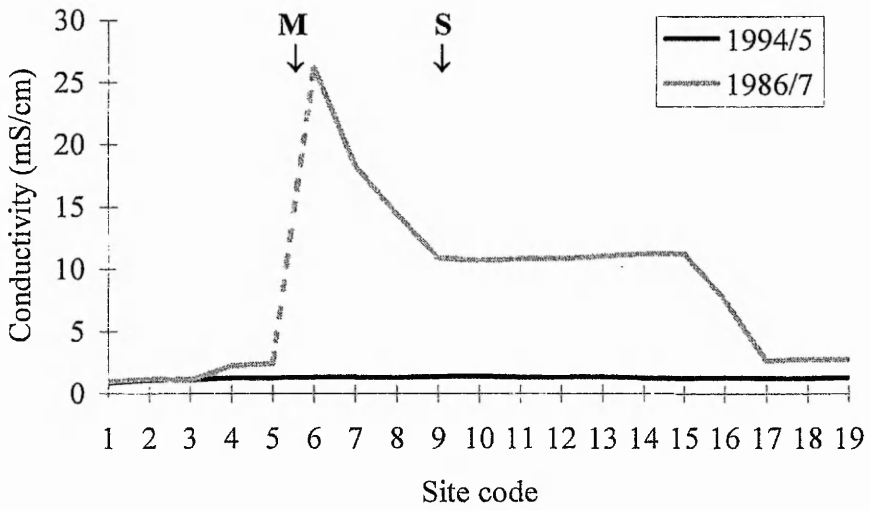


Figure 2.5. Mean Conductivity Values (mS/cm) for Millwood Brook / River Poulter (1994/5) vs. 1986/7. Data for 1986/87 from Bird (1989). Sites of inputs from (Mine (up to 1993) and (S)ewage works indicated by letter.

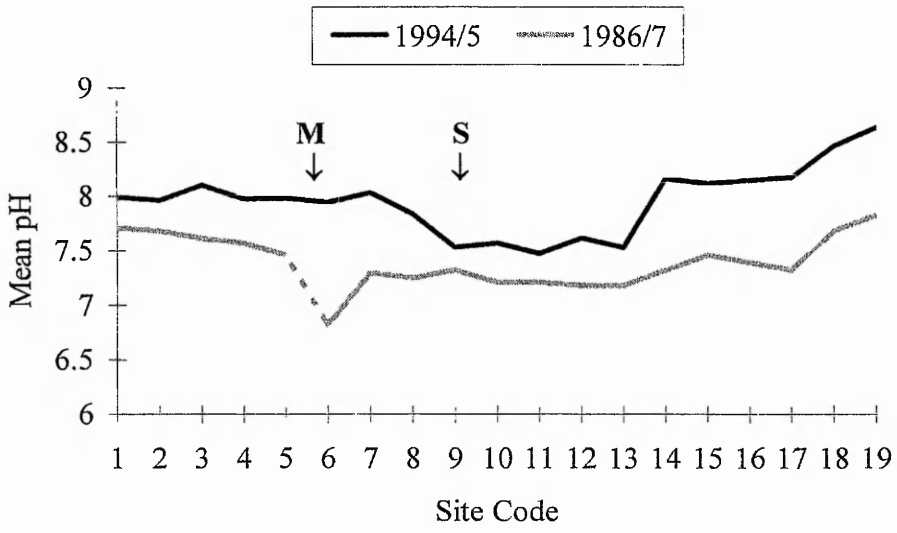


Figure 2.6. Mean pH values for Millwood Brook / River Poulter (1994/5 vs. 1986/7). Data for 1986/87 from Bird (1989). Sites of inputs from (M)ine (up to 1993) and (S)ewage works indicated by letter.

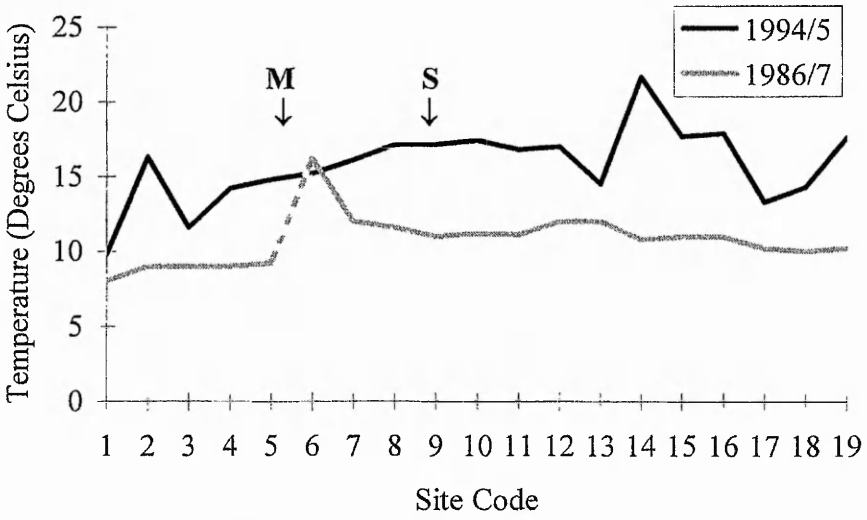


Figure 2.7. Mean temperature Values (Degrees Celsius) for Millwood Brook / River Poulter (1994/5 vs. 1986/7). Data for 1986/87 from Bird (1989). Sites of inputs by (M)ine (up to 1993) and (S)ewage discharge indicated by letter.

These graphs illustrate the fall in chloride concentration and conductivity, but an increase in temperature and pH at most sites (no increase in temperature at site 6, 10m below the discharge point) since the cessation of pumping of the saline minewater from Creswell colliery. The rise in chloride concentration, conductivity and temperature can be observed for the 1986/7 data (Bird, 1989) due to this site being immediately downstream inputs from Creswell mine at site 6. Table 2.2 shows that width and depth for each site are comparable for this and the study of Bird (1989), but the mean flow has decreased at all sites below the mine discharge. The latter is less likely to be a consequence of the cessation of pumping (at 30 - 40 litres per second) in 1993 but due to the drought conditions experienced during the summer, so naturally reducing flow levels. However, a definite reduction is noted below the mine discharge site at site 6. The inputs of effluent from Creswell water treatment works at site 9 continue to add large quantities of organic material to the brook.

The spring Dionex analysis (Appendix II), as compared with Everett (1991) confirm that there is now a lower, stable chloride concentration (ppm) at the 10 sites on Millwood Brook/River Poulter, with nitrate, phosphate and sulphate concentrations no longer increasing at the site of mine discharge, and at levels found in the headwaters. The results of the electron probe microanalysis (Appendix III) indicates changes in water composition of the samples. This technique does not record the presence of several major elements including nitrogen, oxygen, carbon and the calculated percentage assumes that the elements recorded constitute the whole of the sample. Calcium appears to be the main element at Markland Grips, and the mine sediment. This is to be expected as Millwood Brook is a limestone stream, with high concentrations of calcium and low concentrations of other ions, such as nitrate, phosphate and sulphate. The sewage sampled had a reduction in sulphates but an increase in iron content. The increase in iron suggests contamination of the sewage sediment from the erosion of the ochreous sediment (containing iron hydroxide) below the mine, which contained 3.93% iron as compared to 7.87% in 1986/7. Heavy metals such as copper and zinc appear to remain at similar concentrations in this ochreous sediment. The sewage effluent contained high percentages of calcium and silicon, and appeared to introduce small levels of heavy metals into the stream.

2.5.1.2 Qualitative Invertebrate Sampling.

A total of 94 invertebrate taxa were identified from the combined sites, although no site had more than 33 taxa, as compared to 126 and 39 respectively, found by Bird (1989). A species list is presented in Appendix IV. However, Figure 2.8 illustrates the general trend of higher BMWP scores for Millwood Brook, immediately after the mine discharge point, since the cessation of pumping, the exception being sites C, 9, 14, E, 17, 18, 19.

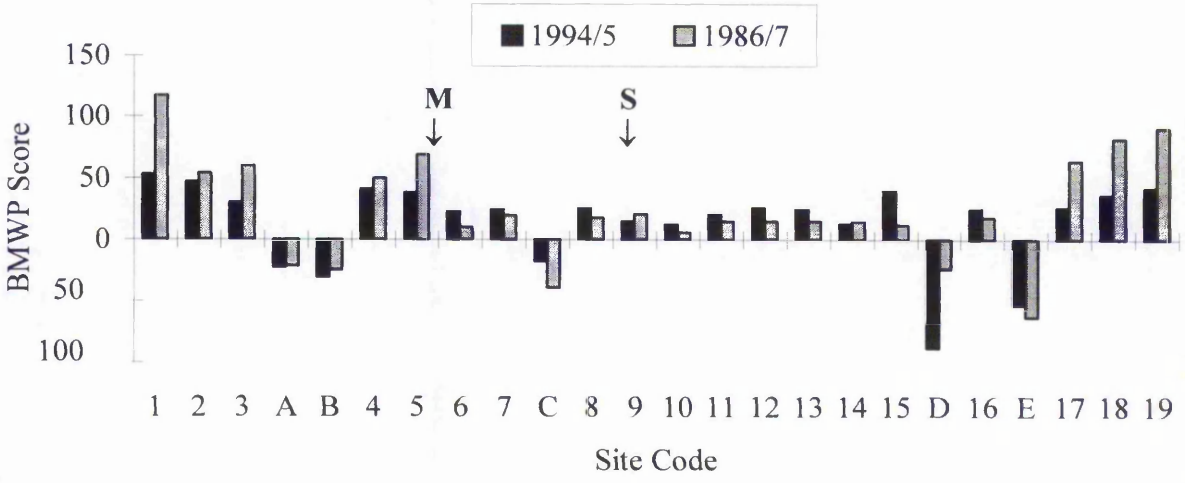


Figure 2.8. BMWP Scores for Millwood Brook / River Poulter (1994/5 vs. 1986/7). Data 1986/7 for 1986/7 from Bird (1989). Sites of inputs from (M)ine (up to 1993) and (S)ewage works indicated by letter.

Each site on the brook is represented above the abscissa; those sites on tributaries are below the abscissa. The Environment Agency assesses freshwater using a General Quality Assessment (GQA). The GQA index is used to classify a river site into one of six grades, according to biological characteristics (BMWP score), chemistry (dissolved oxygen, Biochemical Oxygen Demand and total ammonia), nutrients (nitogen and phosphorous) and aesthetic appearance. The grades for the biological index are: grade 1 (Very good, BMWP >96); grade 2 (Good, 71-95); grade 3 (Fair, 51-70); grade 4 (Moderate, 36-50); grade 5 (poor, 13-35); grade 6 (Bad, 0-12). In the present study, several sites have actually an increased General Quality Assessment biological grade. Sites 6 and 10 have increased from bad to poor. Several sites fell one grade (2, 3, 5 and

C) or two grades (1,17,18,19). There still remains a reduction in BMWP at site 9, a site of discharge from the water treatment works.

Markland Grips scored 64 BMWP points less than for Bird (1989), mainly due to the absence of 6 species of Trichoptera from the samples - only *Limnephilus lunatus* was found. Also absent was *Amphinemura standfussi* Ris, a member of the order Plecoptera. Sites 3, 5 and C scored 21-31 BMWP points less, due to a lack of Trichoptera and Coleoptera representation in these samples, when compared to Bird (1989). Sites 17,18 and 19 on the River Poulter scored 38-48 BMWP points less, due to similar absences. It is to be expected that these sites would obtain at least an equal, if not improved BMWP score as compared to Bird (1989). Sampling errors and/or substratal changes after the spate conditions in winter may account for this. Additionally, Markland Grips ran dry during 1992, so what is now found there is the result of recolonisation, which may have resulted in a poorer species diversity than originally found, as reported for for a similarly dry watercourse by Wright (1992).

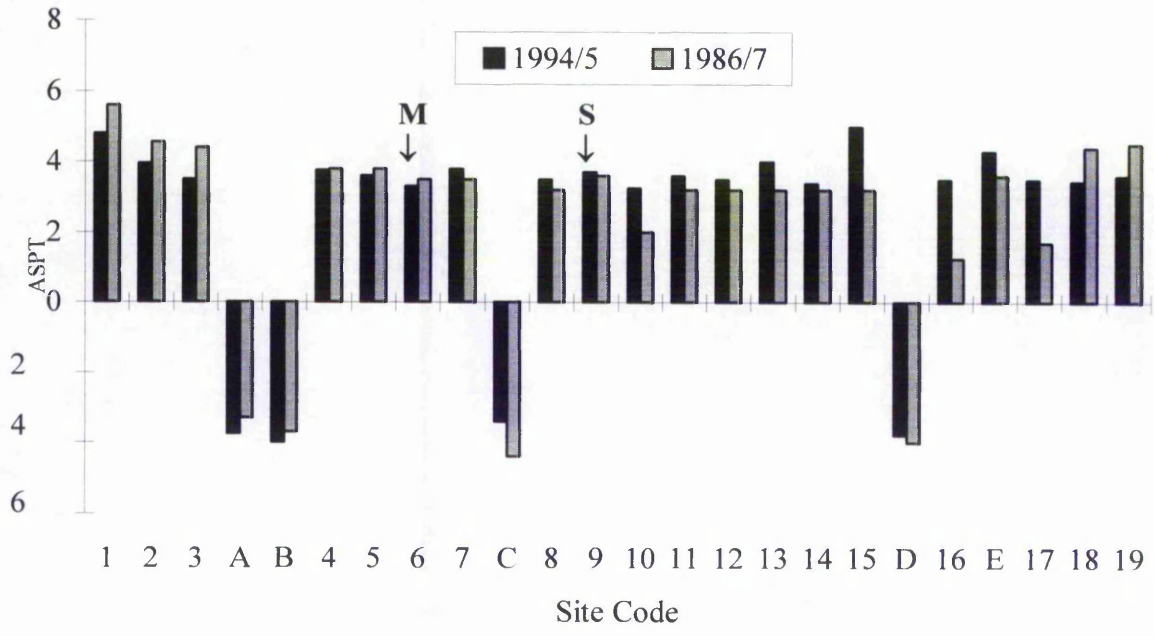


Figure 2.9. Average Score per Taxon (ASPT) for Millwood Brook / River Poulter (1994/5 vs 1986/7). Data for 1986/7 from Bird (1989). Sites of inputs from (M)ine (up to 1993) and (S)ewage works indicated by letter.

Figure 2.9. illustrates the higher Average Score per Taxon (ASPT) for the sites on Millwood Brook, after the point of mine discharge (except for site 6, C, D, 18 and 19). Therefore, although total species found may be less than that for the previous study, the ASPT for sites 7 to 16 (the most immediately affected by previous mine discharge) is greater, hence the average sensitivity of the species has increased which is indicative of a greater water quality. Seasonal BMWP data (Figure 2.10) indicates that BMWP scores from each site differ most greatly between winter 1994 and summer 1995 - probably due to the two extremes of spate conditions in the winter, and very low water levels in drought conditions during the summer of 1995. Corrarino and Brusven (1983) comment on the increased drift and mortality of many insects during drought low flow conditions. Natural patterns of colonisation and sampling errors with pond net sampling can by some way account for the difference in many species found compared with Bird (1989).

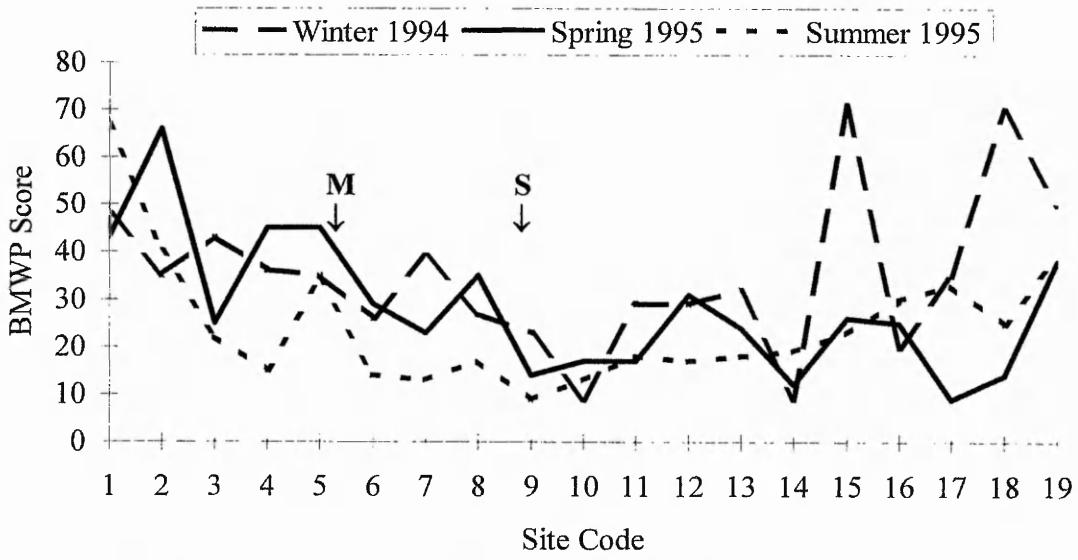


Figure 2.10. Seasonal BMWP values for Millwood Brook / River Poulter. Sites of Inputs from (M)ine (up to 1993) and (S)ewage works indicated by letter.

Several species changes have occurred since cessation of pumping (Appendix IV). Caddis larvae (*Hydropsyche augustipennis* Curtis) and mayfly nymphs (*Baetis rhodani* Pictet) are now found at several sites directly below the discharge point. Molluscs (*Lymnaea peregra* Müller) and flatworms (*Polycelis nigra/tenuis*) also have

recolonised, probably from upstream cleaner sites, and other sites in the area.

Gammarus pulex has recolonised all sites below the old mine discharge in Millwood Brook, and a higher density of *G. pulex* was still found in the clean water 'pocket' around site C, consistently receiving discharge from Crags pond, *via* the treatment works. The enchytraeid worm *Lumbricillus rivalis* Levinsen, continued to dominate the oligochaetes found below the mine. Whereas *G. tigrinus* Sexton was first found at site 12 and often dominated each subsequent site (Bird, 1989), *G.tigrinus* was now only located at sites 18 and 19. There were few obvious changes in substrata, except for site 4 having changed from a rocky substrata to sand/silt and the site 450m below the mine, also siltier, previously pebble/rock. Sites 13 and 15 appear to have changed to a substrate of gravel, from a previously sandy bed. This change at site 15 may have led to a more favourable substrate for colonisation by macroinvertebrates, hence a noticeably greater BMWP score after the mine discharge point, since the study by Bird (1989), as in Figure 2.8.

Figure 2.11 and 2.12 illustrate the rather erratic values for Shannon's diversity (H) for each site. In Figure 2.11, when comparing the three seasons, particularly noticeable are the great deviations at sites 4-7, 12 and 15 for the score. An analysis was performed (Figure 2.12) to calculate the percentage change in winter and summer compared to spring. In spring, emerging nymphs result in a high diversity of macrofauna, hence this is a good season to have for a baseline. Figure 2.12 shows the large seasonal differences in the index. It is unwise to try and attempt to interpret these data given such fluctuations between seasons for each site. In contrast to 1985/6, where below the mine discharge (site 5) the diversity of the fauna was reduced, no pattern of change in diversity is observed past the old discharge site. The extremes of spate conditions in winter, and drought in summer would have reduced the diversity macroinvertebrate communities collected in this sampling programme.

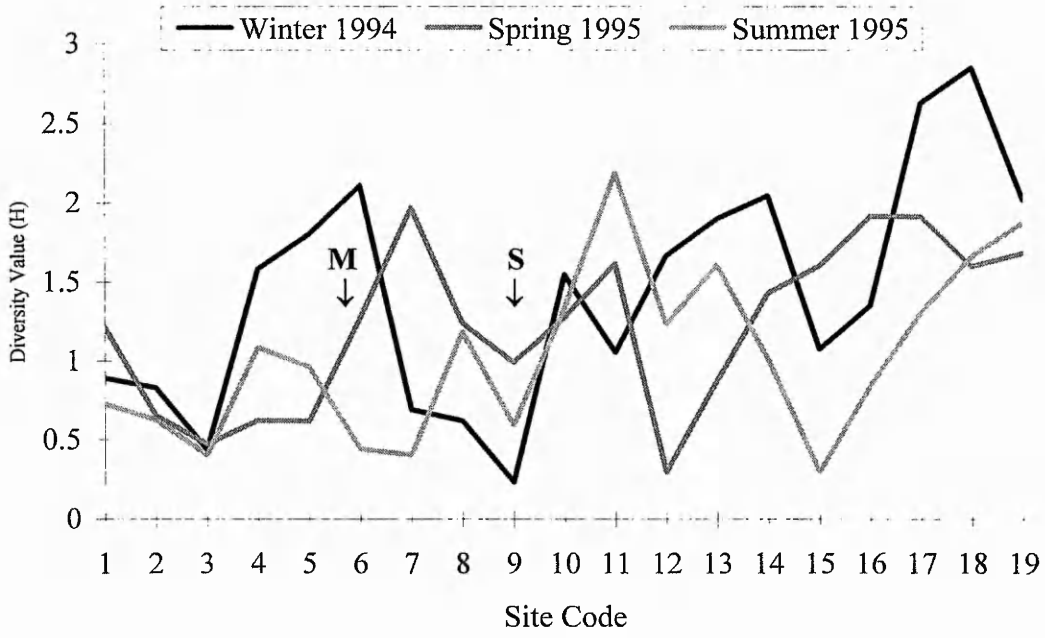


Figure 2.11. Seasonal Shannon's Diversity Values for Millwood Brook/River Poulter (1994/95). Sites of inputs fom (M)ine (up to 1993) and (S)ewage works indicted by letter.

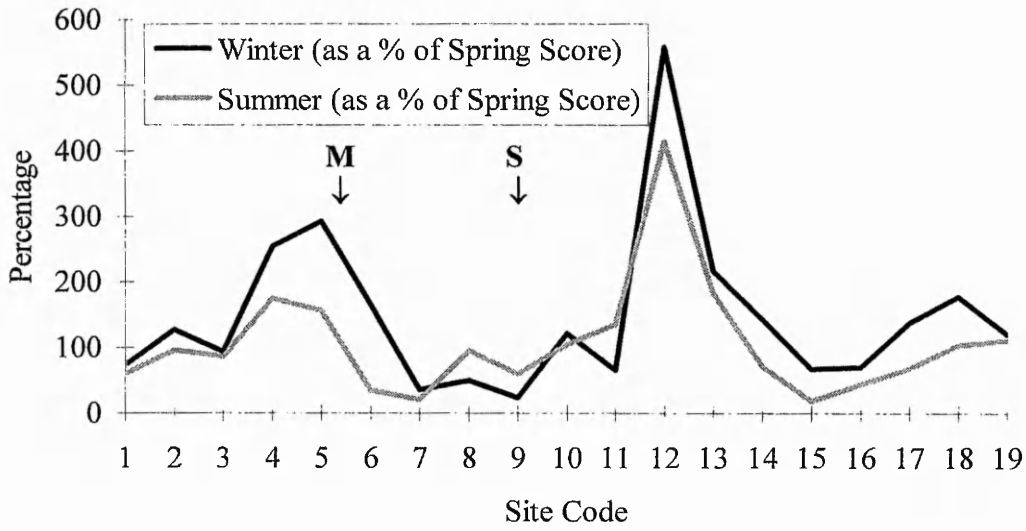


Figure 2.12. Seasonal Shannon's Diversity values for Millwood Brook / River Poulter (1994/95). Scores expressed for Winter and Summer as a % of Spring score. Sites of inputs from (M)ine (up to 1993) and (S)ewage works indicated by letter.

2.5.1.3 Quantitative Invertebrate Sampling.

The abundances of *G. pulex* and oligochaetes in Millwood Brook compared to Bird (1989) are shown in Figures 2.13 and 2.14 respectively (data in Appendix V). Due to the subjectivity of core site selection, caution must be taken in interpreting these data. Whereas *G. pulex* previously disappeared below the mine effluent, current results indicate a recolonisation of the gammarid at all sites, falling at the point of sewage discharge (site 9), then rising again. Oligochaete numbers are significantly reduced for this study period. However, *L. rivalis* maintains its presence throughout the sites sampled, albeit at much lower numbers (0-2420m^{-2} as compared to 0-18621m^{-2} as for Bird, 1989). This is an estuarine species normally present in low numbers in watercourses and the saline mine water discharge provided a suitable habitat for its colonisation. It is thought to be highly tolerant of organic pollution (Hawkes, 1962) and it is also associated with coal washing and sewage outfalls (Learner *et al.*, 1971). Since cessation its levels have fallen. This suggests that the saline mine water provided a suitable habitat for its colonisation. The loss of the estuarine weed species *Enteromorpha* spp. in the brook, with which it was associated, could have also caused a reduction in abundance. However, *Enteromorpha* spp. is still found upstream in Crags

pond. *Paranais litoralis*, another brackish/seawater species (Brinkhurst, 1971) is now only found at 2 sites below the discharge point, as compared with 9 in the study of Bird (1989) (see Appendix V). Tubificidae are only represented by smaller numbers of *Limnodrilus hoffmeisteri* Claparede rather than *Tubifex tubifex*, although the two are often found together (Brinkhurst, 1971). Brinkhurst (1967) considers that the percentage of *Limnodrilus hoffmeisteri* in relation to all other oligochaetes may prove to be a useful indicator of organic pollution; he goes on to suggest that *Tubifex tubifex* is a refuge species which does not do well in competition with other tubificids but is able to survive under more extreme conditions where competition is limited (Brinkhurst, 1969), perhaps the reason why its abundance has dropped since the cessation of highly saline inputs. *Tubifex tubifex* is therefore a typical r-strategist. It is a poor competitor which is absent due to competition with other species, but it will rapidly increase in numbers in favourable conditions.

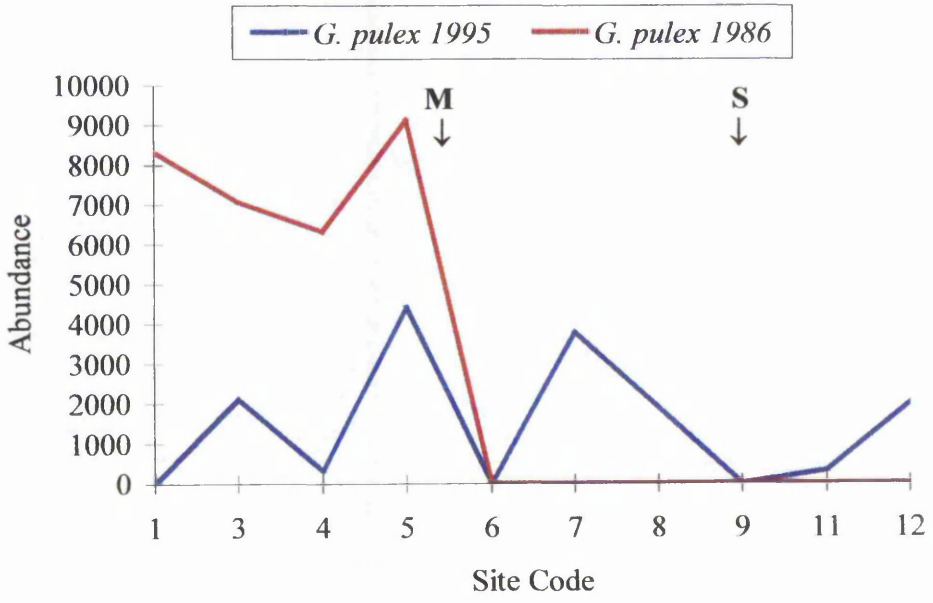


Figure 2.13. Abundance (per square metre) of *Gammarus pulex* in Millwood Brook (June 1995 vs. August 1986). Data for 1986 from Bird (1989). Sites of inputs from (M)ine (up to 1993) and (S)ewage works indicated by letter.

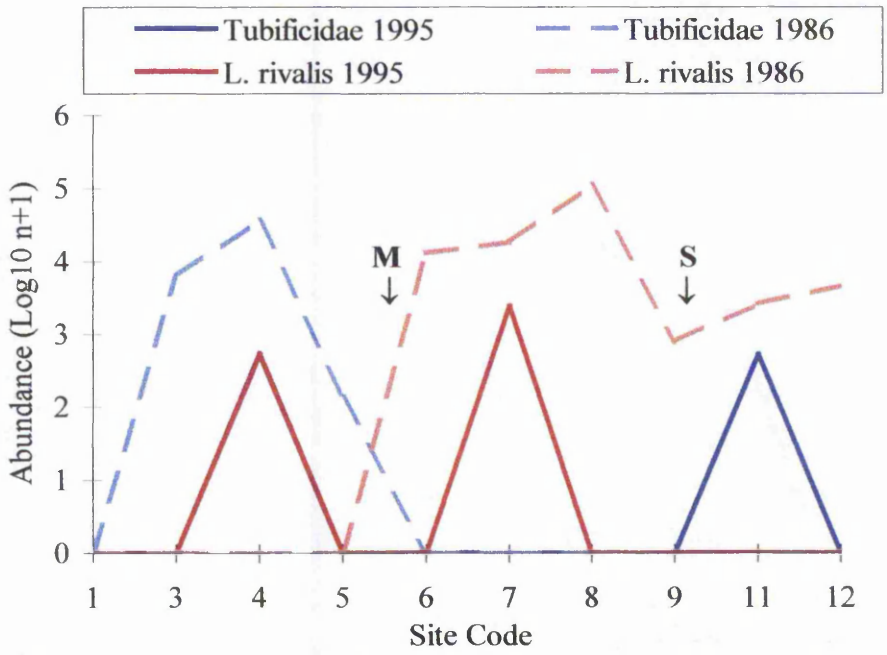


Figure 2.14. Abundance of *Lumbricillus rivalis* and Tubificidae (per square metre) in Millwood Brook (June 1995 vs August 1996). data for 1986 from Bird (1989). Sites of inputs by (M)ine and (S)ewage works indicated by letter.

2.5.2 The River Idle Catchment.

2.5.2.1 Chemical Analysis.

Mean environmental measurements for the River Idle catchment sites are given in Appendix VI. Conductivity values for 12 of the 19 sites (Figure 2.15) have decreased since 1986. Site VW2 has no data available due to the watercourse being dry.

The greatest reductions in conductivity have been at sites S2-S5 (receiving effluent from Shirebrook Colliery, abandoned 1993), CM, P3, RW1, RW2 (at Rufford colliery, abandoned 1993) and at site L1 (above Langwith colliery, abandoned 1989). Langwith colliery is only pumped intermittently, mainly during the winter months. It is geologically linked underground to Creswell and water used to be diverted to Langwith pumps when maintainance was required at Creswell.

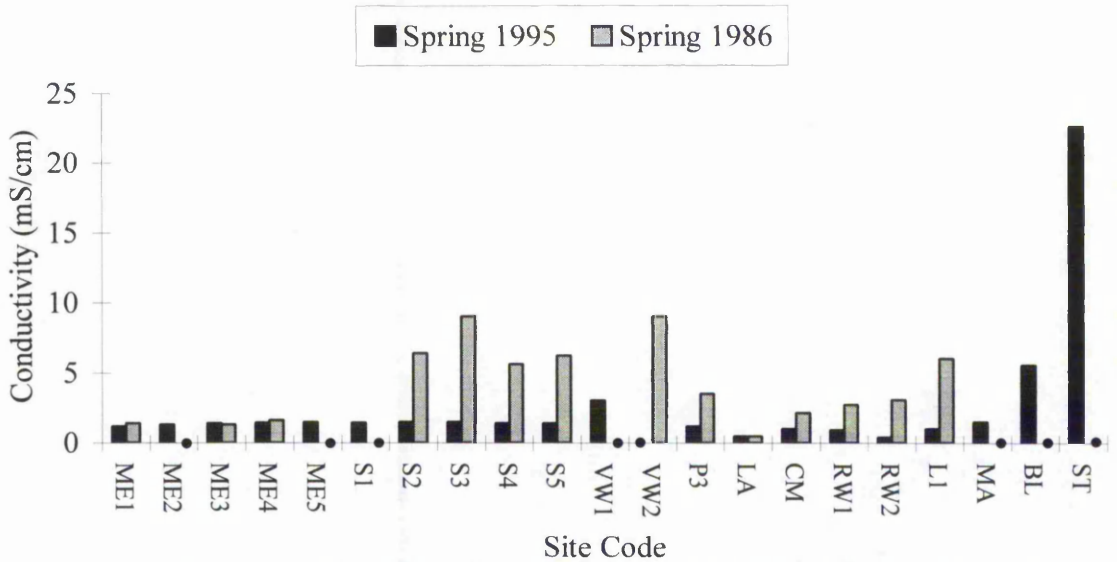


Figure 2.15. Conductivity (mS/cm) for the River Idle Catchment (Spring 1995 vs. Spring 1987). Data for 1986 from Bird (1989). • indicates no comparative data.

A similar pattern of reduction is seen with chloride values (in mM l^{-1}, Figure 2.16). There was a reduction from 636mM l^{-1} to 6.2 mM l^{-1} at Shirebrook Stream, receiving effluent from Shirebrook colliery. Site CM is on the brook flowing into Pennington Flash in Cheshire. This brook was reported to have a chloride value of up to 1500 mg l^{-1} in 1970 by Holland (1976), due to discharges of waste brine from a local salt mine. The reduction in chloride levels found here indicates cessation of this discharge.

From the spring 1995 Dionex analyses (Appendix II), it is found that waterways receiving discharges from operating mines in the catchment, namely Clipstone and Bilsthorpe (sites VW1, BL and ST), have noticeably elevated sulphate concentrations. The salinities and conductivities of sites receiving discharges from Bilsthorpe mine are also markedly greater than other sites. As for Millwood Brook, there has been the recolonisation of species, notably below discharges from the recently abandoned Shirebrook (sites S1-S5) and Rufford (site RW2) collieries. *Baetis rhodani* (Pictet), *Hydropsyche augustipennis* (Curtis) and several new *Simulium* spp. represent the recolonisation of species with flying adults at Shirebrook. Gastropods (*Lymnaea peregra* (Muller) and *Valvata* spp.), flatworms (*Polycelis* spp.) and Crustacea (*Asellus*

aquaticus (L.) and *Gammarus pulex*) are now present at the Shirebrook sites, as are representatives of leeches (*G. complanata* (L.) and *E. octoculata* (L.) at both Shirebrook and Rufford.

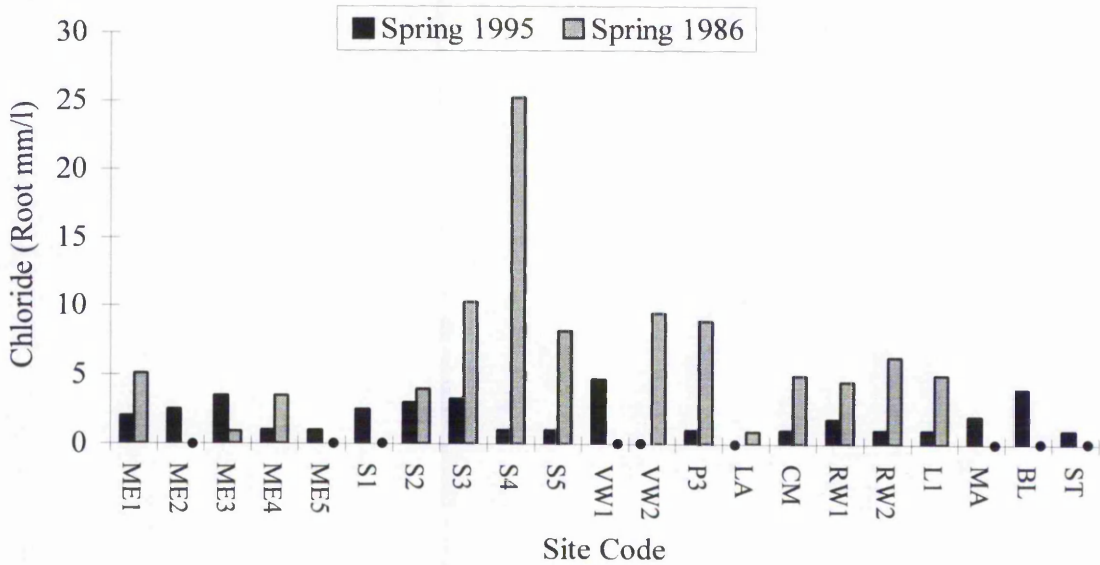


Figure 2.16. Chloride Values (Root mm/l) for sites in the River Idle Catchment (1994/5 vs. 1986). Data for 1986 from Bird (1989). • indicates no comparative data.

2.5.2.2 Qualitative Invertebrate Sampling.

A list of species found in the River Idle catchment can be found in Appendix VII. Bird (1989) only took one sample per site, this study considers 3 samples over 1 year, for use in modelling in chapter 3. Comparing the BMWP Scores for the 19 sites during Spring 1986 with Spring 1995 (Figure 2.17) sites ME1, ME4, S2, LA and L1 had a reduction in BMWP score. Sites previously receiving Shirebrook Colliery discharge have had a BMWP increase of up to nine-fold.

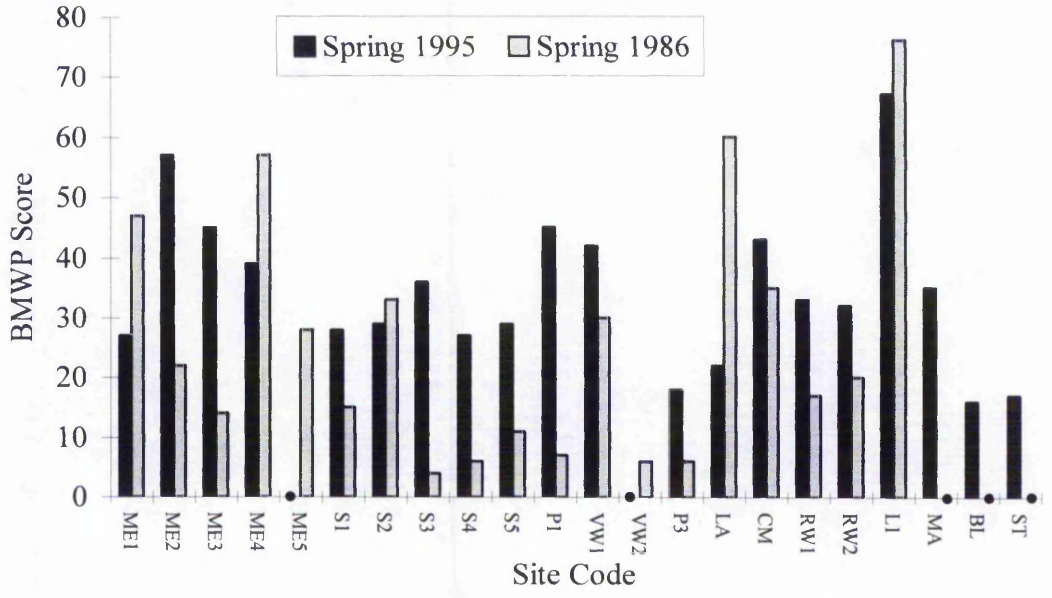


Figure 2.17. BMWP Scores for the River Idle Catchment (Spring 1995 vs. Spring 1986). Data for 1986 from Bird (1989). • indicates no comparative data.

The GQA class for sites S3 was raised from bad to moderate, for sites S4 and S5 and from bad to poor. There were 3 changes in substrates since the previous study; from silt to pebbles and gravel at site S2, from silt to stones and pebbles at site CM and from pebbles/cobbles to silt at site VW2. The former two changes would be expected to have a positive impact on the BMWP, as is indeed observed for those sites, the latter change to the detriment of the BMWP (not observed). The absence of *Gammarus tigrinus* at site CM, formerly present in the study by Bird (1989) may also be explained by the reduction in chloride concentrations in the brook, over the last 19 years to improve conditions for fisheries in the area (Holland, 1976). The sites receiving poor quality discharge from Bilsthorpe colliery (BL and ST, which have no comparative values), exacerbated by poor substrates of gravel and silt respectively, have poor BMWP scores.

At all sites except RW1 and RW2, the ASPT has increased (Figure 2.18), indicative of an increase in the number of pollution sensitive species, hence an improvement in water quality at these sites since 1986.

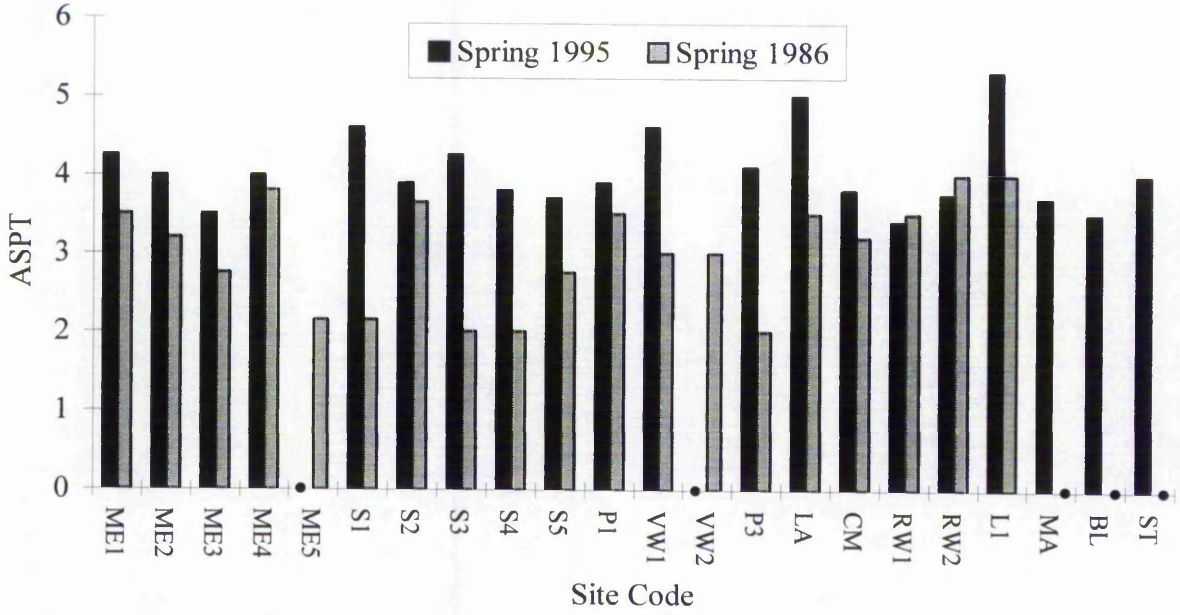


Figure 2.18. Average Score per Taxon (ASPT) for the River Idle catchment (Spring 1995 vs. Spring 1986). Data for 1986 from Bird (1989). • indicates no comparative data.

2.5.2.3. Distribution of Gammarids in the River Idle Catchment.

Figure 2.19. illustrates the changes in Gammarid distribution since Bird (1989). The additional 31 site results are presented in Table 2.5.

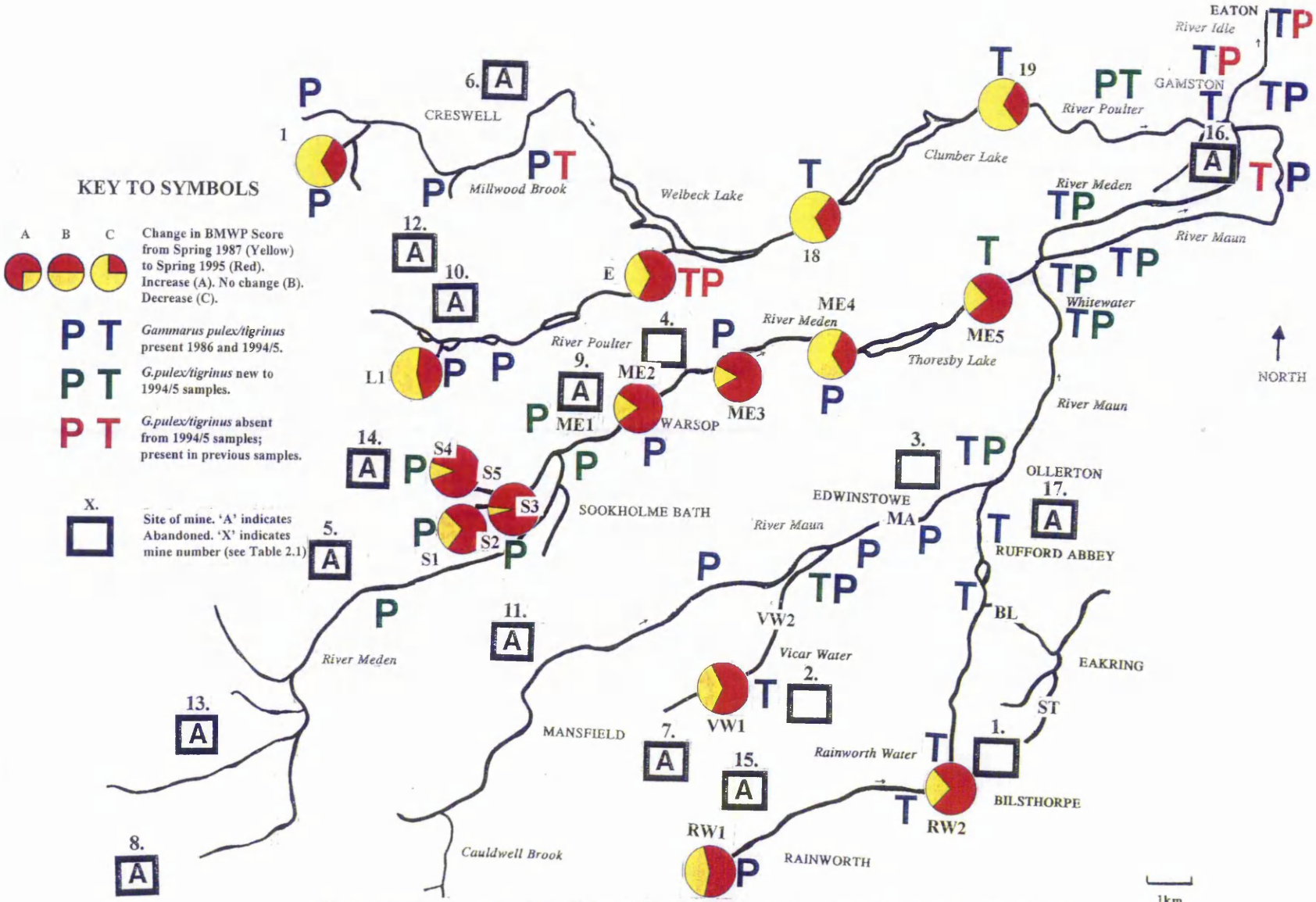


Figure 2.19. A map of the River Idle Catchment showing the changes in BMWP and presence/absence of Gammarids in 1994/5 as compared to Bird (1989).

Table 2.5. Site identification, and environmental data for the 31 sites sampled in the River Idle catchment to determine the presence of Gammarids. 1 sample per site (Winter 1994). Results for Everett (1991) shown in brackets.

Site No.	Grid Reference	Watercourse	BMWP	Evenness	Conductivity (mS cm^{-1})	pH	*G. pulex*	*G. tigrinus*
1.	SK501753	Millwood Brook	30	0.665	0.96	7.51	+	-
2.	SK532739	Millwood Brook	17	0.967	5.71 (1)	7.03	- (+)	- (-)
3.	SK544745	R. Poulter	32	0.328	1.35	7.38	+	-
4.	SK529703	R.Poulter	55	0.202	0.84 (5)	7.17	+ (+)	- (-)
5.	SK672752	R.Poulter	54	0.767	1.18 (3)	8.46	+ (-)	+ (+)
6.	SK481611	R.Meden	9	0.775	0.60	7.67	+ (+)	- (-)
7.	SK510649	R.Meden	38	0.881	1.08	8.19	+	-
8.	SK524651	R.Meden	57	0.544	1.08	8.31	+	-
9.	SK551663	R.Meden	32	0.288	1.55	7.48	+	-
10.	SK546669	Sookholme Bath	32	0.254	1.59 (4)	7.69	+ (-)	- (-)
11.	SK549671	Sookholme Bath	36	0.3775	1.59 (6)	7.83	+ (-)	- (-)
12.	SK681733	Meden	43	0.467	1.22 (6)	0.85	+ (-)	+ (+)
13.	SK703751	Meden	-	-	1.27 (2)	-	- (-)	+ (+)
14.	SK702752	R.Idle	-	-	1.37 (3)	-	- (+)	+ (+)
15.	SK708763	R.Idle	55	0.640	1.33 (2)	8.07	+ (+)	+(+)

Table 2.5. Continued. Site identification, and environmental data for the sites sampled in the River Idle catchment.

Site No.	Grid Reference	Watercourse	BMWP	Evenness	Conductivity (mS cm^{-1})	pH	*G. pulex*	*G. tigrinus*
16.	SK709780	R.Idle	43	0.828	1.31 (1)	8.08	- (+)	+(+)
17.	SK689895	R.Idle	53	0.631	1.47	7.43	+	-
18.	SK656927	R.Idle	50	0.318	1.24	7.72	+	-
19.	SK765962	R.Idle	35	0.484	1.39	7.78	+	-
20.	SK532585	R.Maun	28	0.775	0.36 (1)	7.72	- (+)	- (-)
21.	SK553632	R.Maun	16	0.829	0.99 (1)	7.65	- (-)	- (-)
22.	SK601649	R.Maun	46	0.393	1.05 (2)	7.73	+ (-)	- (-)
23.	SK623662	R.Maun	39	0.742	70.98 (1)	7.75	+ (+)	- (-)
24.	SK649647	Rainworth Water	19	0.530	1.09 (3)	7.68	- (-)	+ (+)
25.	SK647657	Rainworth Water	-	-	1.87 (3)	-	- (-)	- (-)
26.	SK647666	Rainworth Water	25	0.737	1.49	7.78	+ (-)	+ (-)
27.	SK658683	R.Maun	52	0.512	1.10 (2)	7.81	+ (-)	+ (+)
28.	SK663703	R.Maun	24	0.819	1.27 (5)	8.08	+ (-)	-
29	SK666709	R.Maun	0	0	1.29	8.08	-	+ (+)
30.	SK681729	R.Maun	60	0.588	1.20 (2)	8.10	+ (-)	+ (+)
31.	SK713744	R.Maun	-	-	- (2)	-	+ (+)	- (+)

The retreat of *Gammarus tigrinus* from the River Poulter is apparent. *G. pulex* has colonised the sites at the confluence with the River Idle, and Sookholme Bath, where gammarids were previously absent before the closure of Shirebrook Colliery in 1993. These findings correspond with the reduction in chloride and conductivities at these sites, favouring *G. pulex* colonisation. *G. pulex* also was found for the first time at the headwaters of the River Maun (site 22, Table 2.5) and above site B at Creswell Crags (albeit as individuals). *G. tigrinus* was found to be present at sites ME5 and VW2, previously absent. The colonisation of the latter sites is perhaps aided by the apparent change from a substrate of rock to silt, into which *G. tigrinus* burrows. *G. tigrinus* has been lost from six sites: 12, E, CM, RW1, RW2 and site 31 in Table 2.5. Most notably there is the loss from sites RW1 and RW2, on Rainworth water and at the A60 on Millwood Brook, which respectively received discharges from Rufford colliery (abandoned 1993) and Creswell (abandoned 1992). The conductivity values of all these sites have decreased (Figure 2.5 and 2.15).

2.5.3. Results of Millwood Brook species change analysis.

Figures 2.20 and 2.21 illustrate the comparison between the species changes above and below the mine effluent input point at Millwood Brook since the study of Bird (1989). Data for species changes was obtained from comparing species lists in Appendix IV and a similar list in Bird (1989). The sites upstream of the discharge point of Creswell mine will be referred to as unpolluted sites; those downstream as polluted, even though discharges ceased in 1993. There is generally less fluctuation in percentage change in species immediately downstream of the sewage discharge (sites 9 and 10, Figure 2.21). Indeed, the strong influence of organic pollution from the sewage works has always dictated species communities, and saline pollution in this area may not be the dictating variable for species presence or absence. Figure 2.21 illustrates the overall gain by recolonisation of species downstream of the old mine discharge point (sites 5 - 16), indicative of conditions returning to normal after the cessation of polluting discharges. Only the absolute number of species could be compared as only totals for three seasons was available. Therefore it is not possible to statistically compare the difference between these lines. There is a peak of an increase in species of 271% at site

15. This site receives the direct impact of discharges from Whitwell spoil heaps (Figure 3.5a). It is suggested that the abandonment of Whitwell mine in 1986 has, over 9 years, led to a reduction of pollution running off from the spoil heaps. This, combined with the cessation of pollution from Creswell mine upstream has led to the large increase in biological diversity at this site. There are notable losses, however, at sites 17 and 19 much further downstream of the discharge. It is suggested that the negative effects of the flooding and drought on species numbers in these areas override any gain in species due to the cessation of mine discharges.

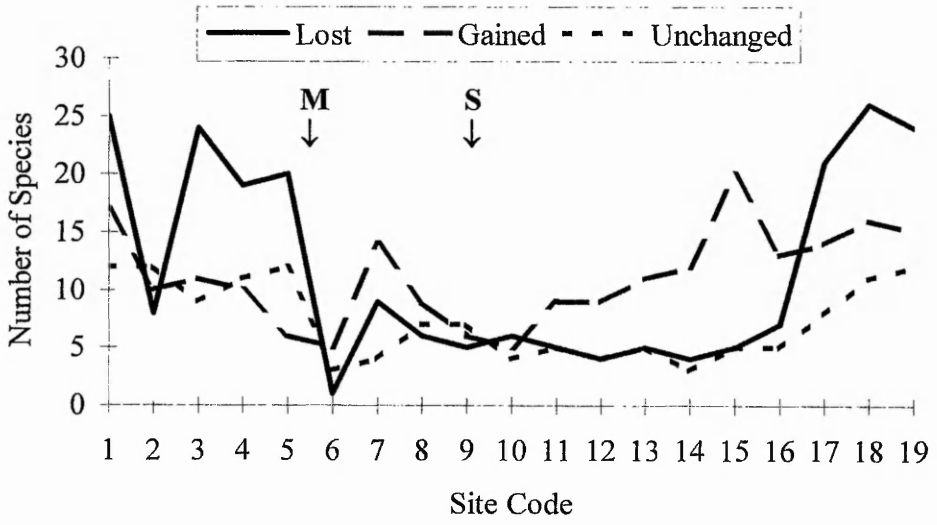


Figure 2.20. Species changes in Millwood Brook/River Poulter since the study of Bird (1989). Sites of Inputs from (M)ine (up to 1993) and (S)ewage works are indicated by letter.

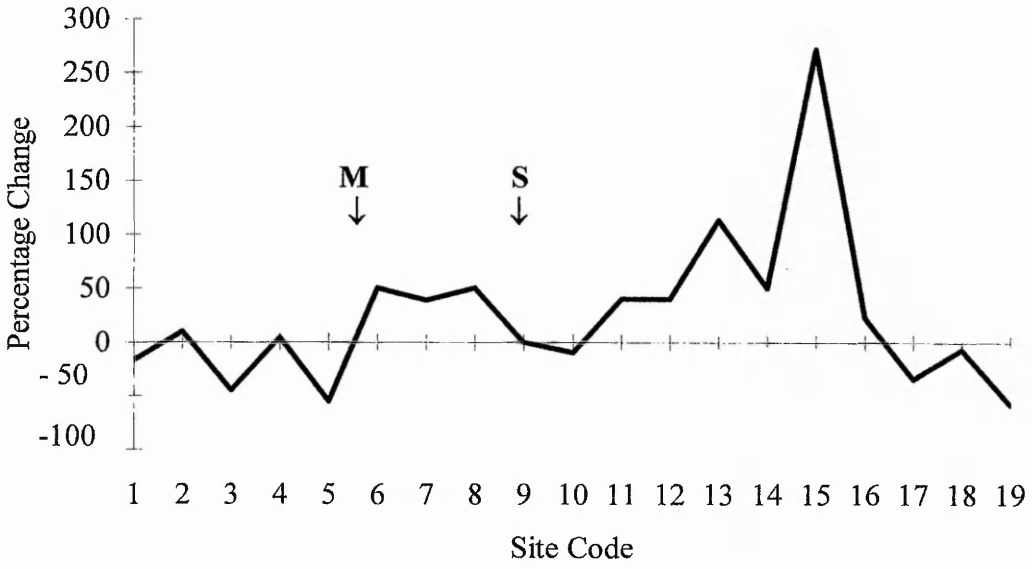


Figure 2.21. Percentage change in species in Millwood Brook/River Poulter since the study of Bird (1989). Sites of inputs from (M)ine (up to 1993) and (S)ewage works indicated by letter.

2.5.3.1 Chi squared analysis of Millwood Brook species data

The results of the chi-square analysis of change in species in Millwood Brook since the previous study by Bird (1989) can be found in Table 2.6. The test was performed to see if there was a significant difference between the mean observed number of species per site (present study) and the expected number of species per site (calculated from Bird, 1989) for up and downstream of the mine discharge. Only a subset of sites were used which were directly on the watercourse upstream, or in the direct path of mine pollution downstream. Although the upstream sites have a greater mean species per site than the downstream sites in each study, Chi-squared analysis suggests no significant difference in the number of species up and downstream of the point where the mine water used to discharge, since the cessation of mine discharges in 1993. The harsh environmental conditions in the period, namely the flood in 1994, and drought in 1995, would be expected to lead to losses at all sites, due to the loss of suitable substrates and macrophytes in a flood, and changes to habitat and food availability in a drought.

It is likely therefore that any recolonisation by species would have been disrupted by these adverse conditions, so each site downstream has only an average of 4.5 species per site. The drought conditions would exacerbate the pollution impact of the sewage discharge directly below the mine, and flooding could have disrupted the substratum for re-colonisation. The upstream sites have more specialised species which are less tolerant to adverse environmental conditions than the fauna in the polluted sites. The loss of habitat at site 1 (Markland Grips) was due to the stream drying: this would have had a considerable effect on sites below it, with the reduction in clean flow of water to the lower sites 2, and 3. Notable species losses include the Ephemeropteran, *Ephemerella ignita* (Poda), several species of Oligochaeta (*Potamothrix hammoniensis* (Michaelsen), *Lumbricillus rivalis* (Levinsen), *Lumbriculus variegatus* (Muller), the Gastropod, *Lymnaea peregra* and the Triclad, *Dendrocoelum lacteum* (Muller).

Table 2.6 Chi-Squared analysis of the change in species in Millwood Brook/River Poulter since the study of Bird (1989).

Change	'Upstream sites'*	'Downstream Sites'**	Total Upstream	Total Downstream
No. of Species Present Study	44	49	56	69
Number of Species Bird (1989)	55	33	76	90
Mean Species per site Present Study (5 sites)	8.8	4.5	11.2	6.3
Mean Species per site Previous Study (11 sites) (Bird, 1989)	11	3	15.2	8.2

Chi-Squared Test (Species Changes)

	Upstream	Downstream
Observed (O)	8.8	4.5
Expected (E)	11	3
(O-E)	-2.2	1.5
$(O-E)^2$	4.84	2.25
$(O-E)^2/E$	0.4	0.75

Chi-Squared***	1.15
Probability	ns

Notes

Test performed on species presence/absence only.

*Upstream sites. Five sites used in Test 1: 2, 3, A, 4, 5. Site 1 (Markland Grips) excluded due to total elimination of all species when dry. Site B (Left Hand Stream) excluded due to its inputs from coal tips.

**Downstream sites. Eleven sites used in Test 1: 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16. Input sites C, D and E excluded. Sites 17, 18 and 19 also excluded as not directly impacted by pollution.

***Degrees of freedom = 1. (2 categories: upstream and downstream).

2.6 Discussion

Since the 1992 proposals for the streamlining of the coal industry in Britain, 7 collieries have been abandoned in the English East Midlands, bringing the total to 13 in the last 15 years. This is a dramatic change over an historically short time period. Due to the lack of legislation until 2000 there is no responsibility for the continued pumping of the abandoned pits to keep them free of water, and as this is costly (approximately £1m per annum in Durham (Younger and Harbourne, 1995)), the pumping ceases. The cessation of pumping has led to ground water rebound with subsequent severe and expensive environmental problems in other coalfield areas such as South Yorkshire, South Wales and Northumbria. These areas are explored in Chapter 4. However, it can be expected that due to the cessation of large, poor quality saline inputs there will be an initial recovery in macrofauna in the River Idle catchment in Nottinghamshire below previous discharge points.

The chemical and invertebrate data presented in this study illustrates the rapid recovery of Millwood Brook since the cessation of mine water pumping in 1993 and elsewhere below similar discharge points in the River Idle catchment. The 'rebound effect' of species recolonising Millwood Brook is shown clearly in Figure 2.21 by the increase in species at sites downstream of the mine discharge point.

The BMWP scores, indicative of water quality, have improved along Millwood Brook, at points below the mine discharge, with an increase in GQA grade in several cases. Average scores per taxon have also consistently increased, indicating recolonisation by more pollution sensitive species. These species previously existed up and downstream of the polluted area and in tributaries, and have now extended their range. Recolonisations of previously disturbed areas occur by upstream larval migration, by larval drift or by adult flight (Townsend *et al.*, 1987, Jacques *et al.*, 1986). Spot sampling studies (Terrell-Nield, 1995) revealed the rapid recolonisation of species with flying adults, with caddisflies and mayflies returning just after the cessation of saline water pumping. Ephemeropterans are considered one of the major invertebrate groups least tolerant of elevated sodium chloride levels (Short *et al.*, 1991), so the cessation of pumping may have contributed towards their return. This study supports these findings and illustrates the further recolonisation by the more sedentary species, notably species

of molluscs, flatworms and oligochaetes, with a reduction in abundance of brackish water oligochaetes. Similar patterns are observed for sites around Shirebrook and Rufford collieries, both recently abandoned. Numbers of the estuarine species *Lumbricillus rivalis* and *Paranais litoralis*, previously dominant below the saline discharge, have decreased since the cessation of pumping. However, Learner *et al.* (1971) criticise the comparison of biological communities up and downstream of a polluted site, as spatial differences in the physical nature of the stream bed, not associated with pollution, may bring about changes in the structure of communities. Cairns *et al.* (1971), who studied a 30 metre stretch of a stream in France, discuss the biological recovery of damaged watercourses as a function of the biological, chemical and physical characteristics of the recovering stream, the severity and duration of the stress and the availability of undamaged areas to serve as sources for recolonising organisms, dependent on distance from the polluted site. The residual effects on non-biological components such as substrate also determine the rate of recovery. It is not apparent whether the release of heavy metals 'locked up' in the sediment below the mine discharge point at Creswell will cause any perturbations in the system, and delay recovery; Chadwick *et al.* (1986) found this problem in a watercourse receiving metal mine wastewater.

The saline minewater from Creswell previously provided dilution for the sewage discharged into Millwood Brook. Considering the value of Creswell Crags as a Site of Special Scientific Interest, the impact of the loss of this diluting effect of the 'nuisance' sewage in terms of amenity value is considerable, even though the biological and chemical quality of the brook has improved overall.

Highly variable seasonal Shannon's diversity and evenness scores were obtained for all sites along Millwood Brook/the River Poulter. Shannon's diversity index has the advantages of using a logarithmic analysis for species changes; ideal for use in the current study as the species numbers change logarithmically, and produces simple, easily comparable scores. However, there are disadvantages, namely that the index is influenced by very common species such as *Gammarus pulex*, and rare species. It would be more useful to use the Environment Agencies abundance scale (1-5, where 1 is 1-9 organisms, 2 is 10-99 etc) to reduce the sensitivity of the index. Sites sampled in the same place at three different times in the present study gave three very different

Shannon's diversity scores. Scullion and Edwards (1980) commented on the insensitivity of this index to changes in water quality in their catchment study. Dills and Rogers (1974) also found great irregularities in the index scores. Indeed, Washington (1984) questions the biological relevance of such information theory indices, and Hughes (1978) indicates that factors other than pollution, such as the sampling method, the time of year the sample is taken, the area of the sample, the taxonomic level used in calculation and the sample size all have significant effects on the index score. Depth and duration of sampling had no apparent influence on the value of the index. These factors are seldom taken account of in similar studies (Hughes, 1978). However, Dills and Rogers (1974) comment on the usefulness of the index to show the seasonal changes in apportionment of individuals among species, not illustrated by simple individual/species counts. It would be pertinent for future studies to explore the use of other indices (see, for example, Marshall and Elliott, 1997) such as the Levins index, which gives a greater weight to dominant items in the sample (in this case, macroinvertebrates).

The alien amphipod *Gammarus tigrinus* has extended its range in the River Idle system over the last 25 years, spreading from the Liverpool docks into the Trent and Mersey canal around the Cheshire salt fields. It extended its range into the River Idle via the Chesterfield canal from the River Trent (Severn-Trent Water Authority Report, 1988b). The reports of Bird (1989) and Everett (1991) gave a general pattern of colonisation of Gammarids in the River Idle catchment. *G. pulex* was confined to the headwaters of the watercourses, where conductivity is low, whereas *G. tigrinus* was found downstream of the mine effluents where conductivity was elevated. The two species can coexist downstream of these inputs as the discharges are diluted. Since these studies, noticeable changes have occurred in the distribution of two gammarids.

G. tigrinus appears to have been 'flushed' from Millwood Brook, from the boundary of its range at site 12 in 1986 (Bird, 1989) to site 18. Terrell-Nield (1995) noted the rapid movement of *G. tigrinus* from site 11 (450m below the discharge) to above the site of discharge (site 5) between September 1991 and March 1993 (a distance of 500m), then the rapid flushing of the species to site 15 (1200m) by spring 1994 after the cessation of pumping. This study highlights the further retreat of *G. tigrinus* to site 18, a movement of 9500m between spring and winter 1994. Although Dorgelo (1974) found salinity to be of primary importance in the distribution of *G. tigrinus*, increases in

water temperature during early summer has been demonstrated as an orientation cue for the upstream migration in Gammarids in a small French chalk stream (Goedmaker and Pinkster, 1981). Therefore, mine effluent at Creswell, flowing at 20^0C (Bird, 1989) may have provided the cue for *G. tigrinus*, cessation of pumping therefore removing this stimulus. Laboratory studies support the theory that migration towards a higher temperature occurs, in preference to a higher conductivity, when *Gammarus tigrinus* is presented with combinations of both in choice chamber environments (Dean, 1998). Indeed, Pinkster *et al.* (1977) indicated that temperature is the most important factor in the reproductive cycle of *G. tigrinus,* as pH, chlorinity and oxygen saturation were all stable within a certain range at the sites assessed. Savage (1982) believed that the success of *G. tigrinus* in Cheshire was due to 2 successive summers with above average temperatures, the lower temperatures in later years led to a rapid decrease in numbers. Supporting these findings, Welton and Clarke (1980) found that there is a decrease in brood development time with increasing temperature for *G. pulex*. These observations of the beneficial effects of increased temperature on *G. tigrinus* are supported by the observations of various authors (see section 2.2.2.4) who note the success of *G. tigrinus* in its natural habitat of high temperatures in the USA. The reduced salinities seem to have reduced the competitiveness of *G. tigrinus* with *G. pulex,* the latter recolonising previously saline sites (Figure 2.19) in Millwood Brook and elsewhere in the River Idle catchment. *G. pulex* also appears to have colonised Sookholme Brook and Shirebrook Stream at Sookholme - although *G. tigrinus* surprisingly did not previously colonise and take advantage of the high salinity. The reduction of chloride concentrations over the period 1986 - 1995 (since the closure of Shirebrook in 1993) appears to have favoured *G. pulex* upon its arrival. There appears to be a change in water chemistry of the site RW2 which receives discharges from Rufford colliery, abandoned in 1993 - a decrease in chloride and conductivity perhaps favouring an increase in BMWP and species diversity, to the detriment of *G. tigrinus* which is no longer present.

G. pulex has recently colonised sites at the confluence with the River Idle in previously exclusive *G.tigrinus* areas. This could be partly due to the closure of Bevercotes mine in 1992, which has several discharge points into the area, and/or a generally enhanced water quality in the catchment. Where conductivities have been

reduced by dilution, the two species may coexist if *G. pulex* is reintroduced *via* a clean tributary. Once *G. tigrinus* is established in an area it is able to maintain populations at low conductivities (Bird, 1989). The broad watercourses with a variety of substrates available at these sites may allow niche segregation. As Bird (1989) observed that *G. pulex* is found under pebbles which provides shelter from the current and *G. tigrinus* digs into the substrate for the same reason, it is possible that they occupy different habitats within the river. Indeed, *G. tigrinus* is generally found in stagnant, very slow running waters in its natural habitat in southern USA (Van Maren, 1974) as is found in its principal habitat along the River Poulter (sites 22-24). If the two species do coexist by niche segregation then they will not be in direct competition.

Younger *et al.* (1995) highlight the benefits of continued pumping. For the responsible party, the option of continued pumping is less expensive than the option of abandoning pumping, primarily due to the saving of the clean up costs of the environmental impacts of the uncontrolled discharge of flooded mine contaminated waters (see section 1.2.1). Temporary cessation of pumping or a reduction of pumping rate were not viable options. The resumption of pumping is often required to maintain a water free environment for pits that are still operating and interlinked geologically to the abandoned collieries. The temporary cessation of pumping, such as for Creswell mine, will lead to a recovery in groundwater levels and their contamination, so that when the pumps are switched on again, they would be pumping initially poorer quality water. Pumping is expected to resume at Creswell within the next few years to protect 4 operating pits to the East (1-4 in Table 1.2). This water may have a greater concentration of iron, as found in discharges in Yorkshire, Northumbria and Wales. Examination of the effects of ferruginous discharges from long abandoned mines in these regions is considered further in Chapters 4 and 5.

2.7 Summary

Since the cessation of saline mine discharges into Millwood Brook in spring 1993, there has been the rapid recolonisation of species of up to 271% below the discharge point. Initially (Terrell-Nield, 1995), species with flying adults such as trichoptera and ephemeroptera have recolonised, but more sedentary species such as

molluscs, flatworms and oligochaetes have now returned. The estuarine salinity tolerant species *Lumbricillus rivalis* and *Paranais litoralis* have been lost. *Gammarus tigrinus*, the saline tolerant amphipod appears to have been 'flushed' from the system and has been replaced by the native *Gammarus pulex*. Following the research of Bird (1989) and a literature review of studies of the natural environment of *Gammarus tigrinus* on the East coast of the USA, it is suggested that it is the loss of warm, saline created by the mine discharge that has resulted in the retreat of the organism. Similar recolonisation of pollution intolerant species, and changes in the distribution of gammarids, has been found elsewhere in the River Idle catchment.

Chapter 3. Mathematical Analysis of Field Data from East Midlands Sites.

3.1 Introduction

Following the detailed survey on the effects of cessation of saline pollution in the River Idle Catchment, the changes that have occurred in macroinvertebrate distribution since the study of Bird (1989) can be modelled mathematically. Bird (1989) conducted data analysis to group sites according to their salinity and physical characteristics; species associated with each salinity group were then identified. Using these species groups, a predictive model was constructed whereby a suggested species list for any site in the East Midlands could be produced upon the input of environmental data which included salinity.

3.1.1 Aims

It is of value to the current study to establish how changes in the species distributions in the River Idle catchment translates into changes of site and species groupings in the model, and to identify the environmental parameters now most influential in determining macroinvertebrate groupings since the cessation of saline inputs.

3.1.2 Modelling and Data Analysis

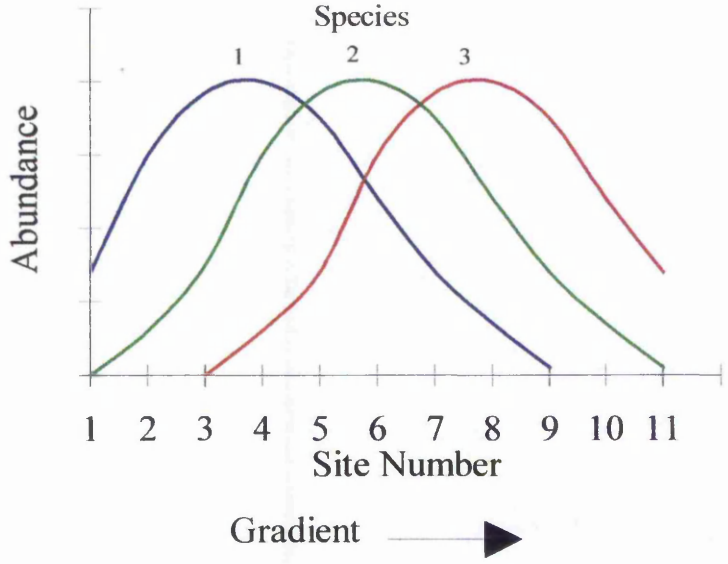
Species normally experience the effects of more than one environmental variable simultaneously. Thus, multivariate analysis is often required to account for the observed variation in abundance or presence / absence of species (Johnson *et al.*, 1992). Scaling, or the ordering of multivariate data, involves investigators attempting to order entities within a data set (eg. quadrat or site) on the basis of the similarity of their characteristics (eg. species presence or absence). The aim is to establish the existence of an underlying order/trend/gradient to the data, and methods used for this are called ordination techniques.

DECORANA (DEtrended CORrespondence ANAlysis) is a commercially available computer program (Hill, 1994) for performing species ordinations by the

method of reciprocal averaging. Sites are placed on two or more mathematically derived axes according to their species content. Therefore a Q-mode analysis is employed; namely determining sample site affinities/relationships based on species attributes. R-mode analysis is the analysis of species affinities based on sample site attributes.

Placing a site on the axes involves deriving its co-ordinates ie. a score for each axes. The site scores are the mean scores of the species (or families) which occur within them. The aim of the axes is to illustrate the 'turnover' of species between sites, so that sites with fewer species in common are further apart. The axes units are measured in 'standard deviations', multiplied by 100 to give the final axes score. A species may be expected to rise to its mode (such as in Figure 3.1) and disappear again in about 4 standard deviations. An ordination of length 4 standard deviations therefore means that species occurring at one end of the gradient are almost completely absent at the other end (Hill, 1994). It follows that sites more than 4 standard deviations apart have few, if any, taxa in common. Therefore samples from sites containing such species will be far apart on the DECORANA axes plot. For the DECORANA ordination, the eigenvalues are a representation of the variance accounted for by each axis and the variance in the sites that axis 1 and 2 explained can be calculated by dividing the sum of eigenvalues for axis 1 and 2 by the sum of all eigenvalues for axes 1 to 4.

Figure 3.1. The hypothetical distribution of three species along an environmental gradient.



As the axes are mathematically related to species turnover, the relationship between the biological communities and environmental variables can be examined by correlating the variables with axis scores.

A problem in generating axis scores, however, is to ensure that the second axes is not related to the first, so as to be separately interpretable. Reciprocal averaging does not address this problem, and scores plotted exhibit patterns such as the 'arch effect', which are mathematical artefacts due to axis 2 scores being related to axis 1. If one environmental gradient is being measured eg. iron pollution, samples of a series of sites experiencing increasing concentrations of pollutant will each contain the peaks of several species, so introducing non-linearity into the data, as illustrated in Figure 3.1. When these sites are plotted on two ordination axes, an arch or 'horseshoe' appears. As there is only one gradient of pollution, the two dimensional structure of the data is a mathematical artefact, not representing any real structure in the data (Hill and Gauch,

1980). The arch size is dependent on the distance between sites on the gradient. Another problem is the 'scale contraction effect'. This is the distortion of the relative distances between sites and species on an axes. Species do not appear and disappear at a steady rate because sampling often 'truncates' a species true range (eg. for species 1 on Figure 3.1, the species entire ecological range is not sampled). Therefore some species will have a very low dispersion amongst sites, simply due to the investigator's choice of samples. DECORANA is a method of 'reciprocal averaging', derived from the fact that species scores are the averages of the sample scores, and, reciprocally, the sample scores are averages of the species scores. 'Re-scaling' by the DECORANA algorithm (DEtrended CORrespondence ANAysis, Hill, 1994) attempts to scale the data to ensure species packing/turnover is constant along the gradient to ensure a smooth turnover of species between samples. Hence the standard deviation of species scores within samples is effectively constant. A summary of the DECORANA algorithm is as follows:

1. Allocate preliminary species scores, set to unit variance.
2. Calculate site score which is the average of the scores of the species that occur within it.
3. 'Detrend' the site scores so that they are unrelated to the second axis. This removes the 'arch effect' by dividing the data of the first axis into segments, and adjusting the sample scores of these segments so that they have a mean of zero. This effectively flattens the horseshoe. The scale contraction effect is avoided by rescaling the axes where necessary, particularly at the ends.
4. New species scores are calculated as weighted mean scores of the sites in which they occur.
5. The new species scores are set to unit variance

Stages 2-5 are repeated until the sample scores stabilise at stage 2.

Eventually in the iterative process above, the scores will stabilise, and may then be taken as the final ordination. The scores can be standardised so that the variance of the new species scores is the same as that of the initial ones. The large amount of data manipulation involved in DECORANA has led to criticism (eg. Wartenberg *et al.*, 1987).

TWINSPAN (TWo-Way INdicator SPecies ANalysis, Hill 1994) is a divisive technique program which directly produces hierarchical groupings of sites by classifying both sites and species. It produces a two-way table to exhibit the relationship between them as clearly as possible. The aim of this table is to throw into relief any salient features of the data, by grouping similar species and similar samples (Hill, 1994). It is considered by Gauch *et al.* (1981) to be the best method when the data set is large and complex. The program also constructs a key to the sample classification by listing the 'differential' species which are particularly diagnostic of each division of the classification, and which may indicate particular environmental conditions. The basic TWINSPAN algorithm is as follows (Hill, 1994):

1. A primary ordination is performed by reciprocal averaging, to identify initial variation in the data.
2. The ordination is divided in the middle to obtain two groups of samples.
3. Species which occur more in one side of the division are identified as 'differential'.
4. These 'differential' species are used to construct an improved ordination.
5. This improved ordination is divided at the appropriate point to produce two new groups at Level 1.
6. Return to 2, using these 2 groups to produce 4 groups in Level 2.

This continues until the algorithm reaches the predetermined level set by the user.

From the sample classification, attributes for each species are defined according to their presence or absence on each side of the sample divisions. From these species and sample classifications, an ordered two-way table is produced. A full mathematical treatment of the DECORANA and TWINSPAN algorithms can be found in Hill (1994). TWINSPAN and DECORANA both have the same method of construction (using eigenvalues; Hill and Gauch, 1980) so they should give supportive results (see Figures 3.2 and 3.3 at this stage). However, whereas TWINSPAN groups samples according to the taxa they contain, ordination techniques such as DECORANA arrange the samples along a gradient without grouping them. The groupings determined by TWINSPAN therefore have to be placed onto the axes produced by DECORANA.

3.2 Methods

3.2.1 TWINSPAN and DECORANA modelling.

Furse *et al.* (1984) indicated the necessity of merging 3 seasons data from species lists, as substantial local variation in time and space and in population levels can result in invalid correlations between biological and chemical factors if seasonal patterns are not accounted for. The species data from appendices IV and VII was entered into the TABLEFIT program (Hill, 1993) which records sample names and species composition (presence/absence) whereby each species is represented by an 8 letter code. TABLCORN (Hill, 1993) was then run to convert this data into Cornell Condensed format, suitable for use by the TWINSPAN and DECORANA computer programs. Standard program documentation was followed. The data collected was grouped into sets for analysis as follows:

(i) The 24 sites on Millwood Brook/River Poulter and its inputs, with macroinvertebrates identified to family level. This was run to establish if the loss of information by defining taxa to family rather than species level has subsequent effects on the variables significantly correlating with DECORANA axes.

(ii) As above, with the macroinvertebrates identified to species level, as far as possible.

(iii) The 24 sites on Millwood Brook/River Poulter and its inputs together with 15 of the additional sites sampled in the River Idle catchment.

For the 24 Millwood Brook sites, the TWINSPAN program was run at default settings to 3 levels of division, beyond which Ormerod and Edwards (1987) argued groups ceased to be ecologically meaningful. This is because that after this, the procedure produces more site groups containing single sites, so losing information on relationships between sites. However, runs at 3 levels of division for the 39 test sites failed to satisfactorily divide them, so 5 levels of division were used to form smaller groups. The minimum group size for a division was set at 2, and the program was set to use species composition (ie. presence/absence). DECORANA was run to default values, except that the option to down-weight rare species was employed to avoid such species having a disproportionate effect on the analysis. Therefore the undue emphasis placed on accidental or casual occurrences of animals is minimised.

For each set of data, the DECORANA axis scores were plotted. Only axes 1 and 2 are shown; further variation in the data set could be displayed but much of the major variation is shown on the first two axes. For the DECORANA ordination, variance accounted for by axis 1 and 2 was calculated from eigenvalues. The TWINSPAN groups are shown as polygons drawn over the site plots. Environmental data held in appendices I, VI, VIII and Tables 2.2 and 2.4 was then correlated to the axes scores using the Pearson's product moment correlation coefficient, using the MINITAB 10.1 statistical package, and the best correlations used to calculate regression equations.

3.3 Results of TWINSPAN and DECORANA analysis.

3.3.1 Family Level Analysis.

Data identifying taxa to family level only was included in the TWINSPAN classification (Figure 3.2) and the DECORANA ordination (Figure 3.3). The environmental variables correlated with the axes is shown in Table 3.1. Arrows on the DECORANA plot indicate a positive or negative correlation with the given variable with the relevant DECORANA axis.

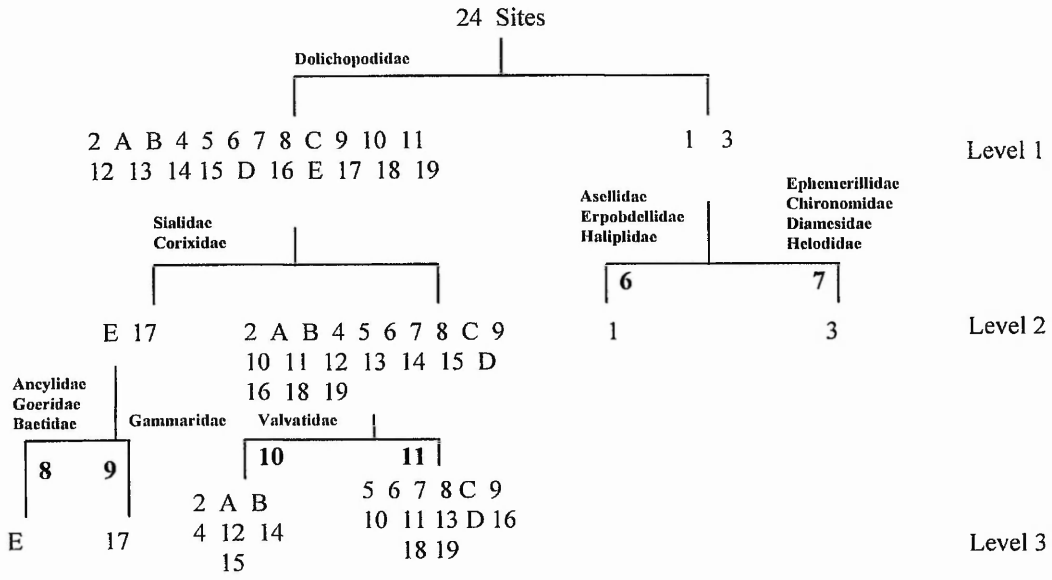


Figure 3.2. Classification of Millwood Brook sites using TWINSPAN with Family level identification of taxa. TWINSPAN Groups of sites are shown from each of three levels of division, and are indicated by number. Indicator families for each division are shown.

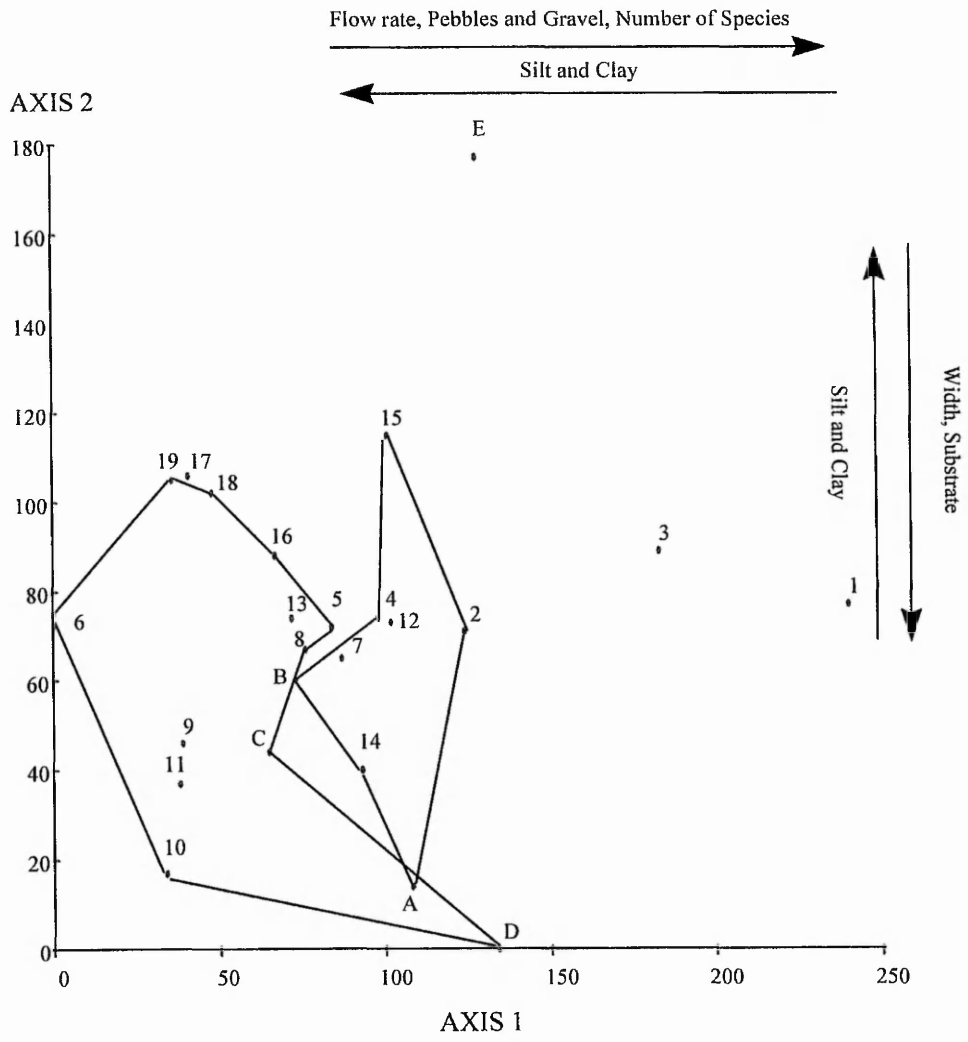


Figure 3.3 DECORANA Plot of Millwood Brook site groups. Ordination using family data.

The eigenvalues for the axes are as follows; axis 1=0.323, axis 2=0.167, axis 3=0.104, axis 4=0.066. From these eigenvalues, axis 1 and 2 explained 74.2% of the variance amongst sites. It is interesting to note that relatively clean sites 1 (Markland grips) and 3 (right hand stream) segregate out at the second level of division. From the ordination, flow rate, percentage cover by silt and percentage cover by pebbles and

gravel were the most highly correlated for axes 1. For axes 2, correlation with percentage silt and clay and substrate phi was significant at 5%. Results of the simple linear regression analysis between DECORANA axes site scores (for family and species level analyses) and variables correlating significantly with the axes (Table 3.2) confirm these correlations as significant.

Table 3.1. Significant correlations (Pearson) between DECORANA scores on Axes 1 to 4 calculated using Millwood Brook data to Family level of classification and Environmental variables measured between October 1994-May 1996.

Variable	AXIS 1	AXIS 2	AXIS 3	AXIS 4
% silt/clay	-0.622***	0.417 *	-	-
Flow (ms^{-1})	0.658***	-	-	-
% pebbles/gravel	0.537**	-	-	-
Substrate phi	0.442*	-0.421*	-	-
Number of species	0.429*	-	0.467*	-
pH	-	-	0.462*	-
Conductivity ($mS\ cm^{-1}$)	-	-	-0.480*	-
Discharge rate ($m^{-3}s^{-1}$)	-	-	0.468*	-
% sand	-	-	-	-0.460*

Notes: r (Pearson's correlation coefficient) is significant at the 0.1% level above 0.625 (***); significant at the 1% level above 0.515 (**), significant at the 5% level above 0.412 (*) or not significant (-).

Table 3.2. Regression Equations Calculated from Millwood Brook/River Idle Catchment Environmental data and DECORANA axis 1 and 2 scores.

Sites, taxa level of identification, regression equations	Variance ratio	Significance
Millwood Brook data. Taxa Identified to FAMILY level.		
Axis 1		
(i) Mean flow rate = 0.0156 + 0.00296 * axis 1	F = 16.78	1%
(ii) % silt/clay = 116 - 0.689 * axis 1	F = 13.9	1%
(iii) % pebbles/gravel = -15.9 + 0.515 * axis 1	F = 8.9	1%
Axis 2		
(i) Substrate = 3.37 - 0.0180 * axis 2	F = 4.74	5%
Taxa Identified to SPECIES level.		
Axis 1		
(i) Mean flow rate = 0.505 + 0.00231 * axis1	F = 12.8	1%
(ii) Number of species = 25.2 - 0.0702 * axis 1	F = 11.27	1%
(iii) % pebbles/gravel = 73.5 - 0.451 * axis 1	F = 9.81	1%
(iv) % silt/clay = 4.2 + 0.516 * axis 1	F = 9.57	5%
Axis 2		
(i) Distance from source (km) = 1.81 + 0.0858 * axis 2	F = 16.4	1%
(ii) Discharge rate = -0.0330 + 0.00450 * axis 2	F = 11.18	1%
Millwood Brook/Test Site data. Taxa Identified to SPECIES level.		
Axis 1		
(i) Mean flow rate = 0.499 - 0.00225 * axis 1	F = 16.84	1%
(ii) % pebbles/gravel= 73.1 - 0.460 * axis 1	F = 12.6	1%
(iii) % silt/clay = -1.6 + 0.474 * axis 1	F = 12.03	1%
(iv) Number of species = 23.3 - 0.0548 * axis 1	F = 7.73	1%
Axis 2		
(i) Discharge rate = 0.407 - 0.0035 * axis 2	F = 10.85	1%
(ii) Distance from source = 9.73 - 0.0760 * axis 2	F = 10.52	1%
(iii) % emergent macrophytes = -6.3 +0.258 * axis 2	F = 8.82	1%

3.3.2 Species Level Analysis.

All the identified taxa were included in the TWINSPAN classification (Figure 3.4). Figure 3.5a shows the clustering of sites on the site map of Millwood Brook and the River Poulter.

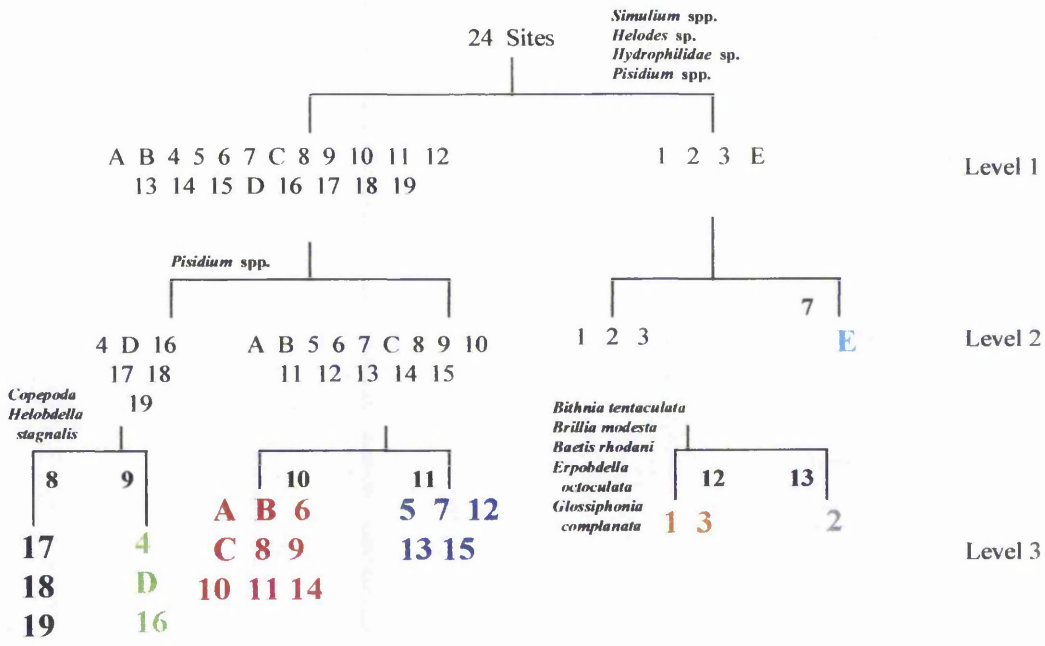


Figure 3.4. Classification of Millwood Brook Sites using TWINSPAN with Species level identification of taxa. Colour linked to group membership. See Figure 3.5a for map. TWINSPAN groups of sites are shown from each of three levels of division, and are indicated by number. Indicator species for each division are shown.

Figure 3.5a. Sketch Map showing Millwood Brook and the River Poulter together with the position of Creswell, Nether Langwith and Whitwell (Terrell-Nield, 1995). Colour of site code indicates inclusion into a TWINSPAN Level 3 end group. Refer to Figure 3.4 for TWINSPAN classification of sites at species level.

KEY: Group 7; Group 8; Group 9; Group 10; Group 11; Group 12; Group 13

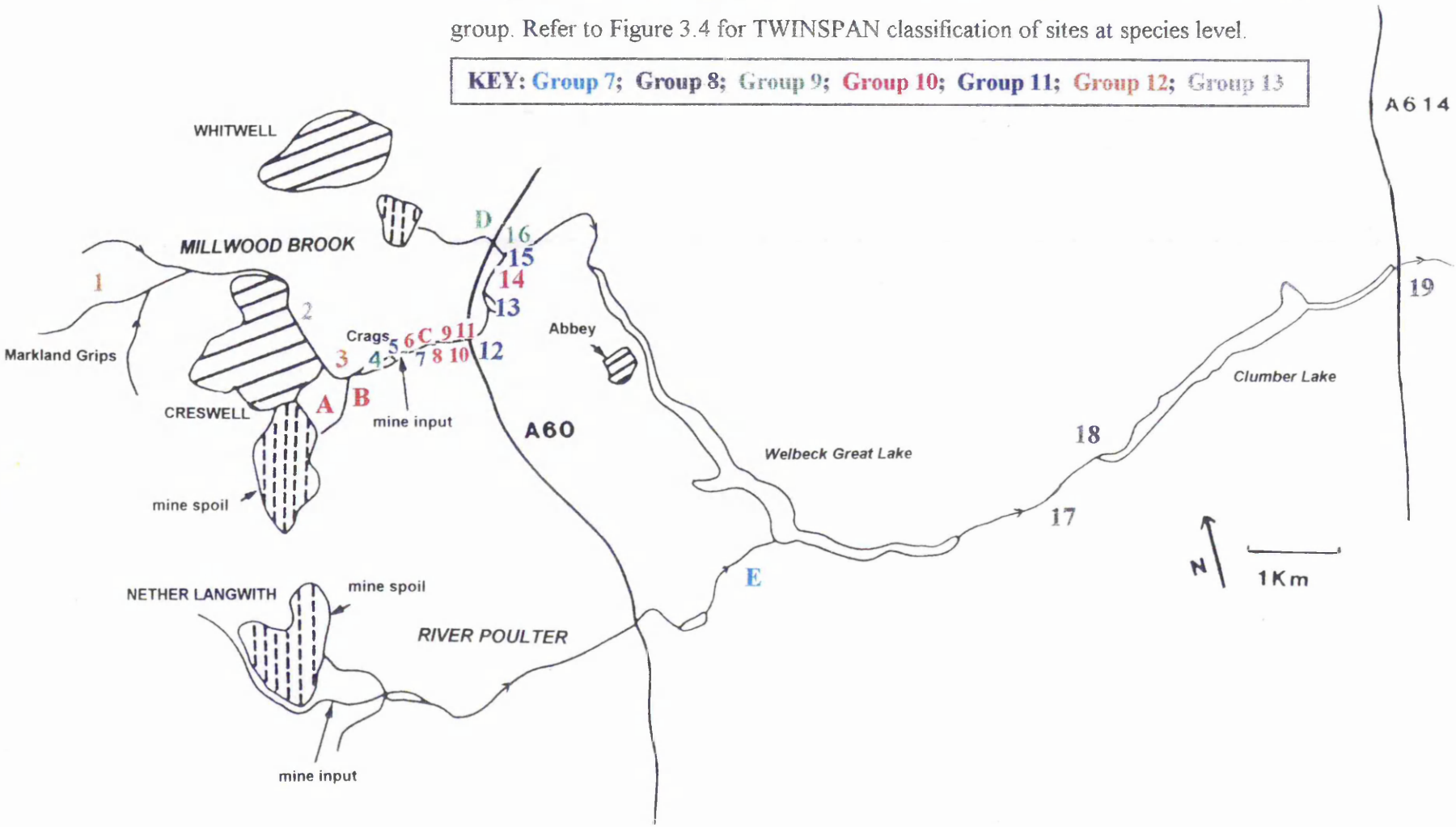
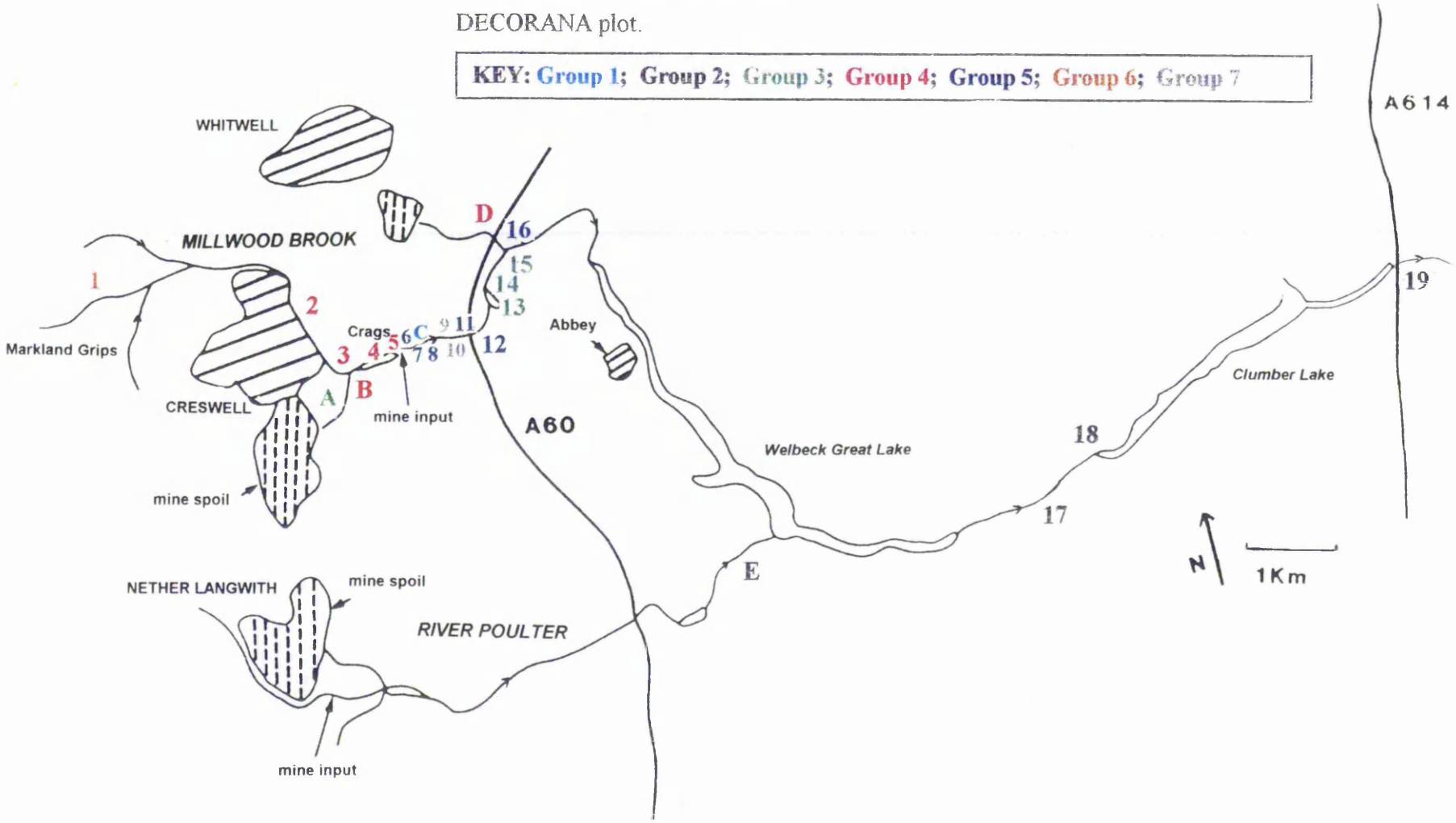


Figure 3.5b. Sketch Map of Millwood Brook and the River Poulter (Terrell-Nield, 1995) showing Bird (1989) site membership to TWINSPAN Level 3 end groups. Colour of site code indicates inclusion into a group. Refer to Figure 3.7 for DECORANA plot.

KEY: Group 1; Group 2; Group 3; Group 4; Group 5; Group 6; Group 7



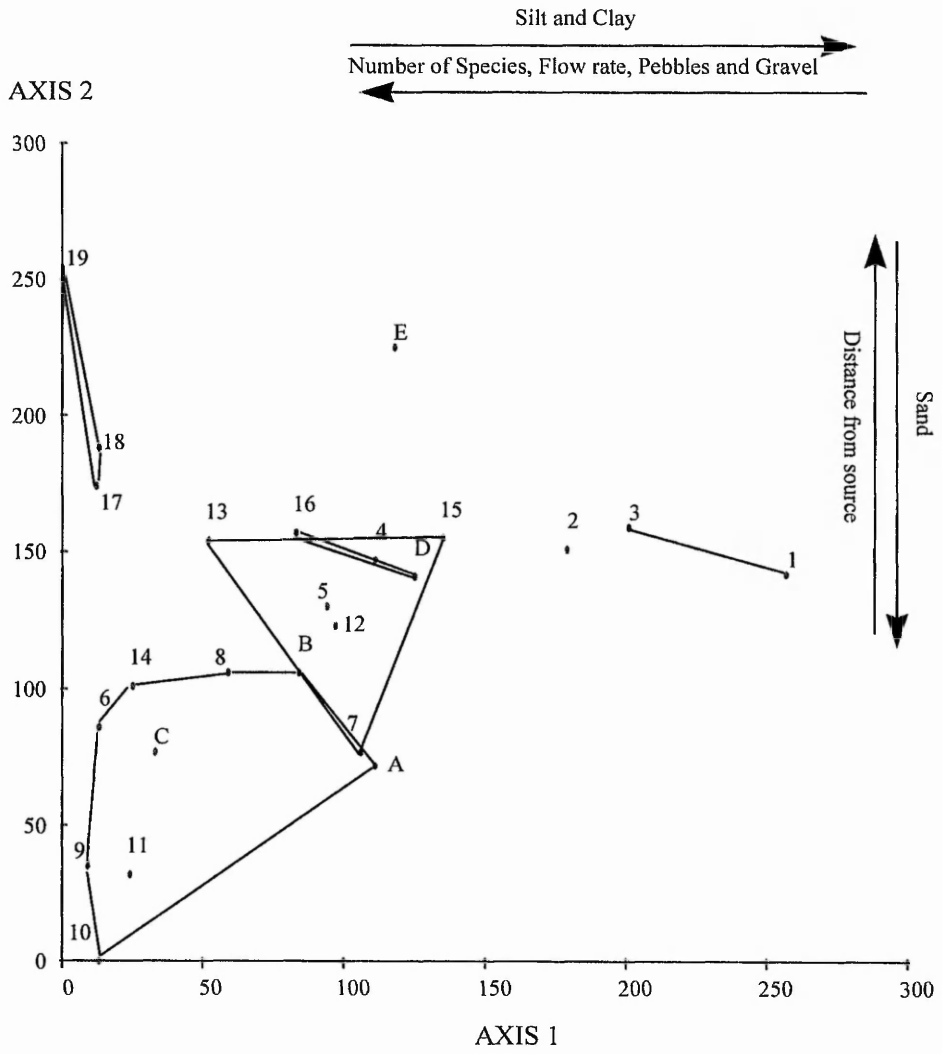


Figure 3.6. DECORANA Plot of Millwood Brook Site Groups. Ordination using species data.

Table 3.3. Significant Correlations (Pearson) between DECORANA scores on Axes 1 to 4 calculated using Millwood Brook data to species level of classification and Environmental variables measured between October 1994-May 1996.

Variable	AXIS 1	AXIS 2	AXIS 3	AXIS 4
Flow (ms^{-1})	-0.606**	-	-	-
Number of species	-0.582**	-	-	-
% pebbles / gravel	-0.555**	-	-	-
% silt / clay	0.551**	-	-	-
pH	-0.544**	-	-	-
% emergent macrophytes	-0.458*	-	-	-
Substrate	-	-	0.443*	-
Distance from source (km)	-	0.654***	-	-
Discharge rate ($m^{-3}s^{-1}$)	-	0.581*	-	-
Depth (cm)	-	0.454*	-	-
Width (m)	-	0.447*	-	-
% sand	-	-	-	0.414*
% boulders / cobbles	-	-	0.534**	-
Temperature (^{o} C)	-	-	-0.461*	-

Notes: r (Pearson's correlation coefficient) is significant at the 0.1% level above 0.625 (***); significant at the 1% level above 0.515 (**), significant at the 5% level above 0.412 (*) or not significant (-).

The DECORANA ordination is presented in Figure 3.6. For the DECORANA ordination, the eigenvalues for each axis are as follows; axis 1=0.390, axis 2=0.238, axis 3=0.153, axis 4=0.114. From these eigenvalues, axis 1 and 2 explained 70.2% of the variance amongst sites. The environmental variables were correlated with the axes (Table 3.3). Flow, number of species, and substrate were most closely correlated with axes 1 (significant at 1%), and distance from source (significant at 0.1%), discharge rate, width and depth closest with axes 2. Axis 1 shows that as silt and clay become greater components of the substratum, the number of species decreases. It is interesting to note the extra information contained in species level data has led to further patterns being found in the data; namely the extra correlations of distance from source and discharge rate with axes 2. It can be concluded that this information was masked using family treatment of data.

The first level of division separated out sites 1,2,3 and E; sites 1,2 and 3 are generally clean sites closest to the source of Millwood Brook. Sites 17, 18 and 19 also were separated at division 3, with the highest number of species, lowest flow and highest pH. Other notable groups were found at level 3 which contained sites in three main ranges of distance from the source, flow rate, pH and species number. Comparing this ordination with that of the previous family ordination, major differences can be observed. However, it can be noted that sites 1, 2, 3 and E still separate out from the other groups.

Comparing the correlations with those of the ordination of family groups, it is interesting to note that although the correlating variables are very similar, the sign of each correlating variable value is reversed. For example, axis 1 correlated negatively and significantly with percentage silt and clay (-0.622) for the classification at family level. However, for the species analysis, the same variable correlated negatively (0.551). This is perhaps an artefact of the DECORANA algorithm used. The actual direction of significance of the correlations between environmental variables and axes scores is not of great relevance; site separation on the axes is of far greater importance.

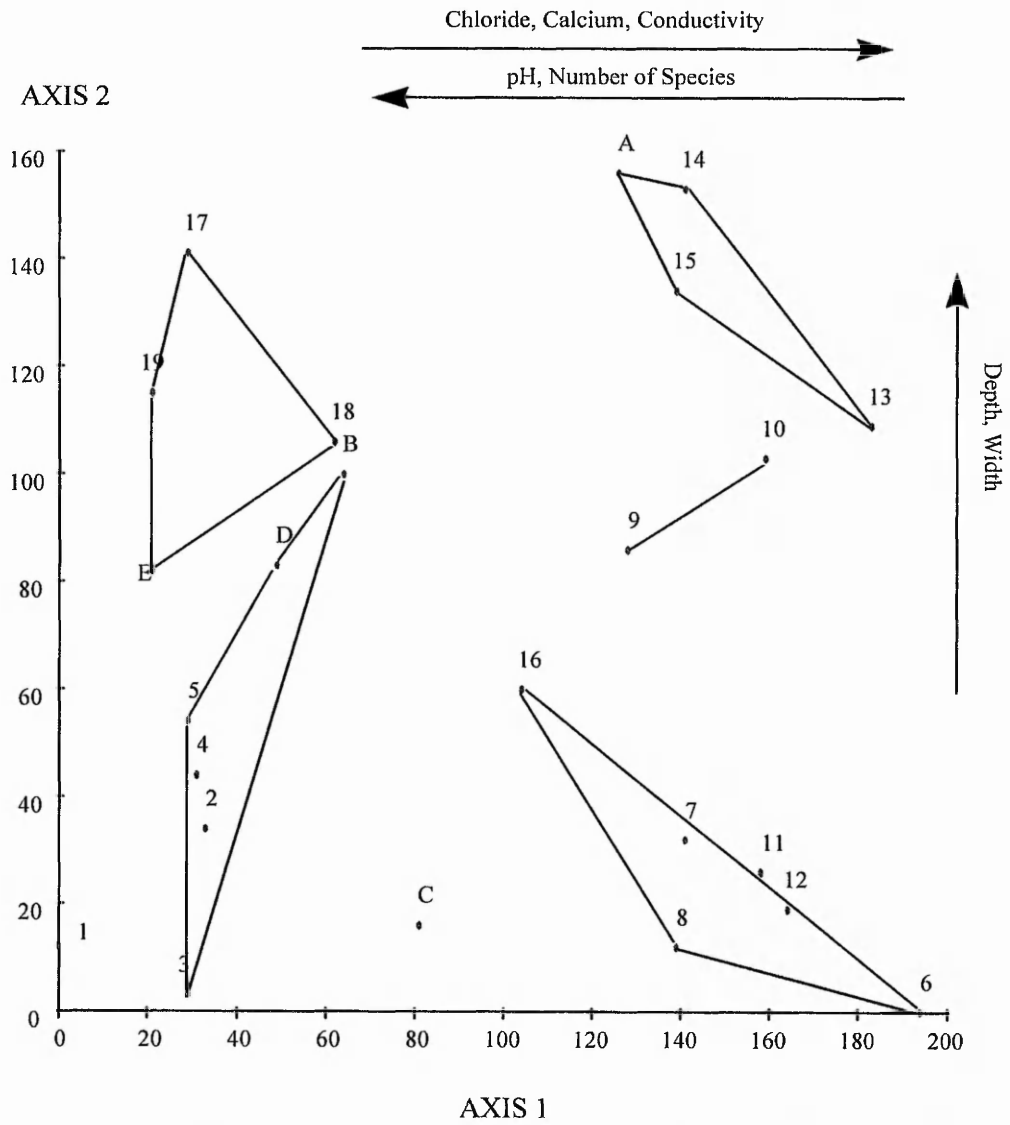


Figure 3.7. Bird (1989) Millwood Brook Site Groups. Ordination plot using species data. Strongest correlations of environmental variables with axes illustrated. See Figure 3.5b for map of end group distribution.

A joint ordination of Bird (1989) and the present data failed to show any site groups containing sites from both studies, probably due to the great differences in species composition between the studies. Incompatibility between mean data for variables between Bird (1989) and the present study, meant that correlations with DECORANA axes had to be performed on separately ordinated data sets. A direct comparison of these results with those of Bird (1989), in Figure 3.5b and 3.7, reveals marked changes in TWINSPAN site groupings. Comparison of Figures 3.5a and 3.5b indicate that the changes are three-fold. Firstly, whereas sites 6 - 15 downstream of the mine discharge clustered into four groups in the study of Bird (1989), the same sites now only cluster into 2 larger groups (Groups 10 and 11). This illustrates how removal of the specific zones created by the mine pollution removes the differences between the sites in terms of specialist species for each zone. Now more uniform conditions downstream creates very similar sites in terms of macrofauna, hence the loss of groupings. Secondly, sites 17, 18 and 19 still cluster together. These sites further downstream were originally less impacted by mine pollution, so the cessation of discharges does not break up the cluster. Finally, sites upstream of the mine discharge (3, 4, 5 and B) clustered together in the study of Bird (1989), now these sites cluster more closely in several groups also containing sites downstream of the discharge (Groups 9, 10, 11 and 12). This is possibly a consequence of the extreme environmental conditions causing loss of species specific to upstream sites, so producing up and downstream sites which are less well differentiated. This is discussed further in section 3.4.

3.3.3 Millwood Brook and test sites analysed at species level of classification.

All the identified taxa were included in the TWINSPAN classification, the results presented in Figure 3.8. Site positions are indicated on Figure 2.19 in Chapter 2. Immediately apparent is that the site at Bilsthorpe (BL) divides off at level 1 from all other sites. It is the only watercourse sampled in the catchment heavily polluted by saline discharges (from Bilsthorpe colliery). Vicar Water (VW2) receiving intermittent saline discharges from Clipstone mine is grouped by itself. Sookholme Bath sites (S2-S5) receive mildly saline discharges from treatment ponds; these are grouped with the

Rainworth Water sites, downstream from Bilsthorpe colliery. The reasonably diverse sites E, 19, ME4, CM and L1 also group together. Also apparent is that sites 1 (Markland Grips), 3 (Right Hand Stream), LA (Lathkill Dale) and P3 (Poolsbrook, Stavely) divided off at the second level of division. Sites 1, 3, and LA are clean streams with a diverse macrofauna; 1 and LA are especially different in species composition to the other sites. P3 is notably organically polluted, being in direct contact with a sewage discharge pipe, and has an unique chemistry outside that of the Idle catchment. When the groups were drawn on DECORANA axes 1 and 2 (Figure 3.9), the groups clearly overlap.

Figure 3.8. Classification of Millwood Brook and R. Idle Sites using TWINSPAN with 'species' level identification of taxa.

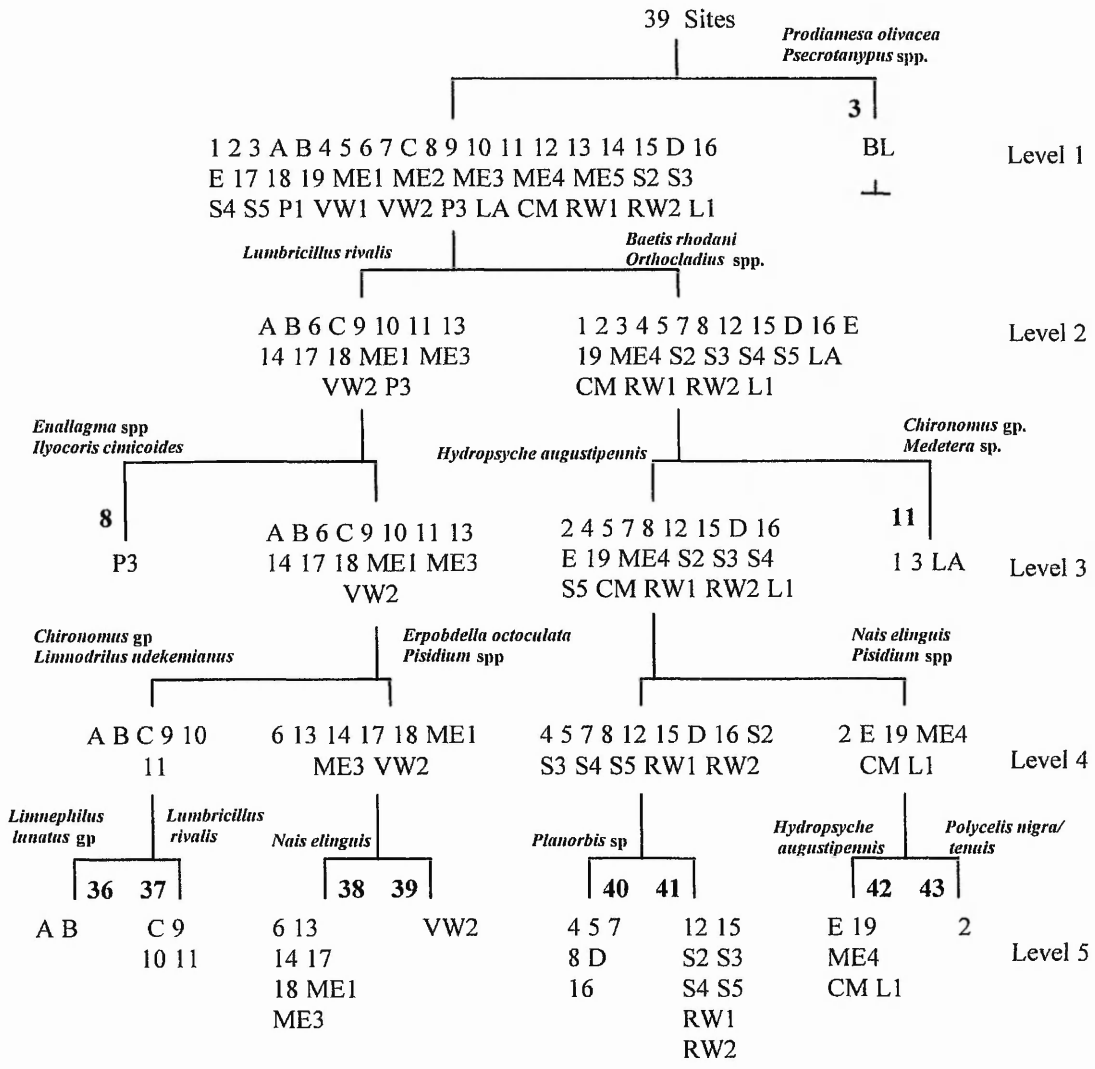


Figure 3.8. Classification of Millwood Brook and River Idle Catchment (Test) Sites using TWINSPAN with 'species' level identification of taxa. TWINSPAN Groups are shown from each of the five levels of division, and are indicated by number. Indicator species for each division are shown.

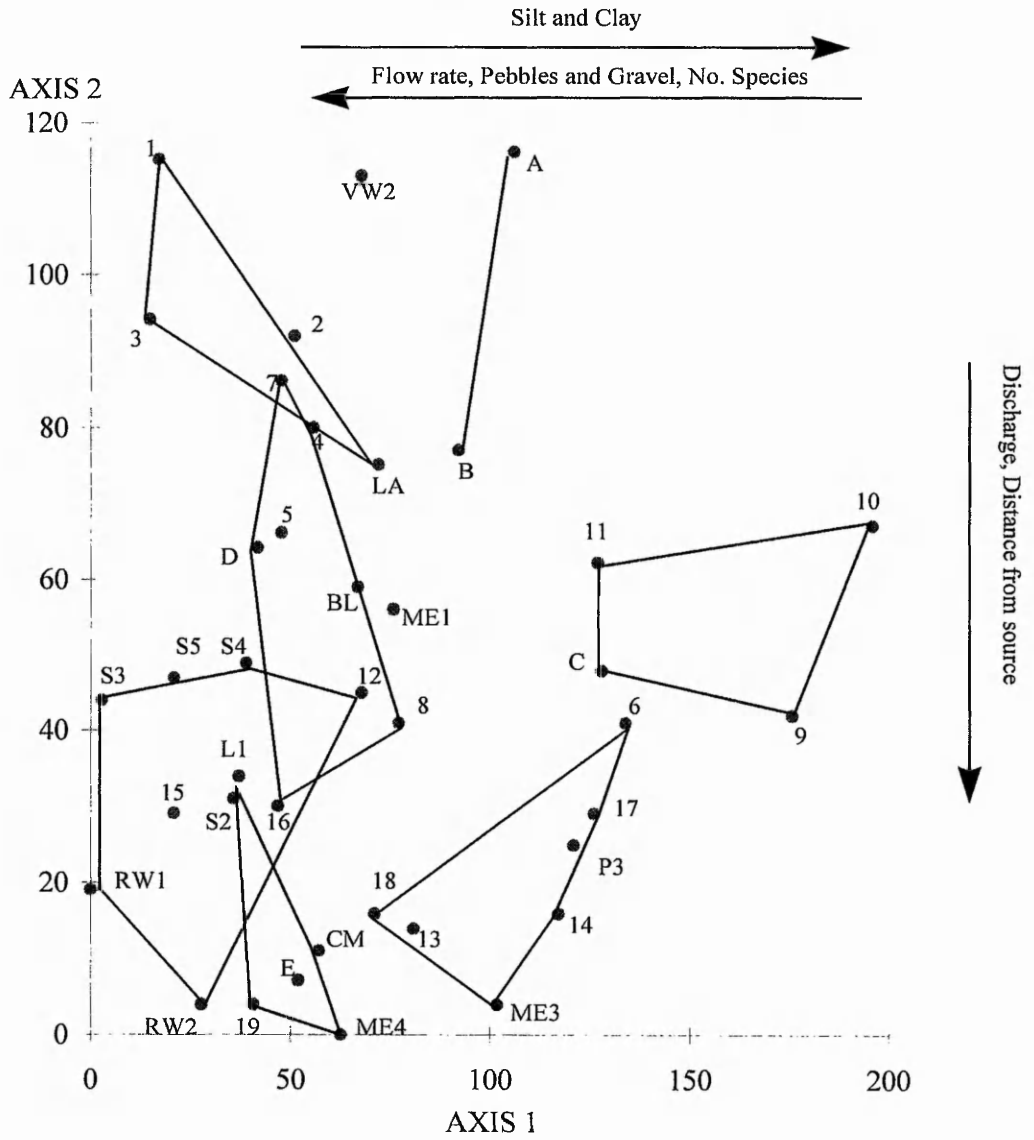


Figure 3.9. DECORANA Plot of River Idle Site Groups. Ordination using species data.

This set of DECORANA scores was correlated with a range of physical and chemical variables and the calculated correlation coefficients are shown in Table 3.4. The correlation analysis shows that flow rate, and substrate characteristics (percentage cover by pebbles and gravel, silt and clay) dictate species groupings on axis 1. Distance from source, discharge rate and percentage macrophytes correlate with axis 2. Axes 3

and 4 correlated more weakly, and there were few significant correlations. The strongest correlations with chloride and conductivity on Axis 4 suggests underlying background influences from these once dominant factors.

Table 3.4. Correlations (Pearson) between DECORANA scores on Axes 1 to 4 calculated using Millwood Brook/River Idle (test) site data and Environmental variables measured between October 1994-May 1996.

Variable	AXIS 1	AXIS 2	AXIS 3	AXIS 4
Flow (ms^{-1})	-0.559***	-	-	-
% pebbles / gravel	-0.504**	-	-	-
% silt / clay	0.495**	-	-	-
Number of species	-0.416**	-0.365*	-	-
Discharge rate ($m^{-3}s^{-1}$)	-	-0.476**	-	-
Distance from source (km)	-	-0.471**	-	-
% emergent macrophytes	-	-0.439**	-	-
Depth (cm)	0.393*	-	0.336*	-
Conductivity ($mS\ cm^{-1}$)	-	-	-	-0.858***
Chloride ($mm\ l^{-1}$)	-	-	-	-0.843***
log Chloride ($mm\ l^{-1}$)	-	-	-	-0.403*

Notes: r (Pearson's correlation coefficient) is significant at the 0.1% level above 0.510 (***); significant at the 1% level above 0.413 (**), significant at the 5% level above 0.320 (*) or not significant (-)

Results of the regression analysis (Table 3.2) confirm the relationships found in this regional study.

3.4 Discussion

It is interesting to note the changes in groupings of the sites in the model, as compared to Bird (1989), discussed in 3.3.2. From correlation analysis, the cause of this appears to be the loss of a chloride gradient from polluting mine discharges in controlling the site groupings. The results support the hypothesis that substrate and river flow parameters now control the species distributions. Flow and substrate are the most influential parameters in all three analyses correlating significantly with axis 1. Discharge rate and distance from source correlate to axis 2 (Tables 3.3 and 3.4). However, due to lack of extreme values and the inherent similarity between the watercourses studied, the correlations are not as high as those of Bird (1989). It is assumed that the incorporation of further environmental variables will aid in discrimination between sites, for example the influence of sewage fungus as a substrate and ammonia in the organically polluted area, as organics are now the main polluting inputs into the catchment and are assumed to be a factor in controlling distribution. Certainly, Wright *et al.* (1984) indicated that individual environmental variables may themselves be highly correlated to measured factors of great importance, such as the contribution made by changing food resources along the river system.

The apparent influence of these more common environmental factors is indicative of a return of a more normal environment to the River Idle catchment, in particular Millwood Brook heading towards a new equilibrium position, with species recolonising accordingly. Creswell mine discharged effluent into Millwood Brook for 98 years until spring 1993 and sampling for this study commenced in October 1994. Therefore, this return to the norm in the macrofauna took less than 2 years; a surprisingly short period of time.

There is a lack of distinct indicator species which represent the different groups; eg. *Paranais litoralis* featured in the study of Bird (1989) as a key organism to indicate highly saline sites. The loss of the saline discharges in Millwood Brook and the Idle catchment means that the groupings are not as obvious or as well defined as previously

(Figure 3.7); clearly overlap in groups can be seen, especially in Figure 3.9. Wright *et al.* (1984) experienced a similar problem in the division of sites; they argued that the changing physical gradients along river systems favour progressively changing assemblages of species from source to mouth so changing TWINSPAN group as one goes downstream. There are only sharply defined zones when there is an input from a polluting source, a tributary or a change in the physical environment. This lack of natural discontinuities between sites means that any classification is bound to be somewhat arbitrary. Moss *et al.* (1987) argue that environmental modelling should produce predictions as weighted probabilities of group membership rather than just allocating one TWINSPAN group. The previous authors suggest that sufficient sampling and incorporating additional environmental variables would improve prediction of stretches of a river to its group. Clearly in this study, extra information for the mathematical representations was require, including macrophytes, substrate type and organic pollution (nitrate/phosphates) which were anticipated as being the most influential factors in controlling the distribution of species now that pollution has ceased.

Due to the intense use of the Idle catchment by industry, many East Midlands rivers have a reduced fauna when compared to sites in the rest of the country; especially in high scoring BMWP groups such as stoneflies (Bird, 1989). This reduction adds to the difficulty in the present study in distinguishing between groups, none of which have a very high diversity of fauna (Appendices IV and VII), and even less so than the previous study of Bird (1989) since which flood/drought conditions have affected communities.

Analyses at 'family' level of identification with fewer significant correlations with DECORANA axes, indicates the problem of accuracy and the loss of information which is provided at the species level. This supports the argument that the use of species data is preferential in such mathematical representations, as families generally persist longer over environmental gradients even though species are changing, so information is lost. However, family level of identification is more commonly used in industry, due to its ease of use, requiring less expertise so reducing time required and cost (Wright *et al.*, 1984 and Furse *et al.*, 1984). Use of family data to explore the effects of coal mine pollution from long abandoned mines is explored in the next chapter.

3.5. Summary

In this chapter, macrofauna communities in watercourses which have experienced the short term effects of mine closure have been analysed mathematically. Macrofauna assemblages in the Millwood Brook and elsewhere in the River Idle Catchment have been ordinated using DECORANA, classified by TWINSPAN and related to physical and chemical factors using correlation analysis. This analysis has shown clearly that since the cessation of coal mine saline discharges in the catchment, there has been changes in the species dependent site groupings, primarily due to the loss of a chloride gradient from the polluting discharges. Since the cessation of discharges, background environmental features such as flow rate and substratum characteristics, dictate species groupings. Recolonisation of species in Millwood Brook, which received mine discharges for nearly 100 years until pumping ceased in spring 1993, has resulted in more heterogeneous groupings of macrofauna below the pollution outlet. The system appears to be heading for a new equilibrium position as a consequence of recolonisation. Similar techniques can be used to explore the longer term effects of coal mine pollution from abandoned coal mines.

Chapter 4. The Ordination, Classification and Attempted Prediction of Macroinvertebrate Assemblages in the Coal Mine Polluted Waterways of Yorkshire, Northumbria and Wales.

4.1 Introduction

4.1.1 Background to the study areas

Following the increase in awareness of the problem of coal mine pollution and new links between the Environment Agency and the coal Authority to implement remediation schemes, several surveillance programmes were conducted to quantify the extent of the problem. These programmes produced ranked lists of mines in order of biological damage caused by the pollution. Sites in three heavily mined regions were targeted: South Wales in 1993 and Yorkshire and Northumbria in 1996 (NRA, 1994; EA 1996). The environmental data collected for these programmes has been used in the modelling studies in the present and Chapter 5 of this thesis.

4.1.2 Aims

The aim of this chapter is to determine if different coal mining regions of the country have factors in common which affect stream macroinvertebrates, thus allowing development of a model which can predict the effects of mine discharges in different regions.

4.1.3 Database Site Descriptions

4.1.3.1 Yorkshire - High and Low Impact Sites

Sites in this region are divided into two subsets: high and low impact, according to the findings of the EA surveillance study (EA, 1996). In Yorkshire, most discharges are associated with shallow mines, abandoned long ago on the Westphalian exposed coal beds. Sites are listed in Appendix IX and XIV and illustrated in Figure 4.1. All sites fall in the River Don, Rother, Calder and Aire catchments. These rivers rise in the west on the upland moors of the Peak District, with a solid geology of Millstone Grit and Carboniferous limestone, and flow to join the Humber estuary in the east.

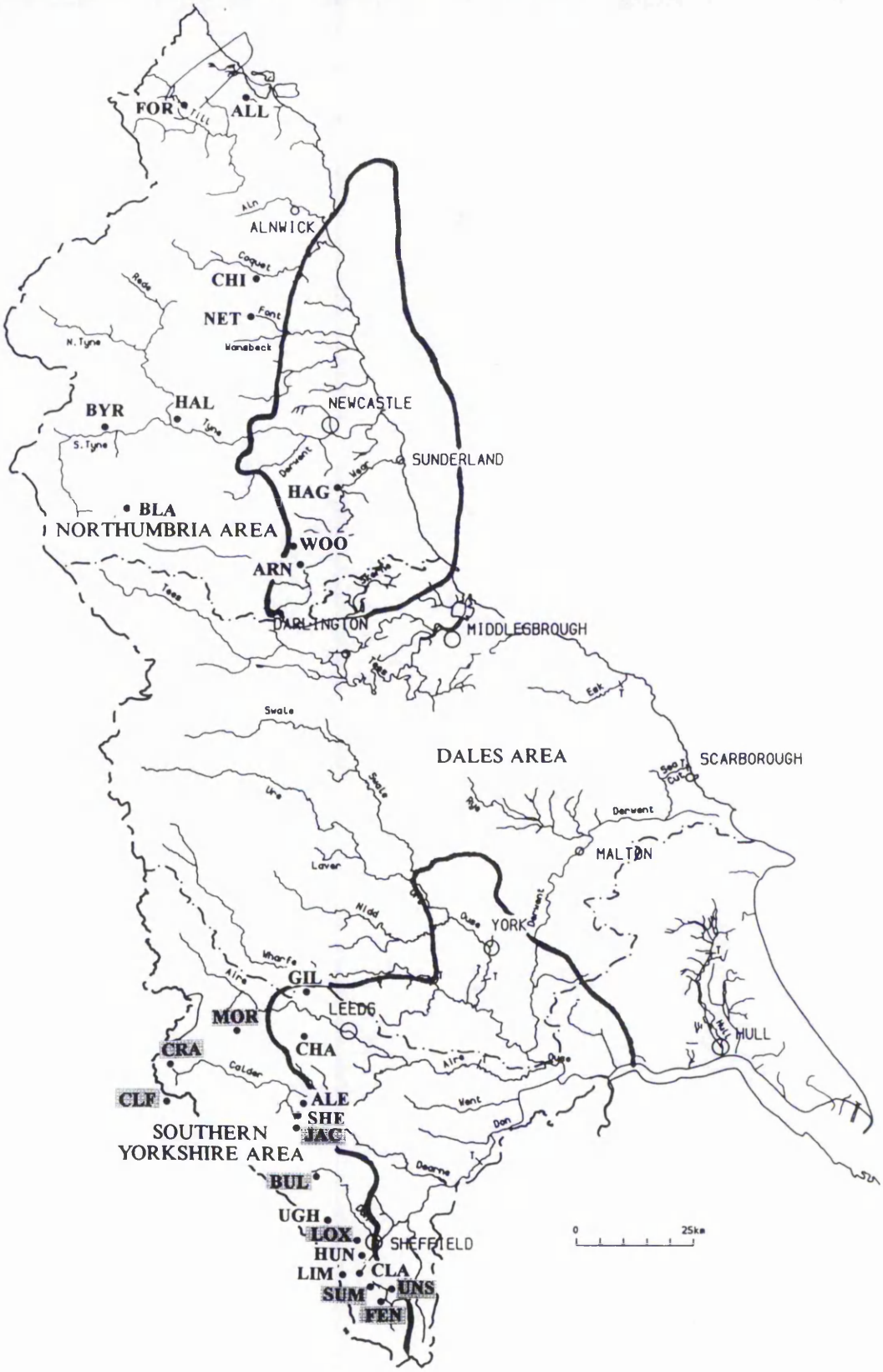


Figure 4.1. Location of mines studied in Northumbria and Yorkshire. Adapted from EA (1996). Codes indicate mine discharges. Outlined areas indicate shallow coalfields only. Yorkshire High Impact sites highlighted in grey. For code descriptions, see Appendices IX, XIV and XIX.

Brown forest and gley lowland soils dominate the region, with low base gley soils in upland areas (Blackman *et al.*, 1963).

4.1.3.2 Northumbria Sites

In Northumbria, most discharges are also associated with long abandoned shallow workings, at the Western edge of the river catchments (EA, 1996). This study used data for low impact sites; site descriptions can be found in Appendix XIX, and are illustrated in Figure 4.1. All sites fall in the catchments of the Rivers Tyne, Wear, Wansbed, Coquet and Till. These rise on the upland Northumberland Moors, with a solid geology of carboniferous limestone, and flow to the eastern coastal estuaries. Grey-brown podzolic soils dominate the region, with acid podzolised soils in the upland areas of the catchments. The main superficial deposit in the region is boulder clay (Blackman *et al.*, 1963).

4.1.3.3. Site Ranking

In the EA study (1996), sites in the survey were ranked primarily on the basis of physicochemical parameters (area of watercourse affected, length affected, substratum quality, iron concentration, pH, dissolved oxygen and total aluminium). However final judgement was made including biological impact, namely percentage reduction in BMWP, change in Log_{10} abundance, area affected biologically and fisheries potential. Five mine waters in Northumbria and Yorkshire with the highest environmental impact were identified:

1. Bullhouse Minewater, Upper Don Catchment (Yorks.)
= 2. Fender Minewater, Rother Catchment (Yorks.)
=2. Edmondsley Minewater, Lower Wear Catchment (North.)
4. Clough Foot Minewater, Upper Calder Catchment (Yorks.)
5. Lambley Minewater, Tyne Catchment (North.)

4.1.3.4. Welsh Sites

Welsh sites, in 11 river catchments, are listed in rank order of impact (determined as for Yorkshire and Northumbria) in Appendix XXIV, and illustrated in Figure 4.2. The solid geology of the catchments is carboniferous Baren productive coal measures and Pennant series sandstone (Blackman *et al.*, 1963). The Blaenpelenna and Gwenffrwd are predominantly conifer afforested at their sources.

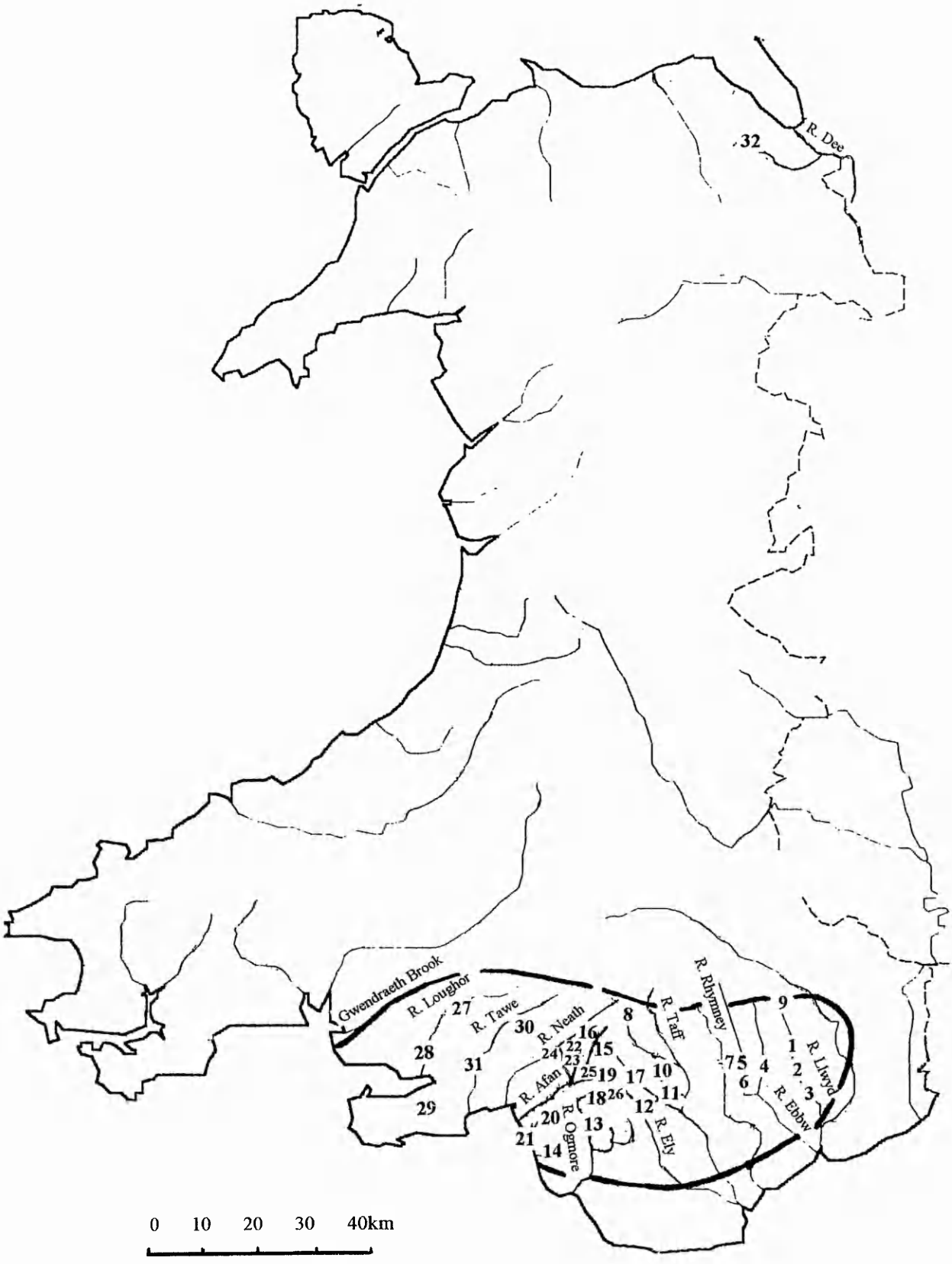


Figure 4.2. Location of mines studied in Wales. Adapted from EA (1994). Codes indicate mine discharges. Outlined area indicates coalfield. For code descriptions, see Appendix XXIII.

The River Taff, Ebbw and Llywd catchments rise in the uplands of the Brecon Beacons and the black Mountains, with an underlying solid geology of Lower Devonian red sandstone. Main soil types include acid brown and upland gley; both low base soils (Blackman *et al.*, 1963).

4.2 Methods

4.2.1 Database Formation

All data (except map information for Yorkshire and Northumbria) was provided by the Environment Agency in raw format, either as field data collection sheets, or laboratory analytical printouts. A separate database was formed for each region and held as spreadsheets for the various analyses in Excel 5.0 (1993, Microsoft Corporation) and SPSS 6.1 (1994, SPSS Inc). For each set of sites, Log to the base-10 transformation was conducted on all variables which required contraction at the upper end of the data set, to provide a normalisation of the data (Elliott, 1977), so to allow all data to be included in the statistical analyses. For Yorkshire low impact sites and Northumbria sites, collected in August and November 1996, means were calculated for all variables. Two subsets had data from a single season only; Yorkshire high impact sites, collected in November 1995/February 1996, and Welsh sites, collected in June 1993. Therefore, Yorkshire low and high impact sites were analysed separately. For Yorkshire and Northumbria, 4 samples were taken around each mine:

1. upstream of the minewater;
2. at the point of mine discharge;
3. the mixing zone of the mine discharge with the receiving water immediately downstream;
4. downstream of the ochre limit in the receiving watercourse ie the 'recovery zone' where no visible ochre indicates more normal conditions can return.

An additional site 5, further downstream, was included for Bullhouse mine. For Welsh sites, samples were taken upstream and immediately downstream only. Sites were allocated codes eg. BUL1 - (BUL)lhouse mine, (1) upstream sample. For Welsh sites, a single site number followed by U(pstream) or D(ownstream) was used.

4.2.2. Environmental variables

Macroinvertebrate samples were obtained by the EA, by the use of 3 minute kick net sampling, with a 1 minute manual search of the sites, and identified and recorded on standard sheets in the laboratory of the Environment Agency. The environmental variables measured are described in Table 4.1.

Chemical data were provided to the EA by external laboratory analysis. Additional variables were calculated for inclusion in the models, namely substrate phi category (section 2.4.3) and distance from source, altitude and slope, calculated from 1:50,000 Ordnance Survey maps.

Missing physical and chemical data were estimated in order to complete the data set. For example, the mean of any existing downstream values was taken to complete the data for a missing data point for a downstream sample. However, sites with large sections of missing data (eg. data missing for a variable for all sites around a mine) were omitted for accuracy in correlation analysis. The effects of this were considered to be minor as no significant correlations were found to be close to the significance cut-off values (Section 4.3).

Table 4.1. The environmental variables used in the analyses together with notes on units and data collection procedures. Standard Environment Agency protocols for analysis are indicated.

Environmental variable	Units of measurement/Category numbers	References/Notes
Information from Maps		
Distance from source	km	Ordnance Survey Maps 1:50,000 and 1:250,000.
Slope of site	m km^{-1}	
Altitude of site	m	
Laboratory Analysis		
Alkalinity	mg l^{-1} $CaCO_3$	Hach Alkalinity test kit
pH	units (+Field pH in Wales)	Palintest digital probe
Conductivity	mS cm^{-1}	Palintest digital probe
Dissolved Oxygen	% (+mg l^{-1} in Wales)	WTW Oxi 320 Oximeter
Suspended Solids	mg l^{-1}	
Sulphate	mg l^{-1}	
Chloride [1]	mg l^{-1}	
Iron (total, filtered)	µg l^{-1}	Filtered=Dissolved Iron (Fe^{2+})
Arsenic (total, filtered) [1]	µg l^{-1}	Total Iron=Fe^{2+}, Fe^{3+}, $Fe(OH)_3$
Lead (total, filtered) [1]	µg l^{-1}	Protocols for all metals in:
Copper (total, filtered) [1]	µg l^{-1}	NRA National Sampling
Zinc (total, filtered) [1]	µg l^{-1}	Procedures Manual Section
Aluminium (total, filtered)	µg l^{-1}	ES021 - 'Field Measurement
Nickel (total, filtered) [1]	µg l^{-1}	Tests'
Manganese (total, filtered) [1]	µg l^{-1}	
Information from Sample Data Sheet		
Temperature	°C	Palintest digital probe
Bedrock	%	Estimated by eye / quadrat
Boulders	%	"
Cobbles	%	"
Pebbles	%	"
Gravel	%	"
Sand	%	"
Silt	%	"
Substrate	phi units	Calculated as in Section 2.4.3
Width	m	30m tape
Depth	cm	Mean depths taken across stream
Colour	units (1-5 where 5=turbid)	Estimated by eye
Ochre [1]	% cover	"
Flow	units (1=Slack 2=Slow 3=Moderate 4=Fast 5=Spate	"
Sewage Litter	units (1-5 where 1=clear)	"

Notes:[1] Data not recorded at Welsh sites.

4.2.3 Data Analysis

4.2.3.1 Ordination and Classification

The sites for each regional data subset were ordinated by Detrended Correspondence Analysis (DCA), using the FORTRAN program DECORANA (Hill, 1994), previously described in 3.1.2 for the River Idle catchment analysis. The positions of sites along axes, and their overall lengths, were given in standard deviations (Ormerod *et al.*, 1987). The relationship between biological communities, as summarised by DCA scores, and environmental variables, was investigated by using the Pearson Product Moment Correlation coefficient to correlate the variables with axis scores. This technique was used despite the data not exhibiting a normal distribution, as it is standard in similar studies (eg. Wright *et al.*, 1984) and used to allow comparison of results.

Classification of sites was conducted using Two-Way Species Analysis, using the FORTRAN program TWINSPAN (Hill, 1994), previously described in section 3.1.2. This classifies solely on the basis of difference and similarities of macroinvertebrate communities. Using both TWINSPAN and DECORANA, families were analysed on a presence/absence basis only, rather than using abundance levels quantitatively in the analysis. This decision was based on the findings of Furse *et al.* (1984) who found that family qualitative groups were better predicted than groups based on quantitative data at all but the first level of division. The TWINSPAN procedure was arrested at levels 3-4, depending on the size of the data set.

4.2.3.2 Multiple Discriminant Analysis

Relationships between the TWINSPAN site groupings and the environmental variables were investigated using Multiple Discriminant Analysis (MDA, as used in RIVPACS modelling – refer to section 1.5.5) using SPSS 6.0 routine 'DISCRIMINANT' (1994, SPSS Inc.). The procedure allocates sites to a TWINSPAN group using the functions derived from environmental variables; prediction accuracy is given as a percentage of sites successfully predicted to the correct TWINSPAN group. The stepwise method of analysis was employed. The analysis takes the form of three steps (for full details see Klecka, 1975) :

1. Determination of the discriminant functions. These functions basically describe the locations of TWINSPAN groups relative to each other on axes in geometric space.

A discriminant function takes the form:

$$D_i = d_{i1}Z_1 + d_{i2}Z_2 + ... + d_{ip}Z_p$$

Where D_i is the score of discriminant function $_i$

d are the weighting coefficients

Z are the values of $_p$ discriminating variables

The weighting coefficients are an indication of the relative importance of each variable in each function derived, and can be interpreted in much the same way as in multiple regression analysis.

2. Analysis. This identifies how many of the discriminant functions generated are statistically worthwhile for inclusion in the model, and whether adding extra functions adds significant extra information to separate TWINSPAN groups. The procedure calculates Wilk's lamda; the larger this is, the less unexplained information is remaining. A chi-square statistic is then produced to test the significance of each successive function generated.

3. Classification. To check the ability of the functions and respective variables to separate TWINSPAN groups, the functions generated can be used to classify the original set of sites, to give the percentage of sites correctly classified, at each level of TWINSPAN division.

4.3 Results

4.3.1 Yorkshire Low Impact Sites

4.3.1.1 Classification

Figure 4.3 presents a dendrogram of the classification produced by TWINSPAN to level 3. Eight site groups were produced by the technique, which contained one to seven sites per group. Indicator families, particularly diagnostic of each level of division, are indicated. Plate 4.1 illustrates the minimal impact of Hunters Bar Mine into Porter Brook, near Sheffield, Yorkshire.

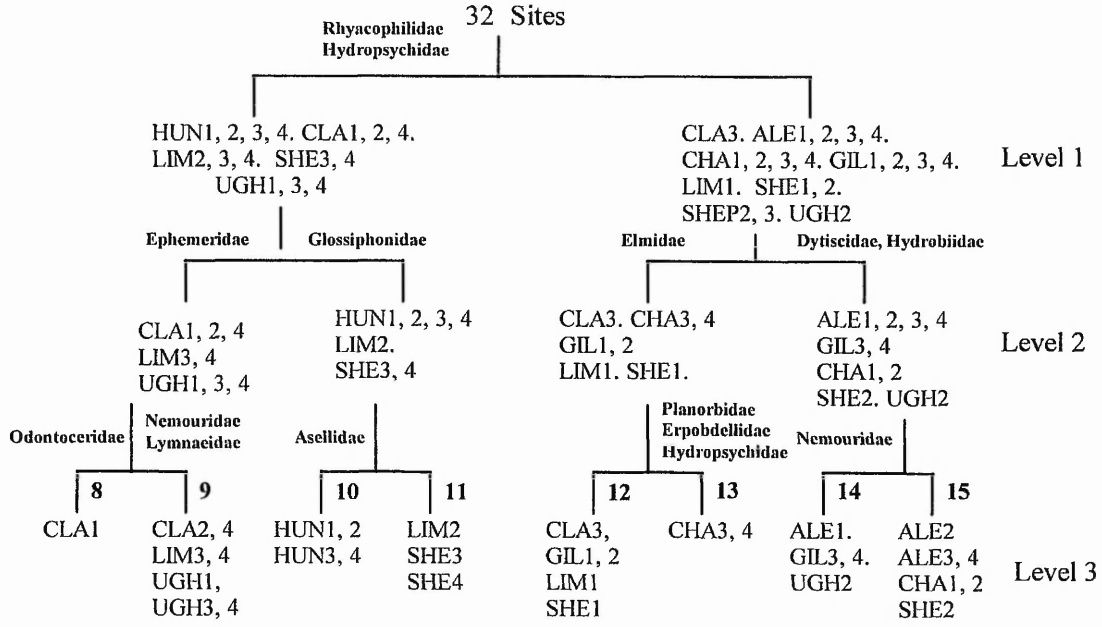


Figure 4.3. Classification of Yorkshire sites using TWINSPAN with 'Family' level identification of taxa. TWINSPAN Groups of sites are shown from each of the three levels of division, and are indicated by number.

Plate 4.1. Yorkshire low impact pollution. Discharge from Hunters Bar mine into Porter Brook, Clough Lane, near Sheffield (site code CLA). A) Upstream (CLA1) and first discharge site. B) Second discharge site immediately downstream (CLA2).

A.

B.

It can be observed in Figure 4.3 that sites at the point of mine discharge (code 2) and at the mixing zone of mine water with the receiving water course (code 3) make up most of the sites in TWINSPAN group 15 which has a low mean BMWP (mean score 29.5, data held in Appendix X). TWINSPAN groups 8 and 9 with a high mean BMWP (mean scores of 104 - 127) however, generally consist of the very low impacted sites at Clough Lane, (Plate 4.1) and in the mixing zone (code 3) and recovery zones (code 4) of Limb Brook and Ughill sites.

4.3.1.2 Ordination

Figure 4.4 illustrates the axis 1 and 2 DECORANA plot for the 32 Yorkshire low impact sites. The eigenvalues for the axes are as follows; axis 1=0.219, axis 2=0.127, axis 3=0.091, axis 4=0.047. From these eigenvalues, axis 1 and 2 explained 71.54% of the variance amongst sites.

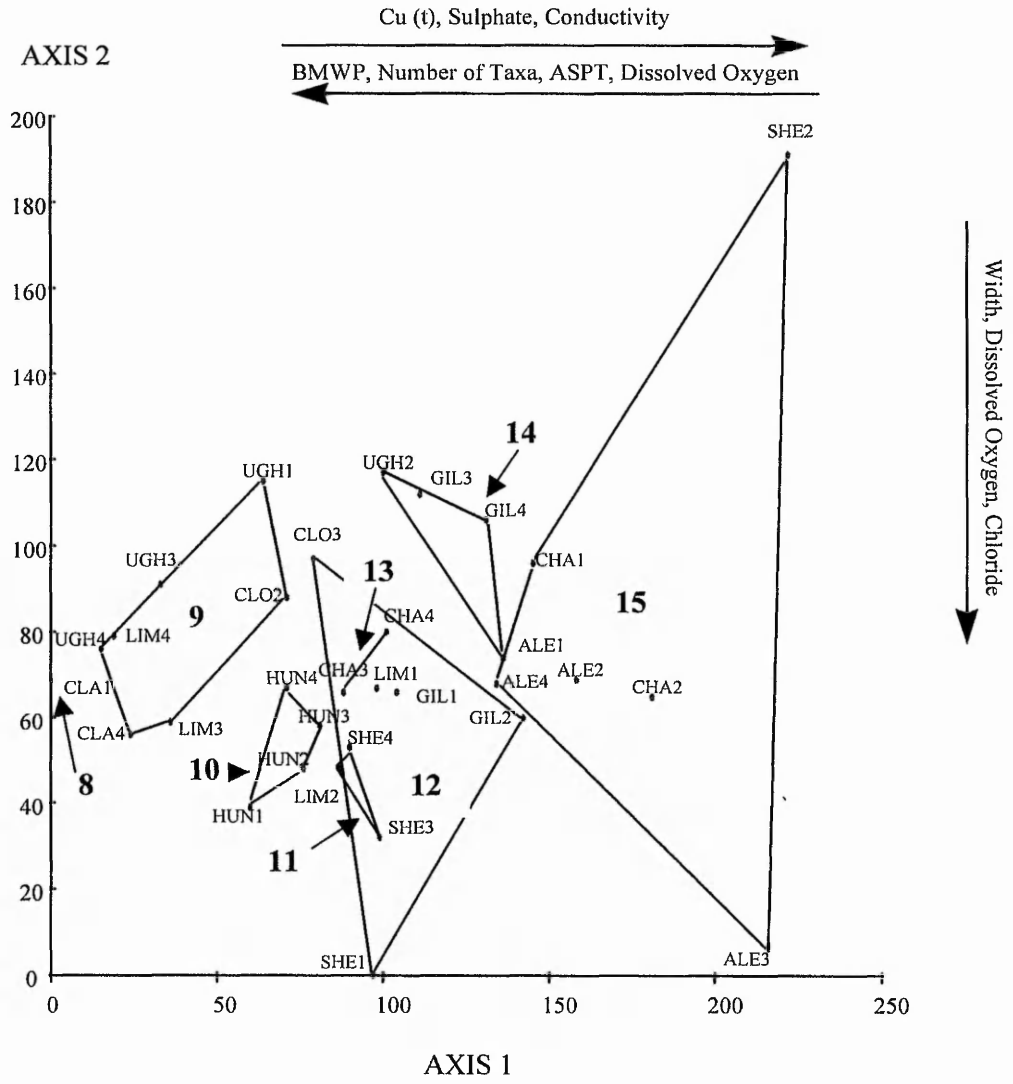


Figure 4.4. DECORANA Plot of Yorkshire Low Impact Sites. TWINSPAN group numbers highlighted.

Table 4.2 illustrates the results of the correlation analysis of the variables with DECORANA axes 1 to 4.

Table 4.2. Significant Correlations of Environmental Variables with Decorana axes for Yorkshire Low Impact site data

Variable	Axis 1	Axis 2	Axis 3	Axis 4
BMWP	-0.780***	-	-	-
Number Taxa	-0.745***	-	-	-
ASPT	-0.751***	-	-	-
Log Cu(t)	0.646***	-	-	-
Log Sulphate (mg l^{-1})	0.601***	-	-	-
Log Conductivity (mS cm^{-1})	0.594***	-	-	-
Log Chloride (mg l^{-1})	0.553***	-0.379*	-	-
Log Mn(t)/(f)	0.523 **/ 0.481**	-	-	-
Dissolved Oxygen (%)	-0.514**	-0.349*	-0.499**	-
Log Fe(t)/(f)	0.474 **/ 0.528***	-	-	-
Log As(t)	0.361*	-	-	-
Width (m)	-	-0.344*	-	-
Log Pb (t)	-	-	-0.527***	-
Log Altitude (m)	-	-	0.427**	-
Log Distance from Source (km)	-	-	-0.397**	-
Colour (units)	-	-	-	-0.378*
Depth (cm)	-	-	-	-0.356*

Notes: r (Pearson's correlation coefficient) is significant at the 0.1% level above 0.525 (***); significant at the 1% level above 0.425 (**), significant at the 5% level at 0.328 (*) or not significant (-). (t)/(f)=total/filtered $\mu g\ l^{-1}$

For axis 1, The most significant negative correlations ($p<0.001$) were those found with BMWP, ASPT and number of taxa . Strong positive correlations were found with the heavy metals copper, also sulphate, chloride and conductivity. Iron and dissolved oxygen correlated less strongly at $p<0.01$, and weak but significant

correlations ($p<0.05$) were found for total arsenic. For axis 2, weakly significant correlations ($p<0.05$) were seen for dissolved oxygen, width and chloride. Axes 3 and 4, reflected the concentration of the heavy metal lead, altitude, distance from source, depth and again dissolved oxygen.

The DECORANA ordination illustrates the changes in macroinvertebrate assemblages in relation to spatial changes in heavy metal concentrations and dissolved ions on axis 1, oxygenation and chloride levels on axis 2, and generally river size and positioning on axes 3 and 4. Significant correlations of dissolved oxygen on the first three axes illustrates the importance of this parameter in dictating community structure.

The shift from low to high biological diversity in the TWINSPAN groups can be related to variables exhibiting correlations with the DECORANA axes.

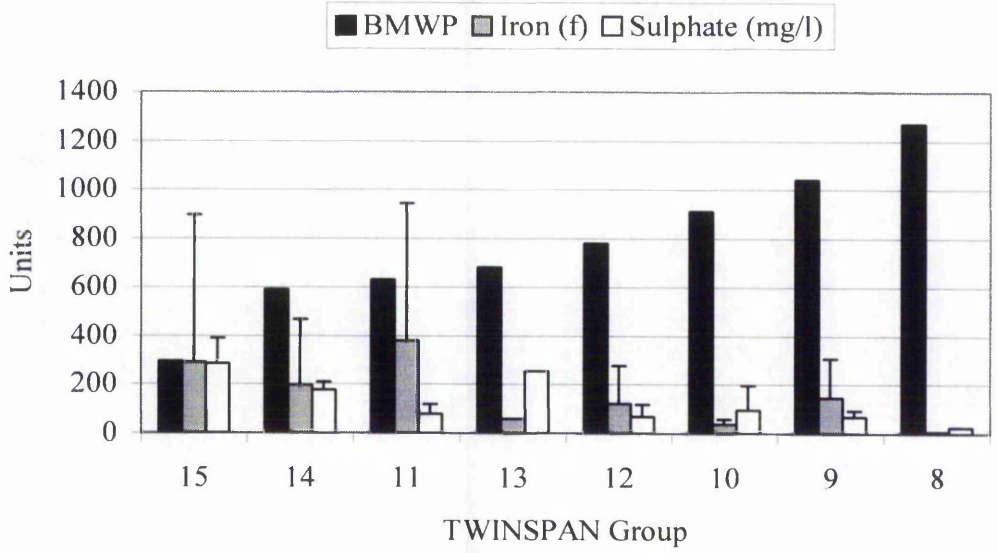


Figure 4.5. Yorkshire Low Impact Sites - TWINSPAN Group Characteristics (mean values +/- 2 SE). Note Iron (filtered) values divided by 10 and BMWP multiplied by 10 for clarity of comparison.

Figure 4.5 illustrates the increase in mean BMWP for each TWINSPAN group, as the iron (filtered) and sulphate concentrations decrease (data held in Appendix X). The mean filtered iron concentrations is high (7903 $\mu g\ l^{-1}$) in the low BMWP group 15, GQA class D; for high BMWP groups, 8 and 9 of GQA class A, values are much lower (80-1468 $\mu g\ l^{-1}$). TWINSPAN groups with mean BMWP

scores falling into the intermediate GQA classes B and C (with BMWP scores of 59-91) have mean filtered iron concentrations of 389 - 1971 μg l^{-1}. Similar patterns are also followed for copper and manganese. Mean sulphate concentrations are high for GQA class E group 15 (283 mg l^{-1}), lower for class B and C groups (68 - 254 mg l^{-1}), but the lowest for class A groups (26-67 mg l^{-1}).

Mean conductivity and chloride values follow a similar but weaker trend of reflecting BMWP scores, namely higher values in lower scoring sites. Values for dissolved oxygen also reflect pollution levels; with a value of only 56% in class E group 15, but rising into the range of 86-90% for the cleaner, class A TWINSPAN groups 8 and 9. An increase in the number of taxa per TWINSPAN group is also observed from groups 15 to 8 in Appendix X, and an increase in the sensitivity of these taxa to coal mine pollution is reflected by the Average Score per Taxon (ASPT) also increasing.

Figure 4.6. (data held in Appendix XI) presents an examination of the proportional representation of taxa between groups in order of increasing mean group BMWP, illustrating a relative decrease in dipterans, whilst ephemeroptera tend to increase.

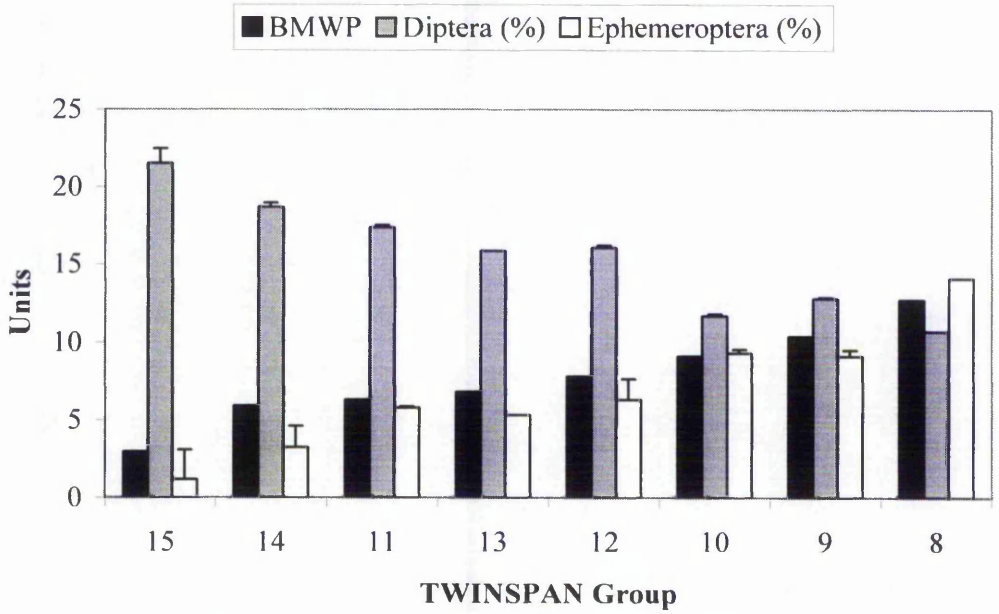


Figure 4.6. Yorkshire Low Impact Sites - TWINSPAN Group Taxa Characteristics (mean values +/- 2 SE after arcsine transformation, shown following back transformation). BMWP values divided by 10 for clarity of comparison.

4.3.1.3 Multiple Discriminant Analysis

The result of multiple discriminant analysis to investigate the variables which most strongly distinguish TWINSPAN groups is presented in Table 4.3. Copper (total) appears to be the strongest discriminator between TWINSPAN groups across TWINSPAN levels. This is to be expected as it correlated strongly with DECORANA axis 1. From the standardised discriminant function coefficients, depth (cm) and conductivity ($\mu S\ cm^{-1}$) are also extracted by the stepwise procedure.

Table 4.3. Results of Stepwise Multiple Discriminant Analysis for Yorkshire Low Impact Sites (for each variable, probability of F<0.01).

TWINSPAN Level	Number of significant discriminant functions[1]	% of sites correctly predicted	Variables selected	Standardised Canonical Discriminant function 1 coefficient
Level 1	1	81.25%	Copper (t)	1.00
Level 2	2	65.63%	Copper (t) Depth (cm) Conductivity ($\mu S\ cm^{-1}$)	1.073 1.114 -0.783
Level 3	2	65.63%	Copper (t) Depth (cm) Conductivity ($\mu S\ cm^{-1}$)	1.25 1.01 -0.157

[1]Wilks Lamda significant to at least p<0.05

4.3.2 Yorkshire High Impact Sites

4.3.2.1 Classification

Figure 4.7 is a dendrogram of the site classification produced by TWINSPAN to level 3. Seven site groupings were produced by the technique. Group sizes ranged from 1 to 8 sites. Plate 4.2 illustrates the high impact of Fenders Bar Mine into Barlow Brook, near Chesterfield, Yorkshire.

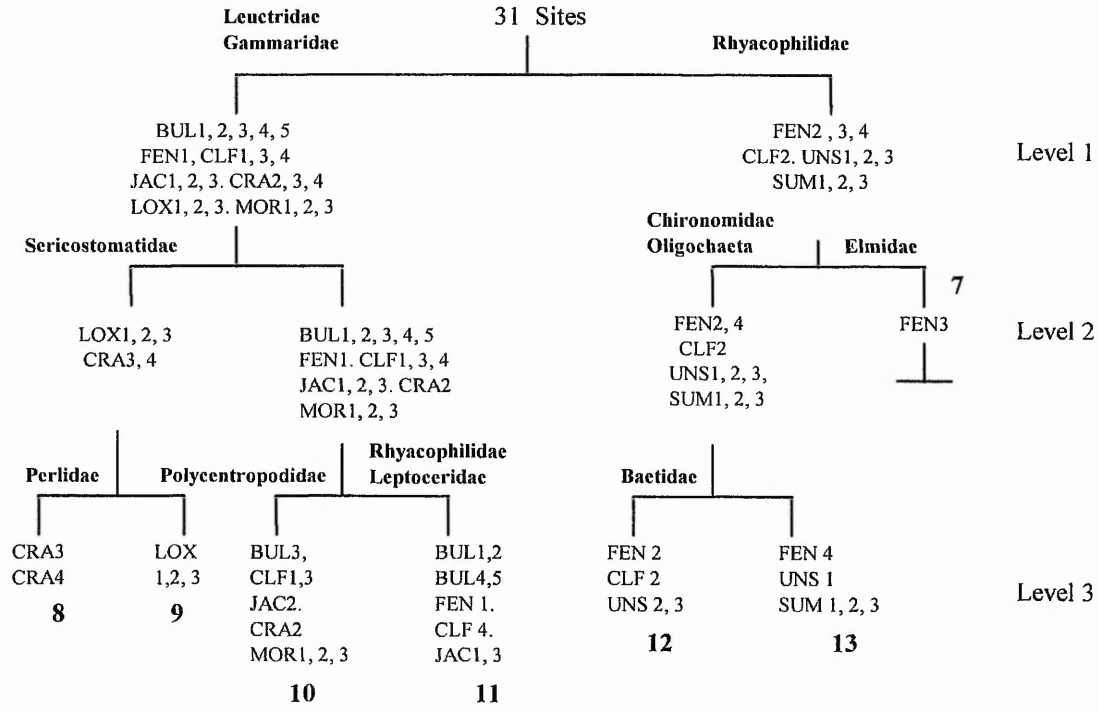


Figure 4.7. Classification of Yorkshire High Impact sites using TWINSPAN with Family level identification of taxa. TWINSPAN Groups of sites are shown from each of the three levels of division, and are indicated by number.

Plate 4.2. Yorkshire high impact pollution. Discharge from Fender mine on Barlow Brook, Barlow, near Chesterfield. A) Upstream (FEN1) and mine discharge site (FEN2). B) Site immediately downstream (FEN3).

A.

B.

4.3.2.2 Ordination

Figure 4.8 illustrates the axis 1 and 2 DECORANA plot for the 31Yorkshire high impact sites, grouped according to TWINSPAN results.

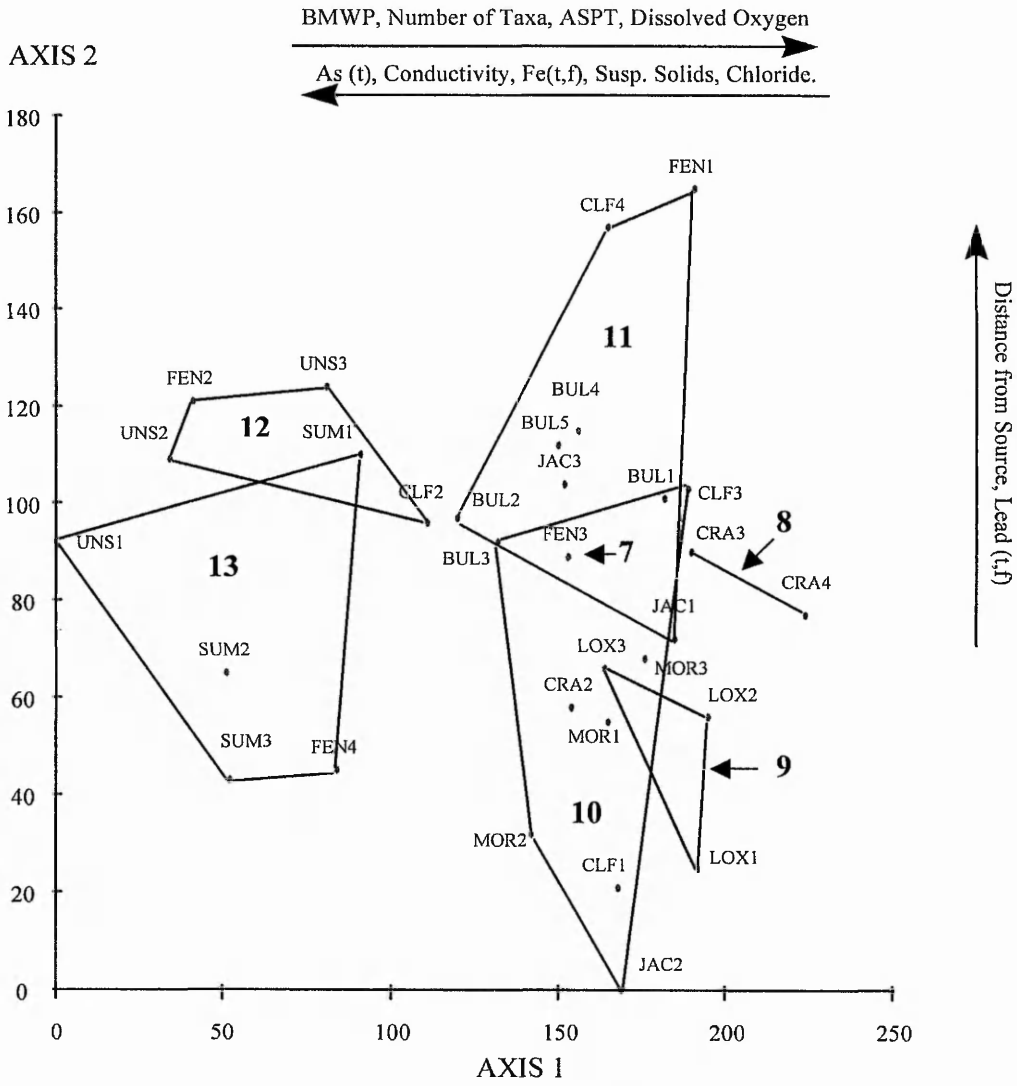


Figure 4.8. A DECORANA plot of the Yorkshire High Impact Sites. TWINSPAN Groups highlighted.

The eigenvalues for the axes are as follows; axis 1=0.196, axis 2=0.142, axis 3=0.099, axis 4=0.0794. From these eigenvalues, axis 1 and 2 explained 65.4% of the variance amongst sites.

Table 4.4 illustrates the results of the correlation analysis of the variables with DECORANA axes 1 to 4. For axis 1, the most significant negative correlations ($p<0.001$) were those with the BMWP, ASPT, number of taxa and also dissolved oxygen, arsenic and total ions measured as conductivity and chloride. It can be noted that whereas biological index values correlated negatively on axis 1 with Yorkshire low impact sites (4.3.1.2), positive correlations are found for the same variables for these high impact sites. This is an artefact of the DECORANA algorithm; there is no reason why the signs should be consistent, what matters is the separation of sites on the axes. The variables, however, are still inversely proportional to pollution variables e.g. iron concentrations. Other noteworthy correlated variables ($p<0.01$ and $p<0.05$) include suspended solids, water colour, ochre smothering and cobbles.

Axis 2 correlated strongly with total and filtered lead, and weakly with the site positioning parameter distance from source. Axis 3 correlates ($p<0.01$) with slope and percentage silt. Further weak correlations on axes 3 and 4 ($p<0.05$) exist with width, depth, aluminium (filtered), and the substrate component gravel. As with Yorkshire low impact sites, the DECORANA ordination illustrates the changes in macroinvertebrate assemblages in relation to spatial changes in heavy metal concentrations and associated changes in oxygenation on axis 1, however, the influence of substrate quality (cobbles (%) and ochre smothering) and water turbidity (water colour and suspended solids) is apparent for these, the most heavily impacted sites in this study. Axis 2 reflects weakly the position of the site in relation to distance from its source. Axes 3 reflects more weakly the influence of site physical dimensions.

Table 4.4. Significant Correlations of Environmental Variables with Decorana axes for Yorkshire High Impact Site Data.

Variable	Axis 1	Axis 2	Axis 3	Axis 4
ASPT	0.856***	-	-	-
BMWP	0.739***	-	-	-
Number of Taxa	0.671***	-	-	-
Log Arsenic (t)	-0.714***	-	0.499**	-
Log Conductivity ($\mu S\ cm^{-1}$)	-0.612***	-	-	-
Log Chloride ($mg\ l^{-1}$)	-0.509**	-	-	-
Dissolved Oxygen (%)	0.509**	-	-	-
Log Alkalinity ($mg\ l^{-1}$)	-0.496**	-	0.355*	-
Log Suspended Solids ($mg\ l^{-1}$)	-0.496**	-	-	-
Log Sulphate ($mg\ l^{-1}$)	-0.488**	-	-	-
pH	0.486**	-	-	-
Log Iron (f)	-0.467**	-	-	-
Cobbles (%)	0.466**	-	-	-
Log Zinc (t)/(f)	-0.419*/ -0.425*	-	-	-
Colour	-0.415*	-	-	-
Ochre (%)	-0.409*	-	-	-
Log Copper (t)/(f)	-0.382*/-0.426*	-	-	-
Log Iron (t)	-0.388*	-	-	-
Log Lead (f)	-	0.563***	-	-
Log Lead (t)	-	0.547**	-	-
Log Distance from Source (km)	-	0.388	-	-
Silt (%)	-	-	0.449**	-
Log Slope ($m^{-1}\ km^{-1}$)	-	-	-0.490**	-
Log Aluminium (f)	-	-	-0.440*	-
Gravel (%)	-	-	-0.439*	-
Log Width (m)	-	-	0.427*	-
Log Depth (cm)	-	-	0.398*	-0.422*
Temperature (^{0}C)	-	-	-	0.357*

Notes: r (Pearson's correlation coefficient) is significant at the 0.1% level above 0.562 (***), at the 1% level above 0.457 (**); significant at the 5% level above 0.354 (*) or not significant (-). (t)/(f)=total/filtered $\mu g\ l^{-1}$

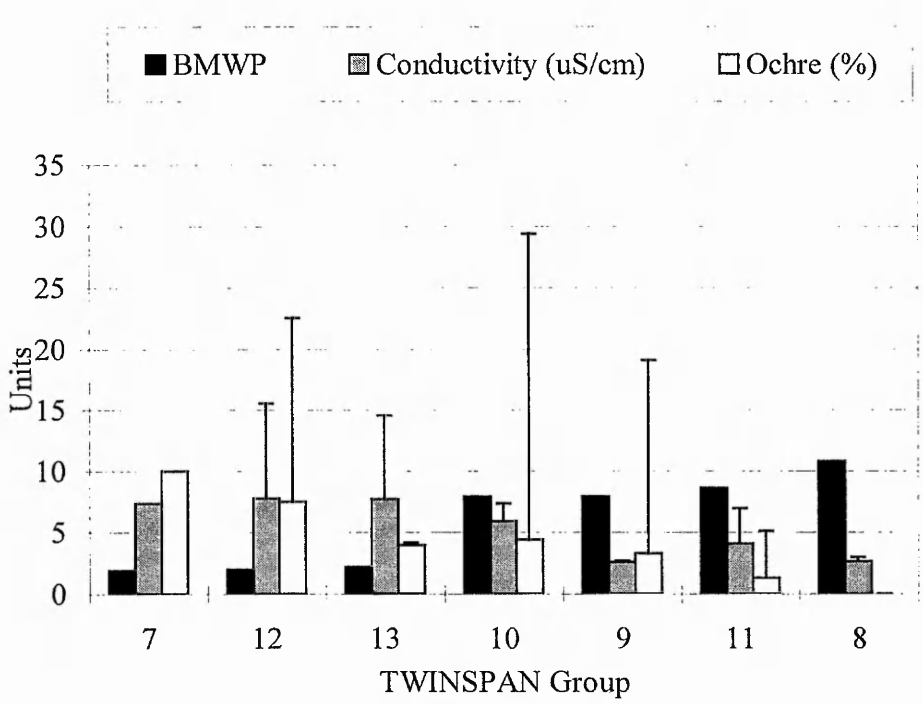


Figure 4.9. Yorkshire High Impact Sites - TWINSPAN Group Characteristics (mean values ±2 SE). Standard errors for ochre values were calculated after arcsine transformation, and plotted following back-transformation. Note BMWP and Ochre values divided by 10, Conductivity divided by 100, for clarity of comparison.

From Figure 4.9, the sites are divided into two major subsets; TWINSPAN groups (7, 12 and 13) with a GQA class of E (with BMWP scores of values 19-22) and groups (8, 9, 10, 11) with GQA classes of A and B (high BMWP scores 79 - 108). As for Yorkshire low impact sites, it can be observed from Figure 4.5 that sites at the point of mine discharge (code 2) and downstream (code 3) feature strongly in the poor BMWP TWINSPAN groups 7 and 12, whilst upstream (code 1) and recovery zone (code 4) sites are distributed amongst the remaining groups, with the greater mean BMWP scores.

Figure 4.9. also illustrates clearly the reduction in mean BMWP value for TWINSPAN groups with increasing mean covering with ochre (%) and mean conductivity (μS/cm) per group. The large degree of variation between sites within a group (as suggested by the error bars) is due to observations at sites recording ochre coverage as either 100% or 0%, rather than a more detailed examination to give a

better estimate of percentage cover. Appendix XV also illustrates that site means for heavy metals, including arsenic, copper, zinc and lead, are generally greater for the GQA class E groups than the other groups, with the exception of TWINSPAN group 7 which contains only one of the most highly impacted sites downstream of Fender mine, so therefore different in character from the other sites. Greater mean alkalinity, sulphate, conductivity, suspended solids, and chloride levels are also observed in these groups.

The change in the proportional representation of taxa between groups, as group mean BMWP increases is shown by the relative decrease in diptera, with an increase in plecoptera in Figure 4.10. (data held in Appendix XVI).

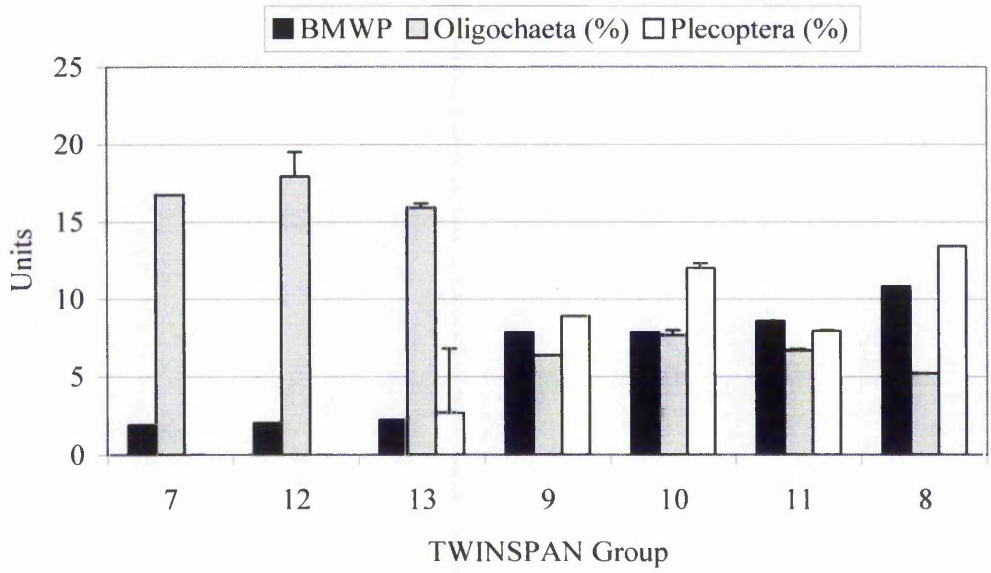


Figure 4.10. Yorkshire High Impact Sites - TWINSPAN Group Taxa Characteristics (mean values +/- 2 SE after arcsine transformation, shown following backtransformation). BMWP values divided by 10 for clarity of comparison.

4.3.2.3 Multiple Discriminant Analysis

The results of multiple discriminant analysis to investigate the variables which most strongly distinguish TWINSPAN groups are presented in Table 4.5. The percentage of sites predicted to the correct TWINSPAN group at Level 3 was more successful for these sites (80.65%) than the Yorkshire low impact sites (65.63%). Heavy metal concentrations were strong discriminators between groups; and the

importance of ochre coverage is apparent at Level 3. This supports DECORANA correlations which suggested that substrate quality, dictated by ochre coverage, was an important factor at these highly impacted sites.

Table 4.5. Results of Stepwise Multiple Discriminant Analysis for Yorkshire High Impact Sites (for each variable, probability of F<0.01).

TWINSPAN Level	Number of significant discriminant functions[1]	% of sites correctly predicted	Variables selected	Standardised Canonical Discriminant function 1 coefficient
Level 1	1	100%	Arsenic (t) Zinc (f) Iron (t) Aluminium (t)	-1.00 1.28 1.07 1.70
Level 2	2	90.32%	Altitude (m) Conductivity ($\mu S\ cm^{-1}$) Lead (t) Zinc (t)	-0.68 0.657 -0.33 0.90
Level 3	3	80.65%	Altitude (m) Conductivity ($\mu S\ cm^{-1}$) Ochre (%) Lead (t) Zinc (t)	1.21 0.682 -1.2 1.42 0.16

[1]Wilks Lamda significant to at least p<0.05.

4.3.3 Northumbria low impact sites

4.3.3.1 Classification

Figure 4.11 presents a dendrogram of the classification produced by TWINSPAN to level 4. Nine site groups were produced, which contained one to eleven sites. Plates 4.3 and 4.4 illustrates the low impact of Halton Mine into Stagshawbank Burn, Halton, Northumbria. Despite the clearly visible ochre on the substratum, the discharge is ranked as low impact as it does not score highly for the other parameters used in ranking (eg area affected).

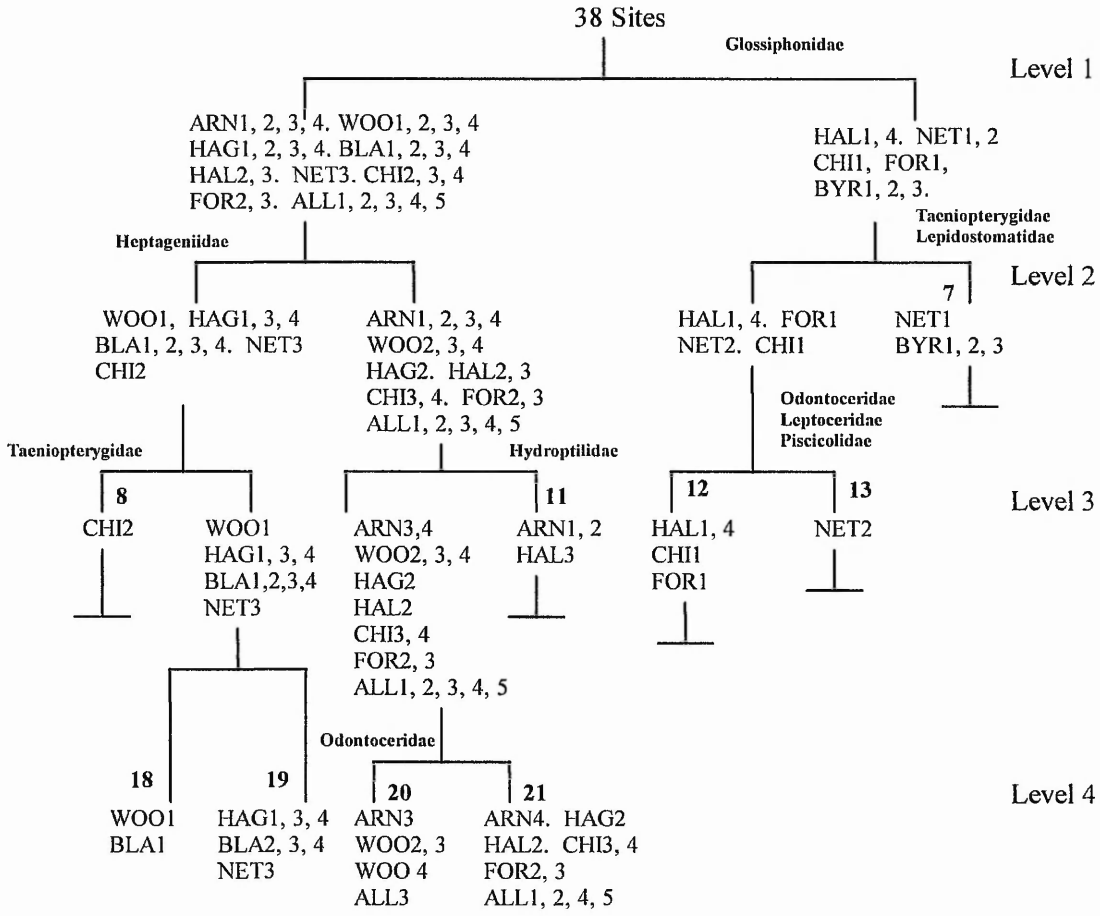


Figure 4.11. Classification of Northumbria sites using TWINSPAN with Family level identification. TWINSPAN Groups of sites are shown from each of the four levels of division, and are indicated by number.

Plate 4.3. Northumbria pollution. Discharge from Halton mine into Stagshawbank Burn, Halton, Northumbria. Upstream (HAL1) and mine discharge site (HAL2).

Plate 4.4. Northumbria pollution. Discharge from Halton mine into Stagshawbank Burn, Halton, Northumbria. Downstream recovery zone (HAL4).

From Figure 4.11 it is apparent that unlike Yorkshire, distribution of up and downstream mine discharge sites are distributed amongst all the TWINSPAN groups, of both high and low mean BMWP, this suggests that the pollution is having less of an overall impact. The TWINSPAN group with lowest diversity (8) however contains a single site, at the point of impact of the Chirm minewater with Maglin Burn.

4.3.3.2 Ordination

Figure 4.12 illustrates the axis 1 and 2 DECORANA plot for the 38 Northumbrian sites. The eigenvalues for the axes are as follows; axis 1=0.209, axis 2=0.148, axis 3=0.087, axis 4=0.046. From these eigenvalues, axis 1 and 2 explained 72.9% of the variance amongst sites. Table 4.6 illustrates the results of the correlation analysis of the variables with DECORANA axes 1 to 4.

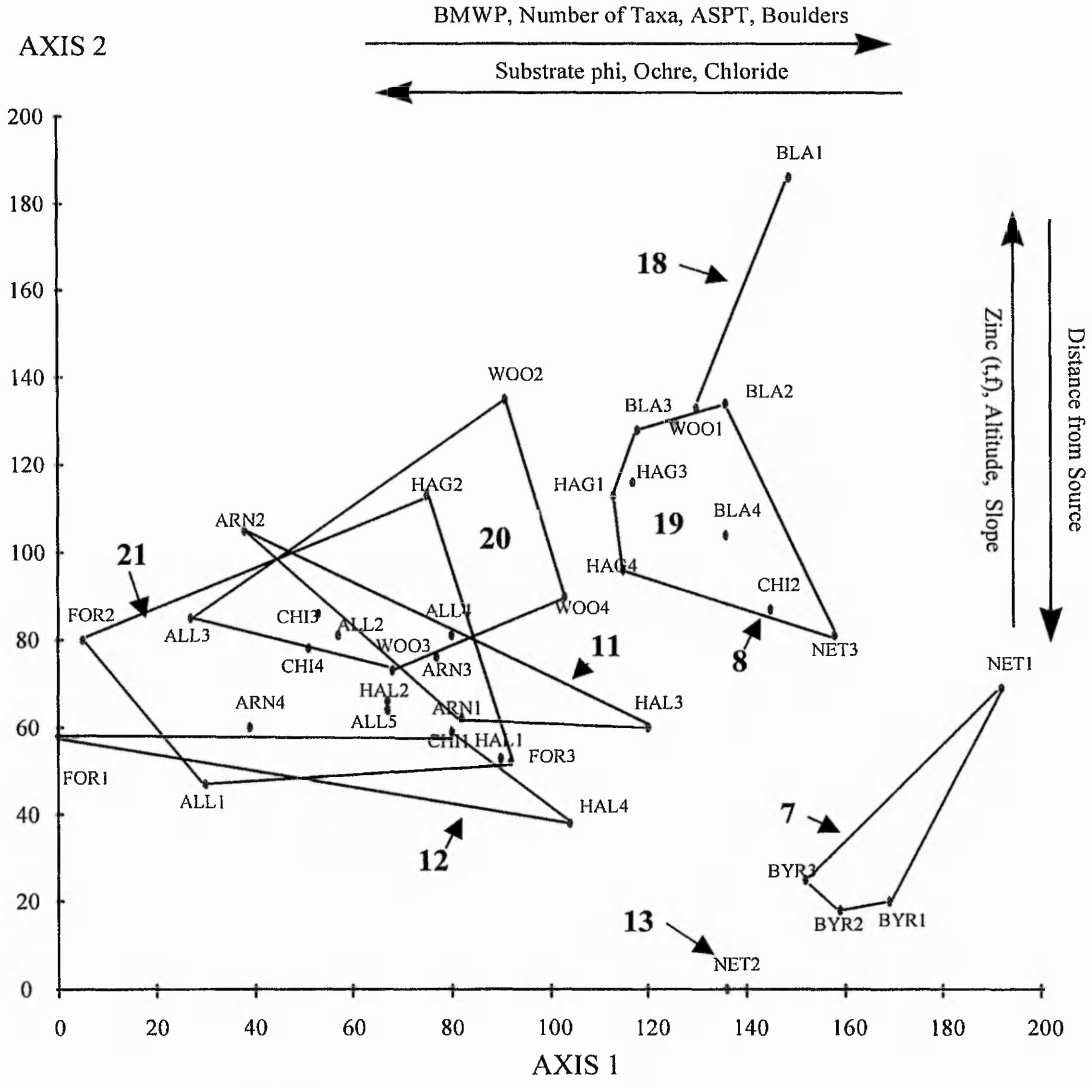


Figure 4.12. A DECORANA plot of the sites in Northumbria. TWINSPAN Groups highlighted.

Table 4.6. Significant Correlations of Environmental Variables with Decorana axes for Northumbria Data.

Variable	Axis 1	Axis 2	Axis 3	Axis 4
ASPT	0.789***	-	-	-
BMWP	0.725***	-	-	-
Substrate (phi units)	-0.69***	-	-	-
Silt (%)	0.69***	-	-	-
Boulders (%)	0.646***	-	-	-
Number of Taxa	0.645***	-	-	-
Log Width (m)	0.558***	-	-	-
Log Mean Chloride (mg l^{-1})	-0.410*	-0.375*	-	-
Log Al (f)	0.372*	-	-	-
Log Altitude (m)	0.363*	0.471**	0.340*	-
Mean Flow (units)	0.341*	-	-	-
Cobbles (%)	0.333*	0.399*	-	-
Ochre (%)	-0.331*	-	-	-
Log Zinc (f)	-	0.583***	-	-0.373*
Log Zinc (t)	-	0.511**	-	-
Log Distance from Source (km)	-	-0.496**	-	-
Log Lead (t)	-	0.431**	-	-
Log Alkalinity (mg l^{-1})	-	-0.351*	-	-
Log Slope (m km^{-1})	-	0.329*	-	-
Log Sulphate (mg l^{-1})	-	-	-0.708***	-
Log Conductivity ($\mu S\ cm^{-1}$)	-	-	-0.631***	-
Log Copper (t)/(f)	-	-	-0.576***/-0.528***	-0.329*
Log Depth (cm)	-	-	0.470**	-
Log Nickel (t)	-	-	-0.429**	-
Log Arsenic (t)	-	-	-0.411*	-
Log Iron (t)	-	-	-0.368*	-
Log Suspended Solids (mg l^{-1})	-	-	-0.334*	-

Notes: r (Pearson's correlation coefficient) is significant at the 0.1% level above 0.513(***), at the 1% level above 0.413 (**), significant at the 5% level above 0.325 (*) (*), or not significant (-).
(t)/(f)=total/filtered $\mu g\ l^{-1}$.

From axis 1 in Table 4.6, the most significant negative correlations ($p<0.001$) were with BMWP, ASPT, number of taxa and substrate characteristics, namely boulder cover, silt, and substrate type. Width of river also correlated strongly. For axis 2, the most significant correlation ($p<0.001$) is with total and filtered zinc, however, notable significant correlations are also found with altitude, slope and distance from source. Axis 3 reflects significant water quality characters such as heavy metal loads (total and filtered copper, and nickel) and ionic concentrations (conductivity, sulphate).

The DECORANA ordination illustrates the changes in macroinvertebrate assemblages in relation to spatial changes in substrate characteristics and more weakly water chemistry on axis 1, and generally position along the length of the river on axis 2.

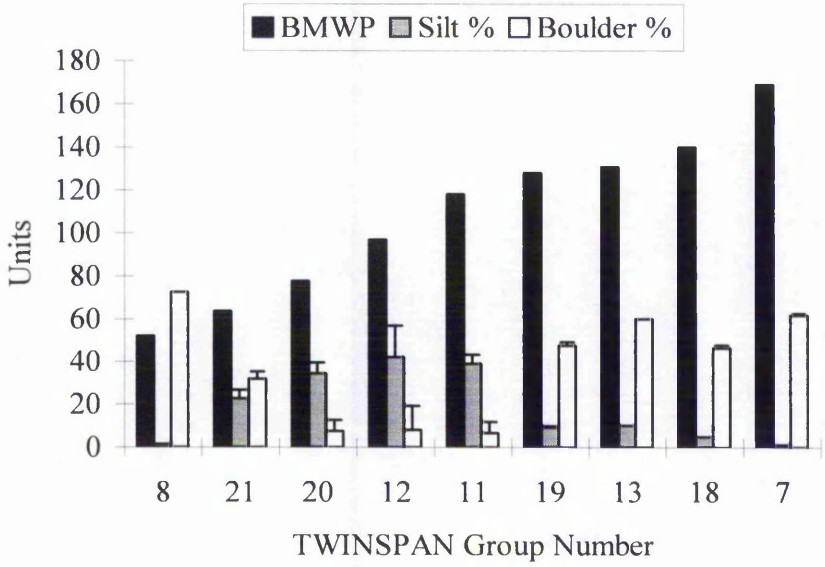


Figure 4.13. Northumbria TWINSPAN Group Characteristics. Boulders (%) and Silt (%). Mean values +/- 2 SE after arcsine transformation, shown following backtransformation.

A clear relationship of BMWP with increasing substratum hardness is illustrated in Figure 4.13 (data held in Appendix XX). The mean BMWP score tends to increase as the substrate becomes harder, with increasing percentage cover by boulders, with a decrease in coverage by silt. It therefore appears that it is the physical nature of the sites in terms of substrate which controls macrofauna groupings, as comparison of the mean chemical variables of the water between groups show no clear trends with

increasing group diversity, as reflected by weak correlations with DECORANA axes for this region (Table 4.6).

It can be observed from Figure 4.13 that in general, the mean BMWP scores for the TWINSPAN group fall into higher quality GQA bands than for Yorkshire sites (Figures 4.5 and 4.9), with only two groups (8 and 21) of grade C, one group (20) at grade B and the remaining 6 groups of grade A. This illustrates the higher diversity of macrofauna in Northumbria when compared to Yorkshire, with more representation of high BMWP scoring plecoptera and ephemeroptera. Indeed, the mean ASPT scores are mostly above 5, and taxa numbers increase across groups to 29. Following this pattern, an examination of the proportional representation of taxa between groups in Figure 4.14. (data held in Appendix XXI), illustrates a relative decrease in diptera, but relative increase in trichoptera as group mean BMWP increases.

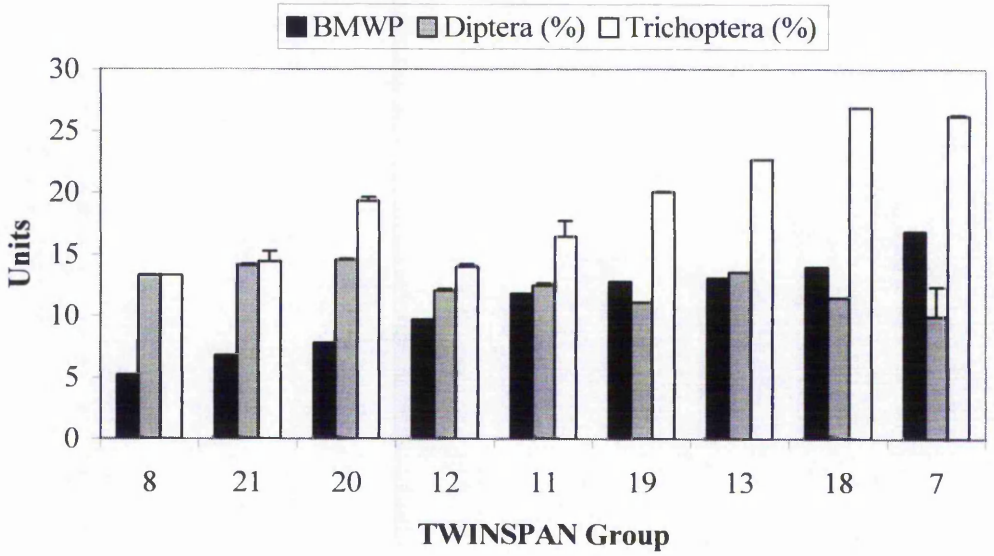


Figure 4.14. Northumbria Sites - TWINSPAN Group Taxa Charactersitics (mean values +/- 2 SE after arcsine transformation, shown following backtransformation). BMWP values divided by 10 for clarity of comparison.

4.3.3.3 Multiple Discriminant Analysis

The results of multiple discriminant analysis to investigate the variables which most strongly distinguish TWINSPAN groups are presented in Table 4.7. supporting findings from DECORANA correlations, the physical parameters substrate and width

were important factors in discriminating across TWINSPAN groups, as is the influence of the heavy metals zinc and arsenic.

Table 4.7. Results of Stepwise Multiple Discriminant Analysis for Northumbria Sites (for each variable, probability of F<0.01).

TWINSPAN Level	Number of significant discriminant functions[1]	% of sites correctly predicted	Variables selected	Standardised Canonical Discriminant function 1 coefficient
Level 1	1	78.95%	Width (m)	1.00
Level 2	3	55.26%	Substrate (phi units) Zinc (f)	0.702 -0.614
Level 3	5	62.16%	Width (m) Zinc (f) Arsenic (t)	0.945 1.284 -0.765
Level 4	6	81.6%	Zinc (f) Sulphate (mg l^{-1}) Alkalinity (mg l^{-1}) Manganese (f) Depth (cm)	0.771 -2.497 2.672 1.096 1.127

[1]Wilks Lamda significant to at least p<0.05

4.3.4 Welsh Sites

4.3.4.1 Classification

Figure 4.15 presents a dendrogram of the TWINSPAN classification performed on the 64 sites in Wales to four levels of division. Thirteen groups were produced, containing 1 to 14 sites per group.

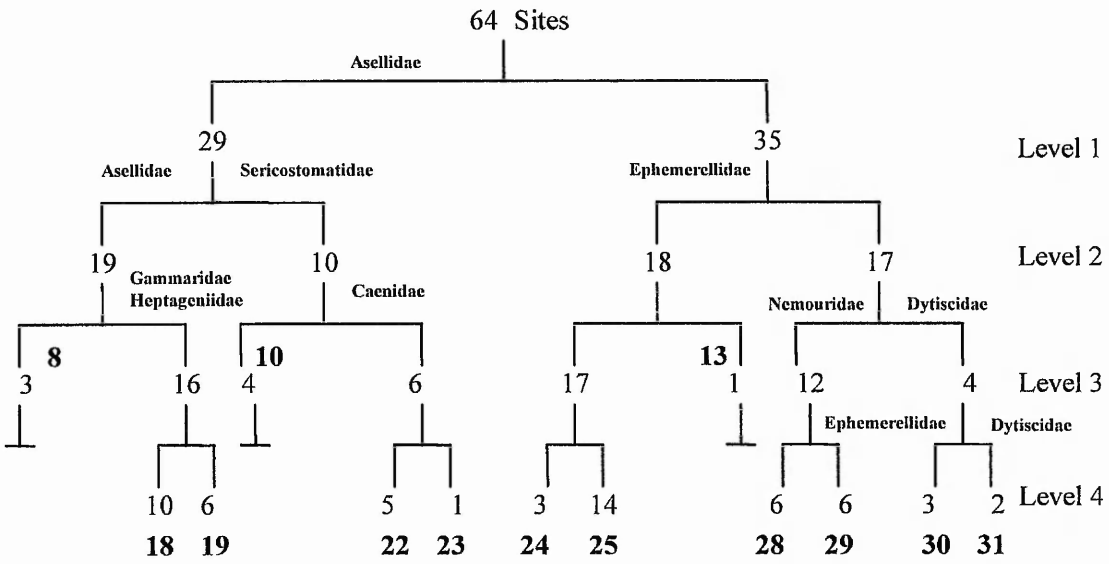


Figure 4.15. Classification of sites in Wales using TWINSPAN with taxa identified to 'Family' level. Numbers of sites in each TWINSPAN group are shown for each of the four levels of division, and group numbers are identified in bold type.

4.3.4.2 Ordination

The resulting plot for the DECORANA ordination of the 64 sites in Wales is illustrated in Figure 4.16; each TWINSPAN group is highlighted by polygons. The eigenvalues for the axes are as follows; axis 1=0.241, axis 2=0.157, axis 3=0.126, axis 4=0.072. From these eigenvalues, axis 1 and 2 explained 68.8% of the variance amongst sites. The correlations of environmental variables with DECORANA axes 1 to 4 are shown in Table 4.8.

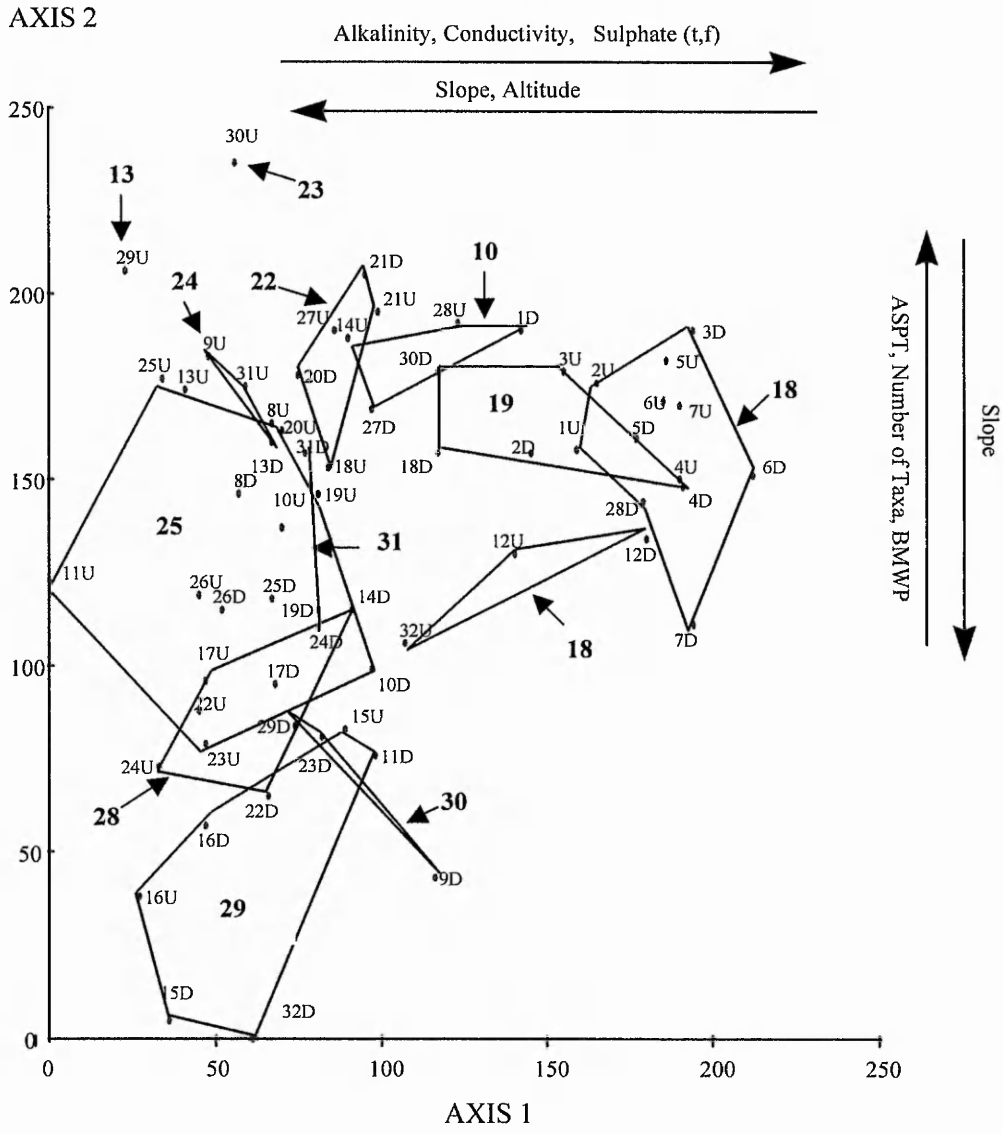


Figure 4.16. DECORANA Plot of sites in Wales. TWINSPAN Group numbers are highlighted.

Table 4.8. Significant Correlations of Environmental Variables with Decorana axes for Welsh Data

Variable	Axis 1	Axis 2	Axis 3	Axis 4
Log Width (m)	0.553***	-	-	-
Log Depth (cm)	0.473***	-	-	-
Log Altitude (m)	-0.247*	-	-	-
Log Slope ($m\,km^{-1}$)	-0.574***	-0.370**	-	-
Log Aluminium (t)	-0.254	-0.306*	-	-
Log Alkalinity ($mg\,l^{-1}$)	0.723***	0.259*	-	-
Discharge (units)	0.767***	0.251*	-	-
Field pH	0.416***	-	0.347**	-
Log Conductivity ($mS\,cm^{-1}$)	0.547***	-	-0.444***	-
Log Iron (t)	-0.295*	-	-0.465***	-
Log Sulphate (f)	0.532***	-	-0.490***	-
Log Sulphate (t)	0.541***	-	-0.470***	-
Log Distance from Source (km)	0.331**	0.262*	0.275*	-
ASPT	-0.310	0.412***	0.670***	-
pH	0.363**	-	-	0.312*
Log Aluminium (f)	-	-0.427**	-	-
Flow (units)	-	0.248*	-	-
Temperature (^{0}C)	-	0.311*	-	-
Number of Taxa	-	0.809***	0.553***	-
BMWP	-	0.752***	0.599***	-
Log Iron (f)	-	-0.304*	-0.448***	-
Log Dissolved Oxygen ($mg\,l^{-1}$)	-	-	0.348**	-

Notes: r (Pearson's correlation coefficient) is significant at the 0.1% level above 0.40 (**), at the 1% level above 0.319(*), significant at the 5% level above 0.246 or not significant (-). (t)/(f)=total/filtered $mg\,l^{-1}$

From Table 4.8, the macrofaunal diversity indices are strongly reflected on axis 2, rather than axis 1, as for all other analyses in this study. Number of taxa, BMWP score and ASPT all correlate strongly ($p<0.001$) with axis 2. Flow rate, filtered aluminium and slope also correlate strongly with axis 2. Axis 1 correlations are strongest with discharge, alkalinity, slope and width. These represent river size and positioning parameters. Water quality is also strongly correlated ($p<0.001$) with axis 1, including sulphate, conductivity, alkalinity and field pH.

Axis 3 reflects the correlations of axis 1. Dissolved oxygen correlated weakly but significantly ($p<0.05$) to axis 3. The DECORANA ordination with subsequent correlations of axes with variables therefore reveals the complex interaction of physical and chemical parameters, controlling macrofaunal distributions in the sites sampled over a large region and wide range of waterway types in Wales.

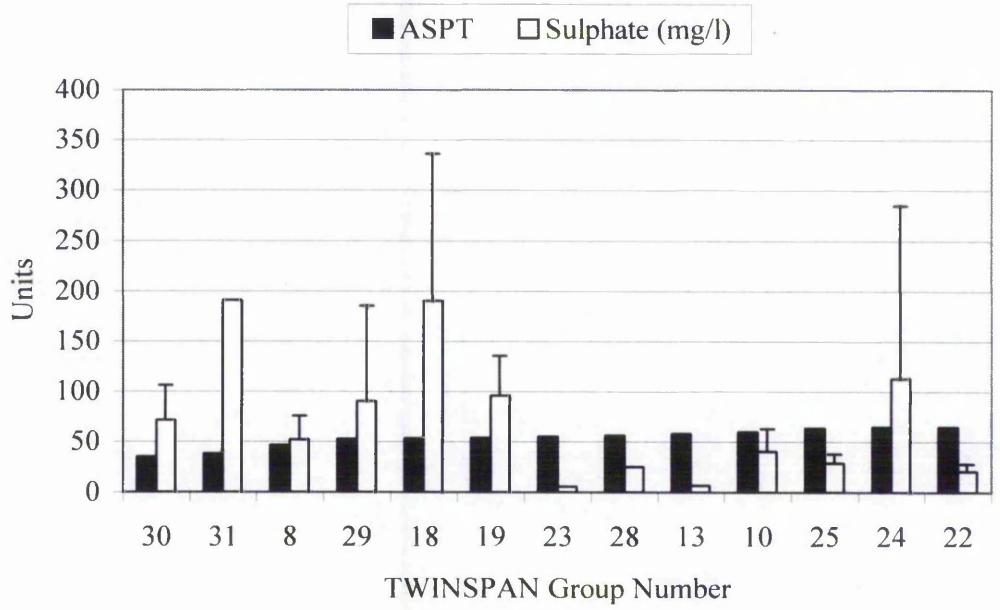


Figure 4.17. Wales TWINSPAN Group Characteristics (mean values +/- 2 SE). ASPT Values multiplied by 10 for clarity of comparison.

No immediate strong patterns can be found in the mean values for environmental variables in each TWINSPAN group, however, arranging groups in order of ASPT (Figure 4.17.) shows the general reduction of sulphate (mg l^{-1}) concentrations with increase in ASPT. Conductivity values (mS cm^{-1}) held in Appendix XXV follow this trend.

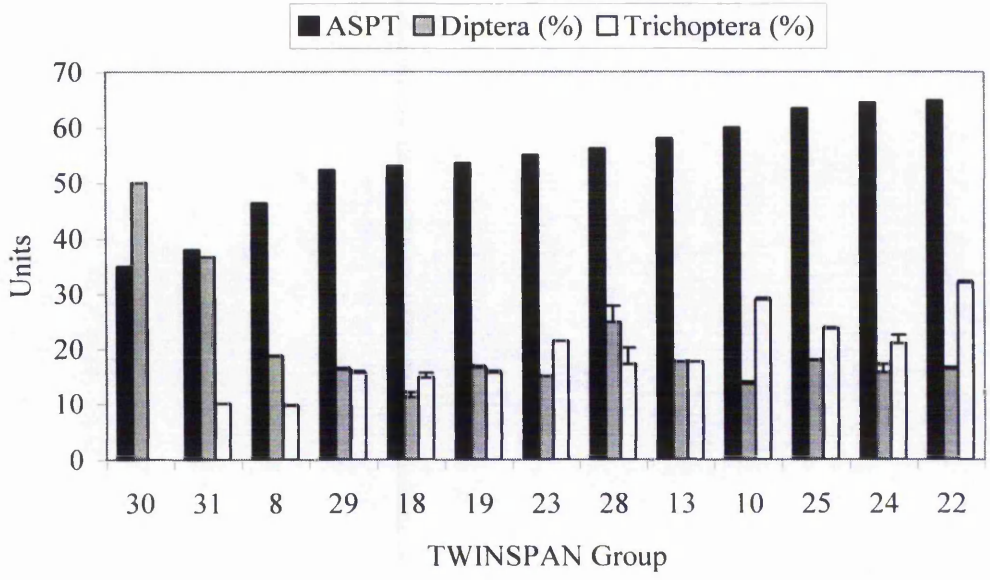


Figure 4.18. Wales TWINSPAN Group Taxa Characteristics (mean values +/- 2 SE after arcsine transformation, shown following backtransformation). ASPT Values multipled by 10 for clarity of comparison.

Arrangement of groups in order of mean ASPT shows patterns in the distribution of taxa between groups. Particularly strong patterns can be seen in Figure 4.18. (data held in Appendix XXVI). Dipterans are most dominant in groups 29, 30 and 31, which have low mean BMWPs and ASPT. Trichoptera increase as mean BMWP increases across groups.

4.3.4.3 Multiple Discriminant Analysis

The results of multiple discriminant analysis to investigate the variables which most strongly distinguish TWINSPAN groups is presented in Table 4.9. Noticeably more variables were selected than in the case of Yorkshire and Northumbria by the stepwise procedure to distinguish between heavily overlapping TWINSPAN family groups, perhaps relating to the variety of watercourses sampled over a variety of catchments. From the discriminant function coefficients, discharge rate (units) and slope (m km^{-1}) are significant discriminating variables across groups. RIVPACS is a similar approach using MDA to predict TWINSPAN group membership, and this system has

been used successfully to predict macrofauna scores on a national basis in England, Scotland and Wales, across many different river catchments. Unlike the current study however, the success of RIVPACS lies in comprehensive species lists, sampled over three seasons, unlike the single season data used in the MDA model for Wales.

Table 4.9. Results of Stepwise Multiple Discriminant Analysis for Wales Sites (for each variable, probability of F<0.01).

TWINSPAN Level	Number of significant discriminant functions[1]	% of sites correctly predicted	Variables selected	Standardised Canonical Discriminant function 1 coefficient
Level 1	1	89.06%	Discharge (units) Slope (m km^{-1})	0.672 -0.639
Level 2	2	71.9%	Discharge (units) Alkalinity (mg l^{-1}) Iron (t)	0.727 0.646 -0.044
Level 3	3	65.6%	Discharge (units) Silt and Clay (%) Conductivity (µS cm^{-1}) Sulphate (mg l^{-1}) Depth (cm) Slope (m km^{-1})	0.985 1.095 -1.23 1.374 -0.211 -0.50
Level 4	4	56.3%	Sand (%) Silt (%) Discharge (units) Conductivity (µS cm^{-1}) Sulphate (t) Sulphate (f) Depth (cm) Aluminium (f) Slope (m km^{-1})	-1.755 1.640 0.608 -0.337 3.811 -3.498 -0.335 -0.227 0.277

[1]Wilks Lamda significant to at least p<0.05.

4.4 Discussion

Clearly, the multivariate analysis has revealed some strongly defined patterns in coal mine pollution factors which appear to influence macroinvertebrate distributions within the regions of Yorkshire, Northumbria and Wales. A multiple discriminant

analysis for each region clearly showed that relationships exist between the biological classification system and discriminant functions derived only from physical and chemical variables. Therefore, measured environmental variables could be used to generate family lists from TWINSPAN groupings. Prediction of correct TWINSPAN group membership to Level 3 for Yorkshire, Northumbria and Wales ranged from 62.2 - 80.7% (using 3-6 variables). This compares favourably to those studies predicting group membership in unpolluted river models. Using family qualitative data to Level 3, and 14 variables, Furse *et al.* (1984) obtained a prediction success of 69.8%. For one season only (spring), using 6 variables, a prediction of 59.7% was achieved. Notably in this study, prediction success was highest in Yorkshire High Impact sites (80.65%), perhaps due to the strong pollution gradient clearly separating tolerant and intolerant families.

However, the correlations found must be interpreted with caution, as Wright *et al.* (1984) stress that as environmental variables correlate with each other (e.g. heavy metals in this study), there is the danger that importance may be falsely ascribed to a variable which is highly correlated to unmeasured factors of actual importance. Such factors may include the contribution made by changing food resources along the river system, as well as geological and climatic factors. There are problems in relating only biotic factors to environmental variables. Biological factors could also be incorporated into models, for example competition and predator-prey interactions. Many factors are also inter-correlated with hydrographic regimes eg. water flow and substratum are linked.

4.4.1. Factors controlling macroinvertebrates in unpolluted freshwater environments in Yorkshire, Northumbria and Wales.

The regional differences in patterns of correlation suggest that there are underlying variables not related to pollution, controlling macroinvertebrate distributions regionally, on to which the influence of local coal mine discharges is projected. Wright *et al.* (1984), in a nationwide survey of unpolluted river systems, found, using species data, that substrate and alkalinity correlated most strongly to axis 1, whereas distance from source, discharge and size (width, depth) characters correlated most highly with axis 2. These overriding variables emerge to a greater or lesser extent in all the

ordinations in the present study, depending on the severity of pollution. As in this study, Wright *et al.* (1984) failed to reveal clearly separated site clusters in their ordinations. They explained this by the 'river continuum concept' suggested by Vannote *et al.* (1980), which argues that there is a progressive change in macroinvertebrates from source to mouth, due to changes in food availability along the river. This is reflected in a shift to collectors, away from grazers and shredders. Collectors are those macroinvertebrates which filter feed, or gather from sediments, fine particulate organic matter. Indeed, the variables slope and distance from source, possibly reflecting these changes, significantly correlated with axis 2 in the Northumbrian model, and axis 1 and 2 in the Welsh model. It tended to be masked by variables directly relating to pollution levels in all regions. It is suggested that a background continuum of macroinvertebrates is present, and onto this are projected the discrete communities found at the point of discharge of a pollutant. In the cleaner upstream sites in Northumbria, with more sensitive taxa such as plecoptera, any polluting input will have a much bigger impact on the continuum than in downstream sites where species have already been exposed to organic inputs and are better adapted to low oxygenation, higher levels of suspended solids and higher alkalinity.

It is useful to examine regional differences in some of these main variables known to control macroinvertebrate communities, to ascertain any underlying regional differences in those communities. Table 4.10 illustrates the mean values of some of the key variables for upstream sites in each region. The large variances for each variable suggested the need for a test of significance. A Tukey test was used to test for significant differences between upstream sites in each region for each variable. This test compares site columns by calculating the difference between the means and dividing it by the square root of the mean squared error (residual) divided by the number of replicates, here taken as 10. A one way analysis of variance was also performed. The results are held in Appendix XXIX. Overall, there was no difference between sites in the one way analysis of variance ($F=0.5134$, $p=0.9978$), but many (about 35%) individual site pairs were different in the Tukey test. There are site differences within each region, and much more so in Northumbria and Yorkshire than in Wales.

Table 4.10. The mean values of some of the key variables for upstream sites in Yorkshire, Northumbria and Wales. Shaded areas represent highest mean score amongst regions.

Variable	Yorkshire (mean +/- 2 x SE)	Northumbria (mean +/- 2 x SE)	Wales (mean +/- 2 x SE)
BMWP	81 (21.6)	119 (18.8)	97 (11.2)
ASPT	4.96 (0.6)	5.65 (0.6)	5.94 (0.2)
Number of Taxa	15.9 (3.3)	21.5 (2.4)	16.1 (1.6)
Width (m)	1.31 (0.52)	2.06 (0.1)	5.02 (1.22)
Depth (cm)	9.94 (3.54)	18.9 (7.08)	17.31 (3.0)
Distance from Source (km)	2.38 (2.05)	2.48 (1.32)	1.5 (1.42)
Slope (m km^{-1})	91.7 (79)	44.4 (19.2)	31.3 (12.4)
Alkalinity ($CaCO_3$ mg l^{-1})	59.7 (35)	101.3 (40.8)	41.0 (11.8)
Conductivity $\mu S\ cm^{-1}$	625 (166.8)	409 (169)	196 (50.4)
Suspended Solids (mg l^{-1})	13.1 (9.4)	3.2 (1.0)	3.7 (0.6)

From Table 4.10, it can be observed that upstream sites in Northumbria generally have the richest faunal diversity, as illustrated by the mean BMWP and Number of Taxa scores. From the databases held in appendix XXIII, it is found that these differences are due to a larger proportion of higher BMWP scoring families present in Northumbria compared to Wales and Yorkshire. For example, present in the Northumbrian sites but absent in those in Wales are Leptophlebiidae and Ephemeridae (Ephemeropteran); Taeniopterygidae, Capniidae and Chloroperlidae (Plecoptera) and Beraeidae, Odontoceridae and Leptoceridae (Trichoptera). These all score a maximum of 10 on the BMWP scale.

Differing topographies and geologies between the areas go some way in explaining these differing distributions. Northumbria has the cleaner rivers in the study, with high alkalinities favouring species. Northumbrian sites sit on carboniferous limestone, contributing high water hardness/alkalinity; however, the limestone associated with coal measures in the Welsh coal field also contribute to neutralising any acidity in discharges. The Northumbrian and Yorkshire rivers are notably further from their source than those in Wales, possibly receiving considerably more inputs (e.g. of urban sewage), so a lot more could have happened to these rivers in terms of pollution events by the time mine effluents can enter them. Supporting this, the Yorkshire rivers also appear to show the greatest influence of pollution other than from coal mining, notably from the higher mean suspended solid values, attributable to organic sewage pollution. The pH of all except a couple of sites in the study fluctuated around pH 6-7, so was not considered a strong factor in controlling family distributions. Similarly, temperature values did not vary widely between sites in different regions, although would be expected to have a strong seasonal influence on family survival.

4.4.2 The impacts of coal mine pollution illustrated in the Yorkshire region.

A comparison of the lesser polluted sites in Yorkshire ('Yorkshire low impact') and heavily impacted sites ('Yorkshire High Impact') should highlight the effects of increasing pollution in the same area. Caution should be applied to this comparison as the higher impact sites were only sampled for one season in the survey. However, it is not anticipated that sampling times would have any effect on the data collected as these were sampled in September/October, whilst the two season sampled sites were sampled in August and November, so seasonal differences in eg. emergence of insects would be negligible. Furse *et al.* (1984) suggest one season sampling could lead to inaccurate environmental data, not catching certain species due to life cycle stage, flood or drought, and possible magnification of data logging / identification errors in the final analysis.

Comparing DECORANA correlation tables 4.2 and 4.4, it can be observed that there is a shift in significant correlations of variables with DECORANA axis 1, with water chemistry variables such as dissolved metal, conductivity, sulphate and chloride, to include substrate characteristics, notably ochre and cobbles coverage. Silt and gravel

coverage are also introduced when considering axis 3. This supports the findings of Dills and Rogers (1974), Greenfield and Ireland (1978), Scullion and Edwards (1980) and Jarvis and Younger (1997), who suggested that it is the effects of ferric hydroxide blanketing the substrata which causes the most deleterious effects on the macrofauna. This has a negative impact for the survival of fish populations e.g. Salmon, *Salmo trutta* (Dalzell, 1996), in the rivers being studied. Reduction in food supply and toxicity caused by the pollution are serious economic implications. Specific effects of the pollution are now explored.

4.4.3 The influence of ferruginous discharges on specific macroinvertebrate families.

From the analysis, it is clear that despite similarities in significant correlations of environmental variables with macrofauna distributions between the models, a precise suite of variables common to all models has not been found. The high conductivities and sulphate concentrations at polluted sites do put macroinvertebrates under considerable osmoregulatory stress. It is pertinent to examine the ranges of dissolved metal concentrations in each region (Table 4.11). The Environmental Quality Standard (EQS) for Iron is 2mg l^{-1}. This standard for was derived by assessing what mean annual levels of total iron for surface waters would support salmon populations (Mance and Campbell, 1988). Above this level, detrimental effects on biota would be expected (having an impact on salmonid populations).

The stresses that metalliferous effluents exert on macroinvertebrates have been well documented. Chironomidae and Oligochaeta are common to all regions at all ranges of iron pollution. Leuctridae was found at extremely high concentrations (78.7 mg Fe^{2+} l^{-1}) in Northumbria. Strong support for high iron tolerance by the tolerant families in this study comes from work by Rasmussen and Lindegaard (1988), who examined pollution by dissolved iron in a Danish river system. The species with the highest tolerance (up to 30mg Fe^{2+} l^{-1}) were Oligochaeta (Tubificidae), Chironomidae, Lymnaeidae (*Lymnaea peregra*) and *Sialis lutaria*. Nemouridae, namely *Nemoura cinerea*, had a tolerance up to 11 mg Fe^{2+} l^{-1}.

Table 4.11. Regional ranges of iron, zinc and copper concentrations.

Region	Minimum - Maximum mean Iron concentration Filtered Iron (mg l^{-1} Fe^{2+}) (and Total Iron, mg l^{-1} Fe^{3+})	Minimum - Maximum mean Filtered Zinc concentration (mg l^{-1} Zn^{2+})	Minimum - Maximum mean Filtered Copper concentration (mg l^{-1} Cu^{2+})
Yorkshire (all sites)	0.003 – 28.8 (0.157 – 31.0)	0.005 - 1.35	0.001-0.0074
Northumbria	0.003 – 78.70 (0.17- 83.6)	0.005-3.50	0.001-0.007
Wales	0.006 – 32.05 (0.003 – 38.45)	N/A	N/A

Acute toxicity testing of macrofauna to iron have been conducted using Lethal Concentration (LC50) tests. For example, Furmanska (1979) found that the 48 hour lethal concentration for *Asellus aquaticus* to iron (Fe^{3+}) was 81.08 mg l^{-1} at 20°C. Copper was 32.08 mg l^{-1} and zinc 54.05 mg l^{-1}. This suggests that it is not the direct toxicity of the metals copper and zinc in the regions which are causing a reduction in diversity, as regional values are much less than these toxic levels (Table 4.11). However, iron concentrations in Northumbria have been recorded at 83.6 mg l^{-1}; toxic according to these results. It is important to be aware that these metals could be inter-correlated with other variables in the analyses presented in this thesis. Substratum quality, dependent on ochre smothering, is suggested as the dominant factor, as substratum factors were found to correlate significantly across all DECORANA models. Ochre correlated significantly in the Yorkshire High impact analysis. Studies have recorded changes in macroinvertebrate communities at iron polluted sites at concentrations of <1-5 mg Fe l^{-1} (Greenfield and Ireland, 1978; Scullion and Edwards, 1980; Rasmussen and Lindegaard, 1988). However, acute toxicity of iron to freshwater invertebrates tends to occur at concentrations in excess of 5mg Fe l^{-1} (Furmanska, 1979; Maltby *et al.*, 1987; Gerhardt, 1992). The latter author confirmed that the ephemeropteran *Leptophlebia marginata* has a high tolerance to iron; animals survived at concentrations up to 50 mg l^{-1} iron (total) for up to 30 days at pH 7. Furmanska (1979) detailed carapace destruction, body necrosis and limb deformation by copper in

Asellus aquaticus. Zinc caused loss of activity and paralysis (due to inhibition of respiratory activity). Iron caused a general loss in activity and paralysis in all organisms under study. However, Furmanska (1979) found in acute and chronic tests, copper and zinc exerted more harm than iron on macroinvertebrates, particularly crustaceans, causing decreased respiratory activity. Supporting this, Schultheis *et al.* (1997) suggested that the observed decrease in macroinvertebrates in a copper polluted stream may be due to direct toxic effects. The authors also suggest that the metal may accumulate in the microbial biofilm of leaves to inhibit microbes colonising the leaf material, which increases the palatability of the material ('microbial conditioning') for shredders such as *Gammarus*, or the metal generates an avoidance response in shredders such as *Leuctra* sp. Jiang *et al.* (1994) found a direct inverse correlation between Cu^{2+} and the Shannon's diversity index. Depression in feeding rates in *Gammarus pulex* as iron concentrations increased from 0-3 mg l^{-1} was found to be a sensitive indicator of water quality (Maltby and Crane, 1994). The latter authors used bioaccumulation and feeding activity data to isolate iron and manganese as the principal toxic agents out of aluminium, iron, manganese, nickel and zinc. Hellawell, (1986) in a review of literature on metal toxicities to freshwater life, gave an approximate order for metal toxicity, the order (metals relevant to this study, from most to least toxic) is Cu, Zn, Al, Ni, Fe^{3+}, Fe^{2+}, Mn. When metals such as zinc, cadmium and copper occur in mine drainage, they may act synergistically at low concentrations, to exert direct toxic effects. Maltby *et al.* (1987) from LC50 tests found that *Asellus aquaticus* from sites exposed to mine waste pollution are more tolerant than those from sites not so exposed. They suggested genetic differences for this and/or phenotypic differences due to differences in acclimation. Maltby (1991) goes on to demonstrate the effects on reproduction of iron pollution; *Asellus aquaticus* produces fewer, larger offspring downstream of a coal mine discharge in Yorkshire, so spending less investment of energy into reproduction.

The general effects of ferric hydroxide on all benthos have been well documented. Deoxygenation occurs in receiving watercourses due to the oxidation of ferrous compounds. Dissolved oxygen concentrations strongly correlate positively with BMWP, ASPT and Number of taxa throughout Yorkshire sites. In field surveys, iron appears to be more toxic at neutral pH than at a low pH, due to the precipitation on gills, and substrate blanketing. Ferric hydroxide in suspension can reduce light penetration, and can precipitate out to smother benthos, acutely restricting invertebrate life. Jarvis

and Younger (1997) observed this to be the main cause in faunal depletion at discharge sites in Northumbria. Scullion and Edwards (1980) recorded an 88% reduction in diversity below a discharge point; they summarised the main disruptive effects of ferruginous pollution: interference with attachment, respiratory and feeding mechanisms (after ingestion) and disrupting plant growth.

Koryak *et al.* (1972) found the reduction of benthic algae and plant detritus by smothering was to the detriment of Plecoptera nymphs, Ephemeroptera, Crustaceans and snails. Maltby and Booth (1991) found that colonisation below discharges by Hyphomycetes fungi was reduced. These 'condition' leaves for shredders e.g. *Gammarus pulex.* The effects of ferruginous discharges on macrofauna can be related to the lifestyles of individual families. Table 4.12 summarises the possible disruptive mechanisms on specific macrofauna families examined in this study.

4.4.4 Some generalisations - towards and index of pollution.

It is useful to examine families, present across all regions, that appear tolerant of all levels of ferruginous pollution. These families could then be used to suggest a replacement of the traditional BMWP system, which is based on organic (sewage) pollution. Table 4.13 illustrates those families which are present across most, if not all, TWINPSPAN groups, so are the most tolerant of all pollution levels. As the TWINSPAN algorithm clusters sites containing similar communities together, common families across all groups will represent a baseline macrofauna to be expected under all pollution conditions. From the table, the predominance across groups of the pollution tolerant Diptera (Chironomidae, Tipulidae and Simuliidae) is clear. The Chironomidae are often found with Class Oligochaeta. Rasmussen and Lindegaard (1988) also found that these organisms, that are able to tolerate organic pollution, can exist in waterways with high iron concentrations. The order Ephemeroptera is represented throughout most groups in each region by the family Baetidae, and Trichoptera by the families Limnephilidae (cased caddis), Polycentropodidae and Rhyacophilidae (caseless caddis). Most surprising is the abundance of the family Leuctridae, representing Plecoptera, scoring 10 points on the BMWP scale. The Plecopteran family Nemouridae are also widely present. Supporting these findings, Scullion and Edwards (1980) found *Leuctra moselyi* (Mort.), *Baetis rhodani* (Pict.) and *Rhyacophila dorsalis* to be tolerant to ferric hydroxide at discharges in a South Wales coalfield. The abundance of *Leuctra* sp. in

coal mine polluted Welsh rivers is probably due to the absence of competition amongst the surviving species (Gower, 1980).

Table 4.12. The possible effects of high iron loadings in ferruginous discharges on specific macroinvertebrates.

Mechanism	Family/Group	Effect of Ferruginous discharge	References
Burrowing.	Chironomidae Oligocheata	Replace non-burrowers Ephemeroptera, Plecoptera and Trichoptera	Scullion and Edwards (1980)
Case Building.	Goeridae Odontoceridae Lepidostomatidae	Physical smothering of materials for case building	EA (1996)
Feeding. Scrapers.	Lymnaeidae Planorbiidae Glossosomatidae Ancylidae	Loss of epilithon (algal layer) on which graze.	EA (1996)
Feeding. Collectors (Filter Feeders).	Sphaeriidae Simuliidae	Interference of iron precipitating out into suspension.	EA (1996) Learner *et al.* (1971)
Filter feeding. Collectors. Net Spinners.	Hydropsychidae Rhyacophilidae	Interference by suspended particles. (note authors found tolerance to this in *Hydropsyche pellucidula*)	Scullion and Edwards (1980)
Feeding. Collectors. Macrogatherers. (sediment)	Ephemeroptera	Blanketing of substrata with ferric hydroxide decimates habitat for feeding.	
Feeding. Detritus shredders.	Gammaridae Tipulidae Limnephilidae Lepidostomatidae Nemouridae	Blanketing of substrata with ferric hydroxide.	
Feeding. Predators.	Perlidae Tanypodinae	Reduction in prey	

Table 4.13. A Comparison of Macroinvertebrate Families common to most TWINSPAN Groups in Yorkshire, Northumbria and Wales. Total number of TWINSPAN groups per region in brackets. Trichoptera and Plecoptera common across groups are shaded (dark and light respectively).

Region	Families present across all TWINSPAN groups	Families absent from only 1 TWINSPAN group	Families absent from only 2 TWINSPAN groups	Families absent from only 3 TWINSPAN groups
Yorkshire Low Impact (8)	Limnephilidae Chironomidae Oligochaeta Gammaridae Sphaeriidae Tipulidae Dytiscidae	Polycentropodidae Hydrobiidae Lymnaeidae Baetidae Simuliidae	Leuctridae Nemouridae Elmidae Hydrophilidae	Glossiphoniidae Scirtidae
Yorkshire High Impact (7)	Lymnaeidae	Hydrobiidae Chironomidae Oligochaeta Tipuliidae	Nemouridae Baetidae Asellidae Simuliidae	Leuctridae Limnephilidae Rhyacophilidae Gammaridae Heptageniidae Erpobdellidae Planariidae Hydropsychidae
Northumbria (9)	Leuctridae Gammaridae Chironomidae Oligochaeta Elmidae	Limnephilidae Nemouridae Tipulidae Baetidae Dytiscidae Simuliidae	Leptophlebiidae Glossiphonidae Sphaeriidae Lymnaeidae Sialidae Hydrobiidae Ephemerellidae Scirtidae	Polycentropodidae
Wales (13)	Oligochaeta Chironomidae Simuliidae	Leuctridae	Baetidae Tipulidae	Polycentropodidae Rhyacophilidae

Indicator families for each level of TWINSPAN grouping, as shown in figures 4.3, 4.7, 4.11 and 4.15 also are useful as indicators of certain environmental conditions. Nemouridae and Baetidae both act as indicator organisms for TWINSPAN site groupings at Level 3 in Yorkshire Low impact and high impact sites respectively (Figures 4.3 and 4.7)

This simple analysis illustrates that the BMWP system of scoring families in organic (sewage) polluted environments has to be modified to account for families such as Leuctridae that are apparently sensitive to organic pollution but tolerant to iron pollution from coal mines, and taxa that are tolerant to organic matter but not as tolerant to iron. This should begin by a shift down in ranking for the plecopterans and trichopterans previously described. These are useful pollution indicator organisms as they have a life cycle of at least one year's duration, so are present in all seasons. Amendments to the old BMWP system is suggested in Table 4.14. This was created by down weighting families 1-3 points on the score scale according to how many TWINSPAN groups they are common to all regions (Table 4.13). Further sampling across all seasons is required to ensure representatives of all families are obtained. Further field and laboratory studies would be required to define the tolerance ranges of these groups and differences in sensitivity of species within each family e.g. Chironomidae and Oligochaeta. For example, Armitage and Blackburn (1985) found differences in distributions within the chironomid genus *Eukiefferiella* spp. around a metal mine in the Northern Pennines, England. *E. ilkleyensis* was restricted to low zinc sites, *E. claripennis* could tolerate medium concentrations, whilst *E. minor* withstood the whole range of metal concentrations.

4.4.5. A Nationwide Model ?

This study has shown regional differences in the factors controlling macroinvertebrate community distribution in coal mine polluted waters. A national model to examine the effects of coal mine pollution, similar to the RIVPACS system for unpolluted sites (section 1.5.5), may be too general for practical use as a management tool on individual rivers and catchments. Therefore region-specific models, as produced in this chapter, would be more useful in the management and understanding of freshwater environments affected by ferruginous mine drainage.

Table 4.14. A Revised BMWP Scoring System for sites affected by ferruginous pollution.

Score	Family (previous score)	Family (new score)
10	Leuctridae	
8		
7	Nemouridae Polycentropodidae Limnephilidae	Leuctridae
6	Gammaridae	Nemouridae Polycentropodidae
5	Tipulidae Simuliidae	Limnephilidae
4	Baetidae	Gammaridae
3	Glossiphoniidae Hydrobiidae Lymnaeidae Sphaeriidae	Glossiphoniidae
2	Chironomidae	Baetidae Tipulidae Simuliidae Hydrobiidae Lymnaeidae Sphaeriidae
1	Oligochaeta	Oligochaeta Chironomidae

Another more simple focus for modelling is to directly predict biotic scores e.g. BMWP, ASPT, and the number of taxa, rather than family lists as TWINSPAN groups. A comparison of traditional regression techniques and novel algorithms to generate such region specific models is explored in Chapter 5.

4.5. Summary

Mathematical representations of macrofauna distributions around long abandoned coal mines were generated by classifying 165 sites with TWINSPAN, ordinating with DECORANA and relating to physico-chemical variables with Multiple Discriminant Analysis. It is concluded from this work that the variables most successful in discriminating between regions depended on the background regional diversity; in the upland, low impact Northumbrian sites, substratum and position along the river dictated family communities. However, in Yorkshire high impact sites, substratum quality, including coverage by 'ochre' appeared to exert a strong influence, whilst water chemistry alone dictated communities in Yorkshire low impact sites. It can be concluded that substratum quality downstream from abandoned mine discharges is paramount in dictating macrofauna communities. The formation and effects of iron compounds as the major factor in polluting events is discussed. Inspection of families common to polluted sites has prompted the suggestion of revisions to the BMWP index to account for iron tolerant families, namely Leuctridae and Nemouridae (Plecoptera) and Polycentropodidae and Limnephilidae (Trichoptera).

Chapter 5. Comparison of Traditional Multiple Linear Regression with the Group Method of Data Handling and Backpropagation for Predicting Biological Scores.

5.1 Introduction

5.1.1 Background to the Group Method of Data Handling and Backpropagation algorithms

Linear multivariate modelling, such as stepwise multiple regression, works under the assumption that the variables being modelled have linear relationships. However, this is rarely the case in ecology. The Group Method of Data Handling (GMDH) is a method of modelling which only extracts patterns and mimics the data, including non-linear relationships (Clareboudt, 1994) rather than attempting to ascribe degrees of relationship between the dependent and independent variables (as do regression coefficients in multiple regression). This method could be considered a useful tool, when the emphasis is on the prediction success rather than the causal factors.

Backpropagation is an artificial neural network (ANN) architecture. Artificial neural networks (ANNs) are receiving increased attention for modelling non-linear systems. Neural networks have the ability to learn, recognise and generalise from patterns contained in ecological data. Whereas a traditional computer program lays down step by step procedures for solving a problem, a neural network does not need a program. Instead it is presented with examples that specify desired outputs for sample cases. The network then adapts its computational behaviour according to the examples. Therefore the network 'learns' from examples, just like the human brain (Kothari and Oh, 1993). ANNs have been used in a wide range of disciplines. However, ecological applications have received attention only relatively recently.

5.1.2 Ecological uses of the algorithms

In aquatic resource management, numerous models have been developed, for example to predict trout population density (Baran *et al.*, 1996) and spawning sites (Lek *et al.*, 1996); and also in fisheries management (Brooks and Probert, 1984).

Backpropagation has been used to predict the ratio of production to mean biomass (P/B ratio) in benthic environments (Brey *et al.*, 1996). More specifically to this study, Walley and Fontama (1998) used backpropagation to predict ASPT scores and macroinvertebrate family numbers more successfully than RIVPACS. To date, these are among the few attempts to use alternative and neural algorithms as a tool for the management of freshwater benthic environments. In the light of this, the present study examines the use of GMDH and backpropagation in predicting biological scores of rivers impacted by coal mine pollution.

5.2 Aims

The main aims of this study are:

1. To determine the value of multiple regression analysis as a diagnostic tool to establish the factors controlling the macroinvertebrate diversity scores in the coal mine polluted waterways of Yorkshire, Northumbria and Wales.
2. To compare the performance of multiple regression and two alternative techniques, Group Method of Data Handling (GMDH) and Backpropagation (BP) to predict macroinvertebrate diversity scores.

5.3 Methods

5.3.1 Data Sets and Pre-processing

The regional data for Yorkshire, Northumbria and Welsh sites analysed in the present study is identical to that used in Chapter 4 (refer to 4.1.3). To compare the ability of neural network algorithms to predict biological scores with multiple regression analysis, the databases were imported into the Neuroshell2 Package 1.5 (1993, Ward Systems Group). The databases were saved in Excel 5.0 (1993, Microsoft Corporation) as a Lotus 123 (.wk1) file, and imported into the package. Various models were then created using the two 'architectures' or 'algorithms' of Group Method of Data Handling and Backpropagation in the Neuroshell2 package. For both procedures, the 'input' (independent) variables and 'output' (dependent) variables were chosen according to those selected by stepwise multiple regression, to allow comparison of results.

5.3.2 Stepwise Multiple Regression (MR)

The technique of Stepwise Multiple Regression (Weisberg, 1980) was performed on the data sets for all 3 regions. This technique was performed by Wright *et al.* (1989) for nationwide U.K. data. Log transformation was conducted on all variables which required contraction at the upper end of the data set, to normalise distributions (Elliott, 1977). The effect on the results was minimal, however the transformation was necessary for the valid use of the regression techniques. Analysis was performed in Minitab 11.0 (1996, Minitab Corporation). An examination of inter-correlations between variables was conducted using Pearson's correlation coefficient, to ensure that any inter-correlations of variables extracted by stepwise regression with other variables in the data sets were accounted for. For each region, the dependent variables were BMWP score, ASPT or Number of Taxa, and the independent variables were physical and chemical parameters. The procedure was terminated when no significant reduction in error mean squares occurred through the addition of more variables. Non significant factors were removed in each model by the software.

5.3.3. The Group Method of Data Handling (GMDH)

The Group method of data handling is a method which attempts to build a polynomial model, which predicts values that are as close as possible to the actual value. Successive layers are built with links that are simple polynomial terms. These polynomial terms are created by using linear and non-linear regression. The benefit of this is that polynomial formulae are widely understood - a polynomial is simply a mathematical expression containing several terms e.g. $x^2+2xy+5y$. The GMDH procedures generate polynomial equations using methods analogous to the 'survival of the fittest' principle, whereby a few basic equations are used to construct a new generation of more complex offspring, only some of which 'survive' to generate still more complex polynomials. The process is stopped when the mathematical equations have generated a model of optimal complexity i.e. not too simple, nor too complex (Farlow, 1984). The model is similar to a network of simple submodels built into the shape of a pyramid (Figure 5.1.). Because many of the polynomial blocks saved during

the iterative process do not contribute to the final model (as seen in Figure 5.1), they are discarded and the remaining polynomials remain to form the 'pyramid' structure seen.

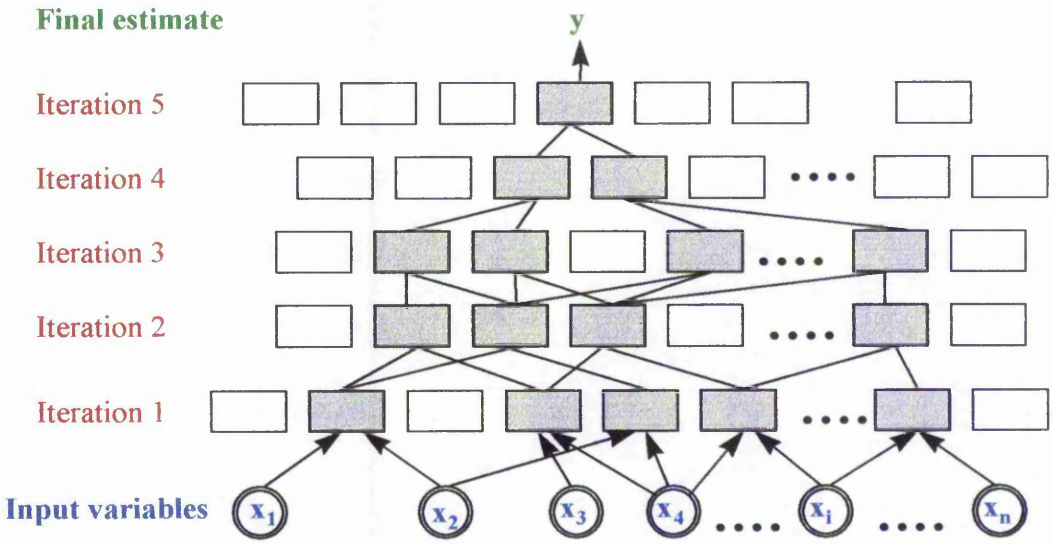


Figure 5.1. Schematic diagram of a GMDH Model. Each box represents a submodel. The shaded boxes represent polynomials contributing to the final model (adapted from Clareboudt, 1994).

The algorithm is as follows:

1. The quality of each model to be generated is specified as an 'evaluation criterion'. The simplest criterion, that is used similarly in linear regression, is the sum over all samples of the squared differences between the actual output and the models prediction divided by the sum of the squared actual values. This is called the normalised Mean Squared Error (MSE). This is the residual error in the analysis of variance.

2. Two of the input variables are selected and a simple set of polynomial terms are generated.

3. All the possible models from these terms are checked and the best model is selected as a 'candidate for survival' with its own value for the evaluation criterion.

4. Another pair of input variables is selected and step 3 is repeated, producing another candidate for survival. This is repeated for each possible pair of n input variables, so $(n^2-n)/2$ candidates for survival are generated, each of which has its own value for the evaluation criterion.

5. Several candidates for survival are selected which give the best approximation to the output variable. These are stored in the first layer of the pyramid (iteration 1).

6. The layer of survivors is then used as inputs to build the second layer of the pyramid. As some inputs are already polynomials, the next layer may contain very complex polynomials.

7. This layer building continues as long as the evaluation criterion is reduced. Model generation is stopped if this is not the case.

A complete description of the algorithm can be found in Farlow (1984). The default 'Smart' Training option was applied to generate all models for each region. This optimises models using the following settings:

a) Model non-linearity was set at high (default) which results in highly non-linear models.

b) Model diversity was set at medium (default) which allows more survivors. It is a compromise between computation time and the necessity of preserving material to create a model.

c) Model complexity was set to medium (default) which avoids 'overfitting', recommended for use when the number of samples in the training set is small, as in this case.

GMDH models were produced using the independent and dependent variables identified by stepwise multiple regression.

5.3.4. Backpropagation (BP)

The mathematical algorithm of backpropagation (Rumelhart *et al.*, 1986) was used to produce a model to predict BMWP scores for Yorkshire high impact sites. The input (independent) variables used were those selected by stepwise multiple regression, to allow comparison of the two techniques in predictions.

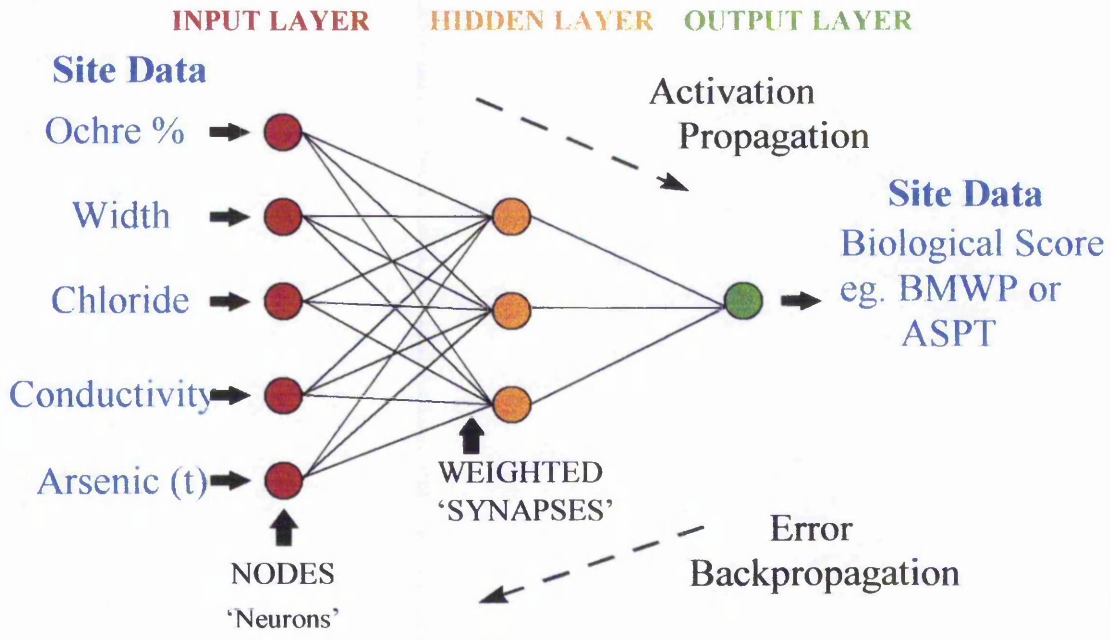


Figure 5.2. Structure of a Backpropagation Network. Example Inputs and Outputs as used in this study are illustrated.

5.3.4.1 Structure and function of a Backpropagation network.

The structure of a typical 3-layer backpropagation network is illustrated in Figure 5.2. The layers have different functions and are called the input layer, the hidden layer and the output layer. The layers contain 'nodes' or 'neurons' which are

interconnected between layers. All the nodes of a layer except the last emit an axon to each node downstream. The input nodes are equivalent to the independent variables used in multiple regression analysis. The hidden nodes detect patterns in the data from the input nodes, and pass this to the output nodes, which represent the dependent variable as found in multiple regression analysis. The 'patterns' found are held in the connections of the network as weights. Input data is scaled to a value in the range of 0 to 1. This ensures that all input data are in a form that the network can handle, and it also ensures that the numbers are within the same range. Input data is passed from the input layer nodes or 'neurons' to the hidden layer, with prediction results produced at the output layer.

The hidden nodes and output nodes process their inputs in two steps:

1. The net input to a node (**j**) is calculated as the summation of every input $\mathbf{o_i}$ multiplied by its weights as follows:

$$\mathbf{x = sum\ (o_i\ .\ wij)}$$

Where **x** is the net input to a node

o is the output from a neuron entering the node **j**

i indexes the nodes that feed the **j**th node

wij is the weight of a connection between the **i**th and **j**th nodes

2. The result needs to be 'filtered' or 'squashed' into a value between 0 and 1. The output is then passed to the next layer. If this is the output layer, then an output value (prediction) is produced.

5.3.4.2. How the network 'learns'

The technique of backpropagation is described as a 'supervised' learning algorithm, in that to learn, the network has to know what output (eg. BMWP score) it should have given. The data for each site are called individual 'patterns', these are presented as a 'training set' to the network, and each pattern is considered in turn, the goal of the process is to minimise the error of prediction over all of the training patterns (the Mean Squared Error). Weights are initially set at random numbers between +0.3 and -0.3, and output is generated as above. The network compares its output with the

actual values in the site data, and from the difference between predicted and actual values an error value is generated (calculated as the sum of squares of errors). This error is then 'backpropagated' to each of the nodes of the hidden layer to adjust the weights, these then update the weights of the input layer, so to minimise the error of the output/prediction in the next forward pass. One 'epoch' is completed when the processes of forward activation propagation followed by error backpropagation is completed for a pattern (site).

The performance of the network is affected by the number of hidden nodes, which has to be set by the user, as the optimum number for an application is not known. Tan and Smeins (1996), suggest that one 'rule of thumb' is to start with a number of hidden nodes between the number of input and output nodes, then proceed from there, by trial-and-error. The optimum number of hidden nodes was determined by this method for the backpropagation networks generated in the present study. One can use parameters such as the learning rate, and the momentum to adjust training time. The learning rate determines how much the weightings of the network are changed in each epoch (Balls, 1996). A large learning rate basically means errors are reduced quickly, reducing the sensitivity of finding patterns in the data, and an optimum model may not be produced. Momentum rate can also be set by the user, which determines the proportion of the last weight change that is added to the new weight change. It ensures smooth changes in weights of the network and prevents oscillations in the changes of weights, so minimisation of error is not achieved (Balls, 1996). For the present study, the learning rate was set at 0.5 (default) and the momentum at 0.9 (default), as preliminary studies identified these to be optimal for the data sets used.

5.3.5 Advantages and Disadvantages of GMDH and Backpropagation

The main disadvantages of GMDH and backpropagation includes the fact that the coefficients of a regular equation that are provided with traditional regression are not produced. Instead, it is possible to determine the relative contribution of each input variable by examining the list in order of contribution factor value (arbitrary units). These are unique to the network and comparable between networks. In GMDH analysis, the contribution of each variable is given in polynomial equations as output for the

models. However, these equations are only approximations of the complex models produced by the algorithm; and are not suitable to publish for use in predictions. This limits the use of GMDH modelling to using the software used to produce the trained models to make any further predictions.

Backpropagation networks can take a long time to learn, depending on the number of training patterns, hidden nodes and learning rate. However, with advances in computing power of desktop machines, this problem is being minimised.

Care is required with both GMDH and backpropagation, so the models are not 'over-trained' ie. memorise all patterns, including 'noise' in the data, so that they are unable to 'generalise' and produce satisfactory predictions for the 'unseen' test data not used in training. Over-training can also be tackled at the training stage, by having a 'test set', which is an extracted set of data from the database, which are modelled alongside the actual main set of data. The training is stopped for the main training set when a predetermined error value for the test set is reached, so an optimum solution for the entire model is produced. Training was stopped in the backpropagation model for Yorkshire high impact sites when the minimum average error for the test data (20% of sites extracted) did not decrease after 20,000 epochs, this was checked at intervals of every 200 epochs. This procedure was repeated for different numbers of hidden nodes (2-30) to compare predictive abilities of the models.

Backpropagation and GMDH neural network algorithms have a number of advantages. They are able to find subtle, non-linear patterns in noisy data, which is present in all ecological results. Because of this, bearing in mind the above limitations, they can be used to generate complex 'expert systems' for many applications, which can make more accurate predictions than traditional modelling techniques. If trained optimally, the models can generalise over data patterns, so increasing predictive success.

5.3.6 Testing of MR and GMDH models

Testing of the Yorkshire high impact site MR and GMDH models was executed by extracting 1/4 of the sites, adjusting the models using the remaining 3/4 of sites to retrain. This 'bootstrap' technique was used successfully by Spitz *et al.* (1996). Predictions were then generated for the isolated sites. This was repeated 4 times. An

additional test was performed using 43 unseen test sites, with data for single seasons across Yorkshire high and low impact sites and Northumbria sites, to assess the ability of the Yorkshire high impact model to generalise across regions. Excel 5.0 (1993, Microsoft Corporation) was used to hold the test site data, equations generated by MR, and codes to extract predictions from GMDH (.def) files, which effectively 'pull' predictions across into the Excel spreadsheet.

5.3.7. Performance Indices

To judge the predictive performance of MR, GMDH and BP models, the following indicators were used:

1. The multiple coefficient of determination (R^2) between observed and estimated values, from the training sets.
2. Analysis of residuals (observed minus expected values of dependent variables)

5.4 Results

5.4.1 Multiple Regression and the Group Method of Data Handling Models

5.4.1.1 Variable contribution to the models

The equations produced by stepwise multiple regression for each independent variable (BMWP, ASPT and Number of Taxa) for each region are presented in table 5.1. It can be observed that in each case, the predictions produced by MR are improved by the use of GMDH, using the same variables, as shown by the multiple coefficients of determination (bracketed in Table 5.1). Improvements varied from 1.1% (Wales BMWP prediction) to 13.2% (Yorkshire low impact sites number of taxa prediction). The number of independent variables retained for the models varied from one (Wales, BMWP prediction) to seven (Northumbria, BMWP prediction). Notably, the heavy metals arsenic, copper and iron featured as influential negative predictors of biological indices common to all regions. From the regression coefficients, total iron appeared to be the single strongest predictor of both BMWP and Number of taxa in Wales.

Table 5.1. Multiple Stepwise Linear Regression equations where the BMWP score and ASPT are the dependent variables and the given environmental parameters are the independent variables. Figures in brackets represent R^2 generated with **GMDH** using the same variables. Polynomial equations produced for the latter can be found in Appendix XXX. Coeff=Regression coefficient (b). CONST=Regression constant, R^2= Multiple Coefficient of Determination. Information on the environmental variables is presented in Table 4.1. Data for Yorkshire high impact and Welsh sites is for one season.

Region	BMWP			ASPT			Number of Taxa		
	Variable	Coeff	t	Variable	Coeff	t	Variable	Coeff	t
Yorkshire High Impact (n=31)	CONST = 3.27			CONST = 8.16			CONST = 20.85		
	As (t)	-1.5	-5.33	As (t)	-5.52	-7.06	Fe (t)	-5.82	-7.0
	Ni (f)	-0.472	-5.96	Sulphate	-1.03	-3.45	As (t)	-27.3	-6.73
	Conductivity	-0.423	-2.69 [1]	Fe (f)	-0.45	-3.26	Temperature	1.54	4.47
							Ochre (%)	-0.03	-3.11
							Zinc (f)	-5.1	-3.0
	R^2 = 0.806 (0.914)			R^2=0.811 (0.917)			R^2=0.874 (0.903)		
Yorkshire Low Impact (n=32)	CONST = 1.687			CONST = 7.38			CONST = 10.43		
	Cu (t)	-0.487	-5.46	Cu (t)	-2.43	-8.42	DO (%)	0.139	5.35
	DO (%)	0.0056	4.88	Depth	-1.09	-2.81	Cu(t)	-9.5	-4.71
	R^2 = 0.680 (0.803)			R^2 = 0.715 (0.827)			R^2=0.668 (0.80)		
Northumbria (n=38)	CONST = 2.018			CONST = 5.576			CONST = 22.88		
	Ochre (%)	-0.0019	-4.01	Silt (%)	-0.012	-4.71	Colour	-3.96	-4.66
	Colour	-0.150	-6.11	Colour	-0.44	-4.14	Width	1.47	4.54
	Chloride	-0.113	-1.53	Pb (t)	1.51	4.17	As (t)	-17.7	3.99
	As (t)	-0.56	-3.91	Cu (t)	-1.01	-2.27 [2]	Ochre (%)	-0.045	-3.31
	Susp.Solids	-0.161	3.10	Depth	0.84	2.17 [2]	Ni (f)	1.98	2.27 [2]
	Depth	0.240	3.10						
	Boulders	0.002	2.47 [1]						
	R^2 = 0.810 (0.870)			R^2 = 0.697 (0.792)			R^2 = 0.754 (0.821)		
Wales (n=64)	CONST = 1.707			CONST = 6.572			CONST = 5.287		
	Fe(t)	-0.234	-4.76	Sulphate (f)	-0.71	-2.78	Fe(t)	-4.91	-5.68
				Fe (t)	-0.38	-2.23 [2]	Alkalinity	3.9	2.57 [1]
	R^2 = 0.268 (0.274)			R^2 = 0.338 (0.380)			R^2 = 0.352 (0.363)		

All t values significant at $p<0.01$ except those significant at $p<0.02$[1], or significant at $p<0.05$[2].

The low multiple coefficient of determination for Wales as compared to the other regions is probably due to the data being more variable, with data for each site collected in only a single season in 1993, over a considerably larger number of river catchments than for Yorkshire and Northumbria. The positive influence of dissolved oxygen is apparent for predictions in the Yorkshire low impact sites supporting the fact oxygenation is critical in controlling macrofauna distributions, and total copper concentrations feature in the models for all three biological scores. Predictions for the high impact sites in Yorkshire, however, include almost exclusively heavy metals and dissolved ions (conductivity and sulphate) to most accurately model biological diversity; substrate factors therefore appear to be masked by water quality in determining diversity at the high impact sites. The exception is the 'number of taxa' prediction, which includes ochre coverage (%) as an independent variable. River physical characteristics, however, feature throughout the Northumbria models. These include substrate characters, width, depth and water colour (the latter indicating the negative impact of water turbidity). The weaker influence of ochre coverage is suggested by a low but negative correlation coefficient.

5.4.1.2 Comparison of performance

A comparison of actual and ideal predictions by GMDH and MR for the Yorkshire high impact sites BMWP predictions is presented in Figure 5.3. Predictions for BMWP values by GMDH fall tightly around the line of an ideal prediction in Figure 5.3A (1:1 ratio ie. prediction is equal to actual value), indicating enhanced predictive ability of these models compared to the under-prediction of BMWP which can be seen in 5.3B. Comparison of the residuals for the model in illustrates the under-prediction of BMWP for most sites by MR in Figure 5.3D, however the much smaller residuals for the GMDH model are apparent in Figure 5.3C.

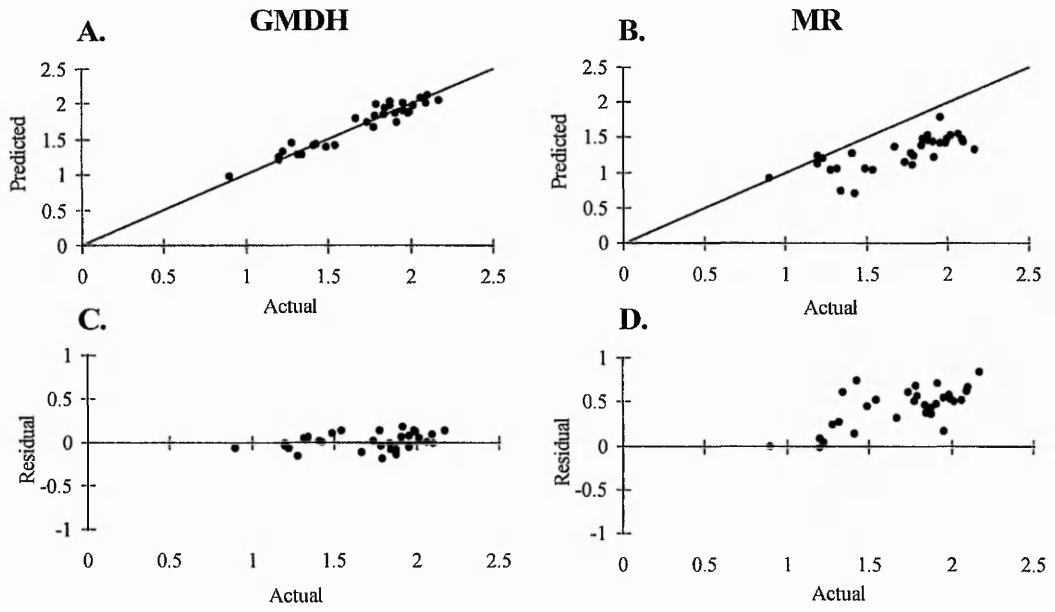


Figure 5.3. Comparison of Group Method of Data Handling (GMDH) and Multiple Regression (MR) in the prediction of Yorkshire High Impact Site BMWP scores. The line on graphs A and B represent the 1:1 slope expected for the predicted score being equal to the actual score. The zero line on graphs C and D represents the zero residual variation that would occur in the event of a perfect prediction.

5.4.1.3 Comparison of predictive abilities

A comparison of the predictive abilities of GMDH and MR models was made four times, using an isolated training set (3/4 of records ie 23 sites) to produce a model to test each time and an independent test set (1/4 of records ie 8 sites). The results are presented in Table 5.2. The GMDH method has a clearly better predictive power in each of the four repetitions of the procedure, reflected in the higher multiple coefficients of determination calculated between observed and predicted values in the GMDH model (R^2=0.9321) as compared to multiple regression analysis (R^2=0.844). Standard deviations for the mean value of the tests is low for both methods.

Table 5.2. Multiple Coefficients of Determination (R^2) of the training set and test set in tests of Yorkshire High Impact site BMWP predictions using GMDH and MR

[1] Actual versus predicted BMWP scores using a subset of 23 sites data [2] Actual versus predicted scores using the trained model for a subset of 8 'unseen' sites data. This repeated for four subsets in both MR and GMDH.

	GMDH		MR	
Subset Number	Training Set[1]	Test Set[2]	Training Set[1]	Test Set[2]
1	0.9282	0.9208	0.816	0.7848
2	0.9398	0.9219	0.729	0.8322
3	0.9023	0.9614	0.774	0.9085
4	0.9413	0.9243	0.857	0.8523
Mean	0.9279	0.9321	0.794	0.84445
SD	0.018043	0.019588	0.055009	0.051226

5.4.2 Backpropagation models

The results for the optimisation of the Yorkshire high impact site model by varying the number of hidden nodes, using the three input variables identified by MR, is presented in Figure 5.5.

It can be observed that the highest multiple coefficients of determination for the training data was achieved with 8 hidden nodes (which can be considered the 'optimum'), producing an R^2 of 0.7803. This compares to 0.806 with MR and 0.9135 with GMDH in table 5.3. Figure 5.5 illustrates how the R^2 fluctuates with changes in the number of hidden nodes. This illustrates the role of the hidden nodes as feature detectors; as the number of nodes increases, so does the number of features detected in the data. These results are erratic; R^2 has been found to stabilise as the number of hidden

nodes pass the optimum (Balls, 1996). This does not occur in the present study as the number of hidden nodes increases from 8 to 30. This is probably due to the very small data set used in training (31 sample sites), which limits the ability of the model to make generalisations across the data.

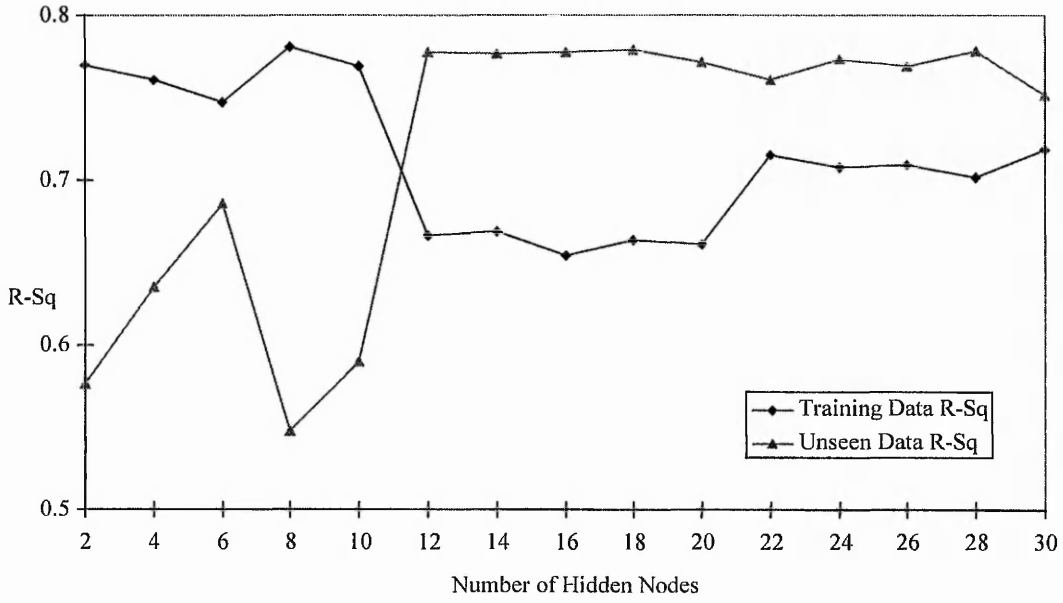


Figure 5.5. A Graph to Show variation in R-Sq for training and 'unseen' test data used in the backpropagation algorithm to model the BMWP for Yorkshire High Impact sites. The algorithm produces a model using the specified number of hidden nodes and the training data, and produces an R-sq for a separate extracted subset of 'unseen' data. Notice the 'levelling out' in R-sq as training progresses.

Table 5.3. Predictions of BMWP across regions (Yorkshire and Northumbria) and seasons by different modelling techniques, trained with Yorkshire high impact site data.

Technique	Training Data Model R^2	Test with 43 sites - R^2
Backpropagation	0.78	0.5481
GMDH	0.9135	0.0404
MR	0.806	0.1504

5.4.3. Ability of different models to make predictions across seasons and regions.

The Yorkshire high impact MR, GMDH and BP models were tested with 43 (single season) test site data, to establish their ability to produce predictions across 'unseen' Northumbria and Yorkshire high and low impact sites (data in Appendix XXXI). From the results in table 5.3, it is clear from the multiple coefficients of determination that the predictions are poor. The GMDH model produces an R^2 of 0.0404, MR an R^2 of 0.1504, but BP the highest with an R^2 of 0.5481.

5.5 Discussion

Multiple discriminant analysis in chapter 4 suggested that TWINSPAN group membership for a particular site could be predicted from environmental variables, so a family list for the group could be generated. Multiple regression in the present chapter has proved to be a useful tool in producing more simple and easily interpretable models to predict biological index scores of coal mine pollution impacted watercourses in Britain. However, in this regression analysis, there is the small possibility that variables could be inter-correlated with other variables in the model. Given the complex non-linear interactions of environmental variables in controlling the distributions of macrofauna around abandoned mine discharges, strong gradients were identified by MR, which translated into highly satisfactory prediction success, as observed by the coefficients of determination; in the range of 0.68 - 0.87 in Yorkshire and Northumbria. Although Moss *et al.* (1987) comments that little is known of the linearity of the BMWP or ASPT score system with environmental variables, the technique has been used to produce similar predictive equations for unpolluted sites by Wright *et al.* (1989). The latter used physical and chemical data for 268 sites from 41 rivers across the UK. Determination coefficients of 0.237-0.297 for BMWP prediction and 0.688-0.714 for ASPT were produced, as compared with 0.268-0.806 and 0.352-0.874 respectively in the present study. This comparison indicates the greater predictive success when pollution gradients are present to produce precise conditions which limit distributions of specific families, which are reflected in the biotic scores.

The technique of Group Method of Data Handling has proved consistently to

enhance prediction success, supporting previous claims of the usefulness of this technique in non-linear modelling (eg. Clareboudt, 1994; Rohde, 1996). The improvements in prediction success by GMDH indicate the ability of the algorithm to simulate the patterns of variation between the variables, and establish highly complex models from this, as it tests for non-linear relationships, inherent to all ecological systems.

The disadvantage of the complexity of the GMDH models, however, is apparent when comparing prediction success with data from other regions, outside the range of source data used to train the model. The Yorkshire High Impact site model successfully predicted the BMWP scores for similar sites in the Yorkshire region (Table 5.2), however, the model produced unacceptably high and low predictions for single season data from Northumbria and Low impact sites in Yorkshire (R^2=0.0404, Table 5.3). Similarly, multiple regression produced an unsatisfactory multiple determination coefficient (R^2=0.1504) when using data outside the range of values with which the model was trained. This highlights the requirements of thorough testing of models, if they are to be used as forecasting tools by the water industry to evaluate the changes anticipated with changing levels of pollution, and to extrapolate outside the source data. To be applicable across the regions, the models must be based on a larger database representing the extreme environmental conditions experienced by macrofauna exposed to both high and low extremes of coal mine pollution. In future studies, the incompatability of data sets due to the differences in variables collected and sampling effort between regions, could be tackled by extensive re-sampling which would then produce a single model for testing. However, Rohde (1996) suggests that GMDH models should be built only for very localised ecosystems. This avoids extrapolations across dissimilar habitats and allows for local differences in macrofauna distributions to be taken into account. This caution in the use of GMDH was first suggested by Green (1988). The latter author claims that GMDH is inferior to regression models, and for noisy, non-linear, multivariate data in small sets, extrapolations can diverge rapidly from observed values. Clareboudt (1994) states succinctly that GMDH 'extracts patterns, mimics the data and nothing else'.

Backpropagation performs the strongest in predicting 'unseen' data (table 5.3, R^2=0.5481). The user has control in the design in the model to stop over-training; from figure 5.5, it can be seen the highest R^2 for the training model is achieved using 8 hidden

nodes. With the test 'unseen' data set, trained alongside this, the R^2 produced for 8 hidden nodes is approximately 0.55. As the number of hidden nodes increases, so does the predictive ability of the training model on unseen data. However, one could argue that there is an unacceptable subjectivity in designing the model, namely choosing the number of hidden nodes, and when to stop training. Indeed, what the model is actually doing to the data inside these 'hidden nodes' has been heavily criticised. Touretzky and Pomerleau (1989), describe a neural network as a 'black box' approach, which fails to provide explanations for the underlying causes and mechanisms of the variables under study. These factors can be deduced from individual regression coefficients using multiple regression.

Despite these limitations, approaches using GMDH and neural network architectures such as backpropagation have been shown in this study to provide powerful alternatives to traditional statistical analysis in the prediction of macroinvertebrate community indices. Stepwise multiple regression remains an excellent diagnostic tool in the exploration of factors controlling distributions of these macroinvertebrates.

5.6. Summary

In this chapter, Stepwise Multiple Regression has been shown to be a very useful diagnostic tool in elucidating the factors that contribute to controlling macrofauna diversity in coal mine polluted watercourses in Britain, given the complex environmental interactions in such systems. From this analysis, heavy metals such as arsenic, iron and copper, appear to be strong factors in determining biological quality in Yorkshire, Northumbria and Wales. The multiple regression supports the previous findings in Chapter 4 that iron and copper are the most significant metals in determining macrofauna distributions, common to models across all regions. These metals were found to have a strong inversely proportional relationship with the biological indices ASPT, BMWP and also number of taxa. The documented toxic effects of these metals to macroinvertebrates are discussed. It is suggested that high iron concentrations in the water lead to the most severe damage by smothering the substratum, clogging feeding apparatus in macroinvertebrates, and high turbidity reducing light penetration for plants.

The novel approach of Group Method of Data Handling (GMDH) has been shown to be superior in predictions of the biological diversity indices BMWP, ASPT and also the Number of Taxa within regional sub-models; and the Artificial Neural Network algorithm Backpropagation was the most successful in predicting 'unseen' data from across all regions, lending itself more favourably to the formulation of a nationwide model using such data. It is suggested that region-specific 'expert systems' could be generated using GMDH and Multiple Regression.

Chapter 6. General Discussion

6.1 Aims and Scope of the Study

The aims of this study were:-

1. To investigate the short term effects of the cessation of mine effluent discharges upon species distribution in the River Idle catchment. Millwood Brook is a good model for this analysis, as it has been sampled intensively prior to mine closure in 1992 (Bird, 1989). The changing status in the catchment of the invading saline tolerant *G. tigrinus* was also to be ascertained.
2. To explore the longer term effects of abandoned coal mine discharges in Yorkshire, Northumbria and Wales, by using multivariate modelling techniques to provide information about the factors controlling macrofauna distributions and so to aid in the management of abandoned coal mines. This was also conducted to contribute towards the understanding of community response to environmental change, as encouraged by Furse *et al.* (1984), who studied unpolluted river sites in Britain.
3. To suggest changes required in the organic-based BMWP index, in order for the index to be of value in monitoring discharges from abandoned mines.
4. To assess the potential use of conventional modelling techniques (multiple regression) and novel techniques (Group Method of Data Handling (GMDH) and Backpropagation) as pollution forecasting tools.

With respect to Millwood Brook, and the upper reaches of the River Poulter, where there is a short time scale (2 years) since discharge of effluent ceased, it has been observed that the watercourse has rebounded enormously, this is shown by an increase of species of up to 271% in sites below the discharge point, highly significant in a chi-squared analysis. Consistent increases in Average Score per Taxon at the sites illustrates recolonisation by less pollution tolerant species. TWINSPAN site groupings indicate fewer, larger groupings of more similar sites in similar stages of recovery downstream of the discharge, in comparison with Bird (1989). This is indicative of a more homogeneous stream system. DECORANA ordination of these groups, and subsequent correlation with environmental variables, revealed a clear loss of the effects of the

pollution gradient (with high chloride and conductivity levels). Now substrate and discharge parameters exert the strongest influence on both family and species distributions. Similar variables were found to control species across the River Idle catchment, indicative of the effects of the loss of polluting discharges from the other 6 mines abandoned since 1992.

This study has gone some way in explaining the reversal of *G. tigrinus* colonisation in the River Idle catchment, and the reduction in range of other salt tolerant species, namely *Paranais litoralis* and *Lumbricillus rivalis*. The loss of the salinity gradient appears to have allowed recolonisation of *G. pulex*. The continued retreat of *G. tigrinus* out of the catchment can be expected. These findings support the intraguild predation theory of Dick and Platvoet (1996); this states that at high conductivities, *G. tigrinus* dominates when its higher reproductive rate is combined with reduced predation pressure by *G. pulex*. However, the loss of pollution in the River Idle catchment means that *G. tigrinus* is excluded by competition from *G. pulex*. However, from studies of the natural habitat of *G. tigrinus* in the U.S. (Van Maren, 1978), the tolerance of the species of temperatures up to 34 °C suggests that the warm saline effluent from Millwood Brook (mean annual temperature of 20 °C) could have provided an upstream migration cue for the species. It would be interesting to continue mapping the retreat of *G. tigrinus* in the River Idle Catchment, and the effects of any subsequent discharges on the colonisation of the amphipod. The investigation of the hierarchy of factors, including salinity, temperature and substrate preference in the colonisation of *G. tigrinus* is another pertinent area of future study.

This study demonstrates that the effects of long term discharges of mine pollution into Millwood Brook have been reversed in a very short time (within 2 years). Water table rebound will eventually lead to future ferruginous discharges from Creswell mine, and it can be anticipated that this will have a negative impact on the biota that has recolonised. The composition of this pollution is likely to be highly variable, as rainfall dictates cycles of pollution generation and discharge from coal mines (Robb, 1994). Any negative effects therefore, will be exacerbated by the lack of a steady environment for pollution tolerant colonisers, unlike the artificial but stable environment created by pumping saline effluent during mine operation, as found by Bird (1989).

The consequences of long term ferruginous discharges from mines has been explored for sites in Yorkshire, Northumbria and Wales. Applying similar modelling

techniques to sites around mines in these areas has revealed the hugely detrimental effect of uncontrolled ferruginous discharges. By elucidating the most important factors controlling the macroinvertebrate distributions around these mines, the models can provide invaluable markers for target setting in any operation to clean up the sites; such operations are receiving increased attention by the Environment Agency. From correlation analysis of the three regions, using TWINSPAN and DECORANA (Chapter 4) and Stepwise Multiple Regression (Chapter 5), a hierarchy of variables to target in remediation has been produced. The negative influence of heavy metals on the macrofauna is evident, and it appears that (in order of importance) iron, copper and arsenic present severe problems to macrofauna assemblages downstream of mines that have been discharging for a long period. To a lesser extent, zinc, nickel and lead also exert an effect. Total conductivity, sulphate, chloride and the reduction in dissolved oxygen as metals are oxidised and precipitate out are prominent factors common across all of the models generated. The significant negative correlation of sulphate across all DECORANA models is to be expected, as this is produced as iron pyrites is oxidised in the mine before ground water rebound (see Box 1.1, Chapter 1.). Although important, these factors appear to be secondary in importance to iron concentrations in the water. These concentrations increase suspended solids (in the form of ferric hydroxide) in the water hence also turbidity (measured in the relative units 'colour') and result in smothering of the substrate in bright orange 'ochre'. These variables all correlate significantly with macroinvertebrate distributions in the Yorkshire High Impact model, however the variables do not significantly correlate in the Yorkshire low impact model. This suggests iron concentrations, ultimately controlling this suite of variables, should be the main focus in any attempt to alleviate the impact of discharges.

These findings assist in the implementation of long term management and remediation strategies for ferruginous mine pollution. The EA is currently working on stripping iron from mine effluent (L. M. Bird, pers. comm). This treatment usually involves diverting pollution from the point of outlet in the watercourse to treatment ponds, lagoons and cascades, where there is the neutralisation of any acidity (with hydrated lime) and the physical settlement of solids (iron floc). The treated water can then be released into the watercourse. Mine sealing with the use of antibacterial agents to prevent oxidation are other methods in use (Down and Stocks, 1978). Other possible options for controlled discharge include pipelines to deliver mine water to offshore

discharge points, or a settling dam for small discharges to release water when river flow would provide good dilution (Robb, 1994).

Such remediation projects conducted by the Environment Agency have begun to tackle minewater pollution in south Wales. One such project is using wetland treatment systems to improve the water quality and appearance of the River Pelenna, located on the Afan catchment, flowing into the bay of Port Talbot. These low cost, low maintenance wetlands help metal oxidation and hydrolysis within the surface and oxygen rich regions of the wetland, resulting in precipitation of metals as hydroxides. Sulphate reduction by bacteria occurring in the low oxygen regions of the wetland substrate also occurs. This results in immobilisation of metals and sulphides.

Lafarge-Redland have applied for planning permission to extend the limestone quarry next to Creswell Crags with possible effects on water table and drainage around the Millwood Brook catchment. It is possible that the localised rising of the water table could lead to ferruginous discharges from the abandoned Creswell mine. In the East Midlands, neutralisation of minewaters occurs naturally due to the high levels of calcium in ground waters from the surrounding limestone, therefore it is anticipated that any future discharges in the catchment will be neutral in pH, similar to those studied in Yorkshire, Northumbria and Wales. This suggests that changes in family groupings detailed for neighbouring Yorkshire could occur in the East Midlands. MDA results showed that TWINSPAN group membership for a site can be predicted successfully (considering all three regions: 62.2% - 80.7%, TWINSPAN Level 3, using 3-6 variables). From this prediction, family lists of expected taxa can be generated for use in forecasting the effects of pollution. The tables of TWINSPAN family and site groups for Yorkshire sites are held in the Appendix XII (low impact) and XVII (high impact). The Environment Agency will monitor any developments affecting the River Poulter system.

Multiple regression has also been explored in Chapter 5 as a tool which could forecast the benefits of such remediation schemes. Consistent improvements on prediction success by use of GMDH, and successful predictions by backpropagation, suggest that these techniques provide an invaluable new resource for the analysis of the complex interactions between the environment and macroinvertebrate communities. These tools do not set constraints on data in terms of normality and linear relationships, so appear ideal for modelling remediation scenarios in one catchment or river system.

6.2 Limitations in the study and Future work.

There are several limitations to the biotic indices and multivariate approaches used in this study to examine macroinvertebrates in relation to water quality. As Moss *et al.* (1987) clarify, the relationships established between environmental variables and macroinvertebrate distribution found should be interpreted as associative rather than causative. However, the significant correlations observed can be used to assist future studies of factors which control macroinvertebrate community structure and function. Studies could include using experimental streams and manipulations in the field. Such a study was conducted by McCahon and Poulton (1991). These authors dosed a soft-water stream in upland Wales with sulphuric acid, aluminium sulphate and limestone slurry to produce zones of pollution. They monitored the responses of *Gammarus pulex* in these episodes, and noted the reduced mortality for animals exposed to liming during dosing. Such dosing could be conducted using iron salts, to investigate effects on biota and substrates.

The main limitation in the present study is the relatively small size of data sets used to construct each region-specific model. Whilst encouraging the exploration of polluted sites using multivariate techniques, Furse *et al.* (1984) used 268 sites sampled over 3 seasons to devise their single model, as opposed to the 165 sites sampled over 1 - 2 seasons to generate individual regional models, as in this study. Merging data sets in the present study was not possible due to Yorkshire high impact sites and sites in Wales only having a sample from one season, and differences in the environmental variables measured. Cao *et al.* (1996) stress that multivariate analysis measures the difference between samples on a relative scale, dependent on the information in the other samples in the data set. A way of reducing errors caused by extrapolating outside the value range (a problem encountered in the comparison of MR, GMDH and Backpropagation techniques in 5.4.3) is to keep models specific to use within a certain river catchment. This reduces inter-region variability. Maximising samples collected within a specific area will reduce prediction error. The study of ferruginous discharges also needs to be extended to include three seasons' data to account for temporal variation in family distributions.

The organic pollution based BMWP system, commonly used by the Environment Agency in water quality assessment, must be modified in several ways if it

is to be of use in nationwide monitoring for and following remediation strategies of ferruginous abandoned mine pollution. A suggested revision is presented in Table 4.14. Firstly, it must take into account the unusually higher tolerance to ferruginous discharge of families such Leuctridae and Nemouridae (Plecoptera), Limnephilidae, Polycentropidae and Rhyacophilidae (Trichoptera) and Baetidae (Ephemeroptera). Secondly, it is unfortunate that the BMWP score is also sensitive to the geological and topographical characteristics of the site. If biological monitoring is to fulfil its potential as a complement to chemical monitoring, it is essential to be able to distinguish between the effects of pollution and natural environmental effects (Wright *et al.*, 1989). The multivariate analyses presented in Chapters 3 and 4 indicate a range of physical and chemical factors, common to different regions in the UK, which can override any effects of pollution in controlling macroinvertebrate distributions. Indeed, Plecoptera, unique to sites studied on fast flowing streams with coarse substrate in Northumbria, were not present in the other regions due to such environmental restraints limiting their natural distributions. Walley and Hawkes (1997) conducted a detailed study to use the characteristics of sites in which certain families are found, together with the abundance of each family, to add sensitivity to the BMWP system and adjusted BMWP scores accordingly. Primarily, the final score is dependent on which set of site types is being studied, e.g. a riffle (coarse substrate) or pool (fine substrate). The authors gave families an indicator value, which takes into account the specificity of the family to certain water quality conditions, estimated from the number of sites of a type in which the family occurs nationwide. This is a similar process to that used to devise the original BMWP system. They also use abundance to calculate the final score; the greater number of individuals belonging to a family is indicative of specific conditions rather than the occurrence of small numbers. This eliminates the effect of bias of individuals, carried by drift, affecting total scores. The latter weighting added to the score increases its sensitivity.

Guhl (1987) criticised the failure of biotic scores to account for differences in tolerances within taxa as broad as families; indeed by simply comparing BMWP scores, more subtle replacements of species with their more tolerant counterparts within the same family is not accounted for. In this respect, reducing community data to a condensed BMWP score at the time of sampling may produce an index which is too insensitive to pollution. It is note worthy that the saline pollution indicator species,

Gammarus tigrinus examined in this thesis scores 6 points using the BMWP system, as does *Gammarus pulex*, intolerant of such pollution. Changes in the BMWP system are clearly required to account for these, and other anomalies found in chapter 4 for distributions of families found in iron polluted sites. It is necessary to downweight *Gammarus tigrinus* in the BMWP table; conversely, *Gammarus pulex* needs to be upweighted.

The goal of biomonitoring should be to detect measurable changes in ecosystems, not minor fluctuations that are quickly dampened. Armitage *et al.* (1974) suggest that the variability of river conditions means that data from pond net sampling are only precise enough to allow gross comparisons between populations of different streams, sites or seasons. Further studies are needed to assess the degree to which pond net samples are a measure of the communities they are assumed to represent (Furse *et al.*, 1981). Despite the limitations, this form of biological monitoring remains a vital tool in providing information for sustained environmental protection (Chave, 1990).

A recent addition to the armoury in predicting macrofauna distributions is the use of artificial neural networks, which has been shown by Walley and Fontama (1998) to provide a greater accuracy in predicting biological scores than the traditional RIVPACS system used by the EA to generate predictions of baseline scores in unpolluted sites for management purposes. The authors use ASPT and Number of Families, themselves less sensitive to sampling effort and seasonal variation, and so more effective for monitoring than BMWP. This study utilises GMDH and backpropagation as powerful tools in modelling non-linear relationships in the ecological data. It can be expected that despite limitations, such tools will receive increasing attention, to investigate pollution remediation scenarios, using pollution related chemical and physical variables.

6.3 Summary

The aims of this study have been largely satisfied. Discharges from abandoned coal mines are clearly complex pollutants, whether saline or ferruginous in nature. The multivariate analysis tools TWINSPAN, DECORANA and MDA have been used to isolate the contributions made by the individual components in the pollution, and where pollution is less severe or absent, the underlying background environmental factors have shown to be significant in the models. The artificial neural network algorithm

backpropagation and GMDH have been shown to perform better than traditional multiple linear regression analysis in predicting biological index scores.

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Appendix I. Mean environmental measurements from Millwood Brook/River Poulter for winter 1994 - summer 1995. 3 samples per site. Results for Bird (1989) shown in brackets.

Site Code	Chloride (mm l^{-1})	Conductivity (mS cm^{-1})	pH	Temperature (°C)
1	1 (1.73)	0.88 (0.91)	7.99 (7.71)	19.9 (8.0)
2	6.6 (2.29)	1.13 (1.2)	7.96 (7.68)	16.3 (9.0)
3	2.8 (2.29)	1.13 (1.09)	8.1 (7.61)	11.6 (9.0)
A	8 (45.24)	1.25 (5.29)	7.36	11.8
B	8.2 (34.85)	1.99 (4.28)	7.37	12.3
4	4.2 (14.34)	1.29 (2.28)	7.97 (7.57)	14.2 (9.0)
5	6.2 (15.52)	1.29 (2.44)	7.98 (7.46)	14.8 (9.2)
6	4.4 (393.2)	1.33 (36.19)	7.94 (6.82)	15.2 (16.2)
7	16 (199.17)	1.33 (18.33)	8.03 (7.29)	16.1 (12.0)
C	6.7 (24.16)	1.39 (3.29)	7.21	14.33
8	5.3 (140.96)	1.29 (14.39)	7.83 (7.25)	17.1 (11.6)
9	6 (105.58)	1.36 (10.87)	7.53 (7.32)	17.1 (11.0)
10	9.8 (104.42)	1.42 (10.696)	7.57 (7.21)	17.4 (11.2)
11	5 (99.44)	1.34 (10.84)	7.47 (7.21)	16.8 (11.1)
12	6 (101.61)	1.33 (10.84)	7.61 (7.18)	17 (12.0)
13	7 (103.35)	1.39(11)	7.53 (7.18)	14.5(12.0)
14	4.5 (106.67)	1.27 (11.23)	8.16 (7.32)	21.7 (10.8)
15	5 (100.17)	1.22 (11.23)	8.11 (7.46)	17.7 (11.0)
D	5.2 (11.03)	1.31 (1.54)	8.32	17.4
16	3.8 (62.64)	1.29 (7.57)	8.14 (7.39)	17.9 (11.0)
E	3.75 (13.61)	1.54 (2.66)	8.21	15.8
17	5.4 (17.42)	1.24 (2.68)	8.17 (7.32)	13.3 (10.2)
18	6.4 (19.17)	1.4 (2.78)	8.46 (7.68)	14.3 (10.0)
19	5.6 (21.47)	1.32 (2.76)	8.63 (7.82)	17.6 (10.2)

Appendix II. Anion determination results (ppm) performed on Dionex DX100. Samples taken in May 1995. 1 sample per site. Results for Everett (1991) shown in brackets.

Site Code	Chloride	Nitrate	Phosphate	Sulphate
1	51.28 (60.76)	1.08 (93.14)	217.86 (30.43)	141.25 (164.42)
3	80.95 (291.03)	85.12 (108.54)	4.07 (91.21)	138.38 (260.01)
5	129.63 (152.34)	78.1 (77.50)	9.19 (77.99)	228.82 (214.19)
6	0.55 (2290.92)	69.08 (74.45)	6.63 (79.02)	218.57 (335.52)
12	131.48 (1663.06)	93.4 (71.19)	7.43 (79.45)	225.54 (209.09)
15	153.35 (751.44)	89.46 (73.05)	6.31 (74.00)	221.95 (228.00)
L1	50.78 (126.85)	0.1 (69.14)	155.06 (83.35)	126.12 (181.41)
E	164.51 (612.21)	0.85 (58.13)	123.81 (32.15)	0.06 (229.51)
18	0 (638.52)	60.81 (60.39)	60.81 (1.41)	213.4 (248.07)
19	0 (665.49)	45.12 (45.97)	0 (34.96)	214.8 (208.75)
S1	136.32	51.76	0	281.44
VW1	0.21	25.1	0.06	260.08
BL	0.33	10.81	0	297.23
ST	19.9	33.19	0	742.74

Appendix III. Percentage composition of evaporated river water from sites 1 and 5 on Millwood Brook, sediment below the mine discharge and sewage effluent. Determined by Electron Probe Microanalysis. 1 sample per site. Results for Bird (1989) shown in brackets.

Element	Site 1	Site 5	Sediment	Sewage Effluent
Na	2.2 (0)	29.33 (41.04)	0.52 (5.70)	1.1 (47.13)
Cl	3.06 (26.83)	34.34 (39.42)	0.4 (6.58)	1.21 (33.328)
Ca	89.97 (26.27)	18.18 (2.31)	92.74 (72.04)	29.132 (5.76)
Si	1.07 (0.61)	0.97 (0.08)	1.78 (2.08)	46.62 (0.85)
Mg	1.06 (12.06)	4.1 (1.87)	0.03 (0)	0.63 (4.41)
S	2.36 (31.61)	11.93 (0.17)	0.19 (0.93)	4.01 (6.99)
Fe	0.19 (0.13)	0.04 (0.06)	3.93 (7.87)	12.42 (0.13)
K	0.09 (2.44)	0.78 (0)	0.06 (0.63)	2.22 (1.42)
Br	0.06 (0)	0.26 (0.45)	0.22 (0.82)	0.61 (0)
Zn	0.23 (0)	0.2 (0.08)	0.05 (0.08)	0.35 (0)
As	0 (0)	0.05 (0.24)	0.04 (0.15)	0.17 (0)
Mn	0.03 (0)	0.01 (0)	0.02 (0)	1.49 (0.02)
Cr	0.02 (0.05)	0 (0)	0.04 (0)	0.08 (0)
Cu	0.11 (0)	0 (0.03)	0.02 (0.04)	0.04 (0)

APPENDIX IV.
SPECIES FOUND IN MILLWOOD BROOK / RIVER POULTER (OCT 1994 - JULY 1995)

TAXA	SITE CODE																						
	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
Porifera								+	+													+		
Copepoda								+															+	+
TRICLADIDA																								
Polycelis nigra/tenuis	+	+	+		+	+	+	+	+	+	+	+	+	+	+		+	+	+	+			+	
Polycelis felina (Dalyell)														+										
Crenobia alpina		+	+			+	+	+		+												+		+
GASTROPODA																								
Valvata macrostoma Steenbuch	+	+	+	+	+																			
Valvata piscinalis (Muller)	+	+				+									+		+				+	+		
Potamopyrgus jenkinsi (Smith)	+	+	+	+	+	+	+	+	+	+	+			+	+	+		+	+		+	+	+	+
Bithynia tentaculata (L.)															+			+						
Bithynia leachii (Sheppard)		+																						
Lymnaea peregra (Muller)	+			+		+	+		+	+	+	+	+	+			+	+	+	+		+	+	+
Physa fontinalis (L.)						+																	+	+
Planorbis sp.	+	+	+	+	+	+	+		+		+			+						+	+			
Ancylus fluviatilis (Muller)		+																			+			
BIVALVIA																								
Sphaerium spp.																							+	
Pisidium spp.	+	+	+			+													+	+	+	+	+	+
OLIGOCHAETA																								
Pristina menoni (Aiyer)						+																		
Paranais litoralis (Muller)											+	+												
Nais elinguis (Muller)	+		+	+			+				+	+	+	+	+	+	+		+	+	+	+	+	
Limnodrilus hoffmeisteri Claparede		+			+							+									+			+
Limnodrilus udekemianus Claparede					+					+			+	+										
Lumbricillus rivalis Levinsen								+	+	+	+	+	+	+		+						+		
Lumbriculus variegatus					+	+				+								+	+	+			+	+
Lumbricidae	+		+				+		+		+					+		+	+	+			+	
NEMATODA																						+	+	
HIRUDINEA																								
Theromyzon tessulatum (Muller)																					+			+
Glossiphonia complanata (L.)		+				+	+	+	+	+					+	+		+		+	+		+	
Helobdella stagnalis (L.)																						+	+	+
Erpobdella octoculata (L.)		+	+			+	+	+	+		+				+	+		+	+	+	+	+	+	+
HYDRACARINA	+		+																+					
CRUSTACEA																								
Asellus aquaticus (L.)		+	+	+	+	+	+	+	+	+	+	+			+	+	+		+	+	+	+	+	+
Gammarus pulex (L.)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+
Gammarus tigrinus Sexton																		+					+	+
EPHEMEROPTERA																								
Baetis rhodani (Pictet)		+	+	+			+	+		+	+	+				+	+		+	+		+		+
Ephemerella ignita (Poda)		+																						

	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
PLECOPTERA																								
Nemoura cinerea (Retzius)	+																							
Isoperla grammatica (Poda)	+																							
HEMIPTERA																								
Velia sp.	+																							
Sigara dorsalis (Leach)																					+	+	+	+
Corixa punctata (Illiga)												+									+	+		
Callicorixa wollastoni var. *caledonica* (Kirk.)																						+	+	+
COLEOPTERA																								
Dytiscidae (larva)						+																+		+
Haliplus sp. (larva)			+																		+			
Haliplus sp. (adults)																		+						
Laccophilinae sp.		+																			+			
Hydrophilidae (larva)																					+			
Helodes sp. (larva)	+	+																			+			
Elmis aena																	+							
Elmis maugei																					+			
Noterinae (larva)	+																							
NEUROPTERA / MEGALOPTERA																								
Sialis lutaria (L.)					+																+	+		
TRICHOPTERA																								
Rhyacophila dorsalis (Curtis)																					+			
Glossosoma sp.					+																			
Hydropsyche augustipennis (Curtis)							+		+		+			+				+		+	+		+	+
Goerinae sp.																		+			+			
Limnephilus lunatus gp.	+	+		+	+	+			+									+	+					
Leptoceridae sp.																		+						
LEPIDOPTERA																								
Larva					+						+							+						+
DIPTERA																								
TIPULIDAE																								
Tipula couckei Mg. /*lateralis* Egg.	+					+	+		+						+		+			+				
Tipula montium gp.																			+					
Tipula flavolineata Mg.									+															
Dicronata sp.	+				+	+			+									+			+		+	
CHIRONOMIDAE																								
Tanypodinae																								
Psecrotanypus spp.					+	+		+		+	+	+	+				+				+	+	+	
Macropelopia spp.		+		+	+	+		+			+					+	+		+	+	+	+	+	+
Procladius spp.								+									+							
Tanypus vilipennis (Kieffer)																	+							
Pentaneura monilis (L.)														+		+	+	+	+	+	+	+		+
Orthocladiinae																								
Brillia modesta Meigen															+	+		+						
Crictopus spp.											+	+			+		+	+					+	
Eukiefferiella spp.		+						+																
Cardiocladius spp.			+																					
Orthocladius spp.	+		+															+		+	+			+
Camptocladius spp.		+																						
Limnophyes spp.										+														
Chironominae																								
Pseudochironomus prasinatus (Staeger)																								+

	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
Chironomus gp.	+			+						+		+	+	+								+	+	
Cryptochironomus spp.																								+
Paratendipes spp.																								+
Microspectra spp.										+						+	+				+		+	
Diamesinae																								
Prodiamesa olivacea (Meigen)	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
SIMULIDAE																								
Simulium costatum Friedrichs	+																							
Simulium equinum L.	+																	+						
Simulium erythrocephalum Den Geer	+																							
Simulium ornatum Meigen	+	+																+						
Simulium spp. (Pupa)	+	+				+																		
Simulium spp.			+																					
OTHER DIPTERA																								
Stratiomyidae sp.																			+					
Tabanidae		+																				+		
Limnophora spp.							+	+						+						+				
Ceratopogonidae spp.							+												+	+	+			
Dipteran larva								+																
Dipteran cocoon							+				+										+		+	
Medetera spp	+		+																					

Appendix V. Abundance (m^{-2}) of *Gammarus pulex*, Tubificidae, *Lumbricillus rivalis*, *Lumbriculus variegatus* and *Nais elinguis* at three Millwood Brook sites estimated from three core samples per site in June 1995. Results for Bird (1989) shown in brackets.

Site Code	*G. pulex*	Tubificidae[1]	*L. rivalis*	*L. variegatus*	*N. elinguis*
1	0 (8318)	0 (0)	0 (0)	0 (0)	0 (0)
3	2104 (7080)	0 (6401)	0 (0)	526 (0)	0 (0)
4	316 (6310)	542 (37153)	526 (0)	2841 (0)	2210 (0)
5	4420 (9120)	0 (135)	0 (0)	0 (0)	0 (0)
6	0 (0)	0 (0)	0 (13183)	0 (0)	0 (0)
7	3788 (0)	0 (0)	2420 (18621)	105 (0)	210 (0)
8	1894 (0)	0 (0)	0 (112200)	0 (0)	0 (0)
9	0 (0)	526 (0)	0 (794)	0 (0)	210 (0)
11	316 (0)	0 (0)	0 (2630)	105 (0)	842 (0)
12	1999 (0)	0 (0)	0 (4732)	0 (0)	0 (0)

Notes.

[1]Bird (1989) found *Tubifex tubifex*, absent in this study.

Appendix VI. Mean environmental measurements from the River Idle catchment for winter 1994 - summer 1995. 3 samples per site. Results for Bird (1989) shown in brackets.

Site Code	Chloride (mm l^{-1})	Conductivity (mS cm^{-1})	pH	Temperature (°C)
ME1	3.8 (26)	1.2 (1.4)	8.4	14.8
ME2	10.3	1.25	7.94	14
ME3	9 (8)	1.29 (1.3)	8.2	12.8
ME4	5 (1.1)	1.29 (1.6)	8.12	13.8
ME5	1	1.27	8.66	16.3
S1	11.5	2.25	8.17	18.3
S2	9.3 (0.9)	1.57 (6.4)	7.9	12.6
S3	10 (0.5)	1.52 (9)	7.92	14.1
S4	2.4 (0.6)	1.33 (5.6)	7.6	13.9
S5	4 (0.6)	1.33 (6.2)	7.68	14
P1	8.6	2.31	7.79	15.2
VW1	17.7	1.85	8.09	22
VW2	12 (0.4)	1.12 (9)	7.74	n/a
P3	1 (1.0)	1.13 (3.5)	8.76	17
LA	11 (3)	0.56 (0.5)	7.34	8
CM	1 (0)	0.97 (2.13)	7.27	15.4
RW1	5.8 (0.9)	0.81 (2.7)	8.32	18.8
RW2	1 (0.9)	0.47 (3.06)	7.69	15.5
L1	1 (1.4)	0.89 (6)	7.72	13.2
MA	9	1.16	8.15	18.3
BL	44.3	5.31	8.45	21
ST	125.2	8.14	12.96	19.5

APPENDIX VII.
SPECIES FOUND IN RIVER IDLE CATCHMENT (OCT 1994 - JULY 1995)

SITE CODE

TAXA	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1	MA	BL	ST
Copepoda		+			+																	
TRICLADIDA																						
Polycelis nigra/tenuis	+								+	+												
Polycelis felina (Dalyell)			+	+									+									
Phagocata vitta		+													+	+						
GASTROPODA																						
Valvata macrostoma Steenbuch						+																
Valvata piscinalis (Müller)					+			+									+	+	+			
Potamopyrgus jenkinsi (Smith)	+	+	+		+	+	+	+	+	+	+	+	+		+	+	+				+	+
Bithynia tentaculata (L.)					+																	
Bithynia leachii (Sheppard)														+								
Lymnaea peregra (Müller)	+				+	+	+	+	+	+	+		+	+		+	+		+		+	+
Lymnaea palustris (Müller)																				+		
Physa fontinalis (L.)					+												+				+	
Planorbis sp.		+			+									+		+			+			+
Ancylus fluviatilis (Müller)					+					+	+								+			
Theodoxus fluviatilis					+						+											
BIVALVIA																						
Sphaerium spp.					+																	
Pisidium spp.		+	+	+	+	+			+	+	+		+	+	+	+	+		+	+		
OLIGOCHAETA																						
Nais elinguis (Müller)	+	+	+	+	+	+	+			+			+	+		+		+				
Limnodrilus hoffmeisteri Claparede				+		+	+													+		+
Limnodrilus udekemianus Claparede		+																				
Lumbricillus rivalis Levinsen				+		+	+	+			+		+	+				+		+		
Lumbriculus variegatus					+			+							+	+	+	+	+			
Lumbricidae						+		+	+		+	+	+				+	+				
NEMATODA				+																		
HIRUDINEA																						
Glossiphonia complanata (L.)		+	+	+	+	+		+	+		+	+		+	+	+	+	+	+			
Theromyzon tessulatum (Müller)					+																	
Helobdella stagnalis (L.)													+	+		+		+		+		
Erpobdella octoculata (L.)		+	+	+		+		+	+	+	+			+	+	+	+	+		+		
Piscicola geometra (L.)															+				+			
HYDRACARINA																	+					
CRUSTACEA																						
Asellus aquaticus (L.)	+	+	+	+			+		+		+	+	+			+	+	+	+		+	
Gammarus pulex (L.)	+	+	+	+	+	+	+	+	+	+	+			+	+		+	+	+			+
Gammarus tigrinus Sexton						+							+	+								
EPHEMEROPTERA																						
Baetis rhodani (Pictet)			+	+	+			+	+	+	+	+				+	+	+	+	+		
Cloeon dipterum (L.)														+								
Ephemerella ignita (Poda)																			+			
Caenis sp.					+																	
Ephemera danica (Muller)															+							

	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1	MA	BL	ST
ODONATA																						
Enallagma spp					+						+			+								
HEMIPTERA																						
Velia sp.																+						
Corixidae sp. (nymph)												+										
Callicorixa wallastoni var *caledonica* (Kirk.)					+	+								+								
Sigara dorsalis (Leach)					+									+		+					+	
Sigara falleni (Fieber)					+																	
Corixa dentipes (Thoms.)															+							
Aphelocheirus montandoni Horv.					+						+											
Aphelocheiridae														+								
Ilyocoris cimicoides (L.)														+								
COLEOPTERA																						
Hydrophilidae (larva)		+														+			+			
Dytiscidae (larva)						+					+			+			+	+	+	+		
Haliplus sp. (larva)												+							+			+
Haliplus lineatocollis (Marsham)					+																	
Deronectes depressus/elegans (Fabricius)					+	+								+		+				+		
Ilybius sp. (larva)																						+
Helodes sp. (larva)																		+				
Elmis aena (Müller)	+			+																		
Elmis maugei		+				+													+			
Agabus didymus	+																					
Agabus sp.											+											
Platambus maculatus (L.)				+																		
Gyrinus natator (L.)												+										
Scarabaeiform larvae																		+				
NEUROPTERA/MEGALOPTERA																						
Sialis lutaria (L.)			+								+			+		+					+	
Sialis fuliginosa																			+			
TRICHOPTERA																						
Hydropsyche augustipennis (Curtis)		+		+			+	+	+							+	+	+	+	+		
Limnephilus lunatus gp.	+	+				+	+	+					+		+				+			
Leptoceridae spp.																			+			
Tinodes waeneri (L.)								+							+							
Philopotamus montanus (Donovan)															+							
Arthripsodes cinereus (Curtis)															+							
Polycentropus sp.															+							
LEPIDOPTERA																						
Larva							+			+							+	+	+			+
DIPTERA																						
TIPULIDAE																						
Tipula couckei Mg. / *lateralis* Egg.													+			+	+	+	+		+	
Tipula montium gp.												+										
Dicronata sp.	+	+	+	+			+	+					+				+					
CHIRONOMIDAE																						
Tanypodinae																						
Psecrotanypus spp.				+	+							+				+				+	+	+
Macropelopia spp.	+		+			+	+	+	+		+			+		+		+	+	+		+
Pentaneura monilis (L.)	+	+	+			+	+			+						+			+	+		
Clinotanypus nervosus (Meigen)																		+				
Orthocladiinae																						
Cricotopus spp.	+	+	+	+	+					+						+	+		+			
Eukiefferiella spp.		+					+															

	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1	MA	BL	ST
Orthocladius spp.		+					+	+	+	+						+	+	+				
Rheocricotopus spp.																		+	+			
Limnophyes spp.														+								
Metriocnemus spp.																+						
Psectrocladius spp.			+																			
Chironominae																						
Chironomus gp.					+									+	+							+
Cryptochironomus spp.																	+					
Microspectra spp.			+											+		+		+		+		
Kiefferulus spp														+								
Diamesinae																						
Prodiamesa olivacea Meigen	+	+	+	+	+		+	+	+	+	+				+	+		+	+	+	+	+
SIMULIDAE																						
Simulium spp.								+		+									+			
Simulium equinum L.			+	+					+			+								+		
Simulium reptans L.		+										+	+		+			+	+	+		
Simulium erythrocephalum Den Geer				+	+							+				+			+	+		
Simulium ornatum Meigen				+	+		+	+	+							+	+			+		
OTHER DIPTERA																						
Empididae																			+			
Tabanidae						+	+															
Pericoma spp.		+																		+		
Limnophora spp.			+	+															+	+	+	+
Ceratopogonidae spp.		+		+												+						
Dipteran cocoon		+					+															

Appendix VIII. Additional environmental data collected from the River Idle catchment for use in computer modelling.

Site Code	Discharge rate ($m^2 s^{-1}$)	Nitrate (ppm)	Phosphate (ppm)	% emergents	% pre-emergents	% boulders	% pebbles	% sand	% silt/clay	phi	SS ($mg\ l^{-1}$)
1	0.121	74	0.5	16	4	0	0	0	100	8	16
2	0.118	57	1.5	80	0	40	60	0	0	-3.5	9.4
3	0.196	49	5	88	0	16	84	0	0	-3.97	13.8
A	0.004	58	0	24	0	8	0	0	92	6.74	0.8
B	0.115	63	15	0	0	0	0	0	100	8	2
4	0.278	56	8	8	0	0	0	0	100	4	9
5	0.097	58	1.5	0	0	50	40	10	0	-4.975	6.6
6	0.06	50	7	0	0	50	0	0	50	0.125	5.4
7	0.06	50	3.5	0	0	30	50	20	0	-3.55	24.6
C	0.196	59	8	0	0	20	80	0	0	-4.15	18.6
8	0.123	43	4.5	0	0	0	0	0	100	-0.625	9.2
9	0.81	63	6.5	0	0	0	0	0	100	8	6.4
10	0.041	61	12	0	0	0	0	0	100	8	8.4
11	0.193	63	6.5	0	0	0	0	0	100	8	6.8
12	0.1585	56	7.5	0	0	30	45	25	0	-3.2875	9.8
13	0.233	6139	4	0	0	0	10	30	60	5.075	8.6
14	0.1428	8854	4.5	0	0	0	0	0	100	8	9
15	0.25	61	7	0	0	0	85	15	0	-2.4625	2.8
D	0.127	88	7	0	12	0	100	0	0	-3.25	2.6
16	0.252	62	33	15	0	0	90	10	0	-3.25	4
E	0.3992	61	16	0	0	0	100	0	0	-3.25	3.8
17	0.715	34	0	0	0	50	0	0	50	0.125	4.8
18	0.99	32	0.5	0	0	0	0	0	100	8	28.44
19	0.8304	19	0	0	0	0	100	0	0	-3.25	13
ME1	0.594	63	63	0	75	0	15	85	0	1.2125	3.2
ME3	0.563	56	1	0	75	40	0	60	0	-1.9	2.4
ME4	0.615	54	1.5	0	80	0	80	20	0	-2.2	6
S1	0.032	12	9	0	0	0	50	0	50	2.375	2.6
S2	0.066	7	0	0	0	0	100	0	0	-3.25	5.2
S3	0.355	77	0.5	0	50	0	100	0	0	-3.25	0.4
S4	0.229	70	15	0	25	15	85	0	0	-3.925	2.6
S5	0.061	17	7.5	0	0	0	100	0	0	-3.25	2.8
VW2	0.033	25	1	0	75	75	25	0	0	-6.625	5.2
P3	0.1166	19	0	0	0	100	0	0	0	-7.75	6
LA	0.4864	50	50	0	50	100	0	0	0	-7.75	2.5
RW1	0.042	84	15	0	25	75	0	0	0	0.6975	2.6
RW2	0.037	19	14	0	0	0	40	30	30	1.7	4.4
L1	0.285	21	0	1	0	15	65	20	0	-2.875	1.8

Appendix IX. Site Descriptions for Yorkshire Low Impact Sites (Source: Environment Agency)

Site Code	Grid Reference	Mine/Catchment	Watercourse
HUN1	SK333855	Hunters Bar	Porter Brook
HUN2	SK332858	"	"
HUN3	SK331858	"	"
HUN4	SK334858	"	"
CLA1	SK301849	"	Porter Brook
CLA2	SK299848	"	"
CLA3	SK300849	"	"
CLA4	SK301849	"	"
ALE1	SE238148	Alexandra Drift/Dearne	Flockton Beck
ALE2	SE240148	"	"
ALE3	SE244148	"	"
ALE4	SE246147	"	"
CHA1	SE202307	Charles Pit	Raikes Beck
CHA2	SE205309	"	"
CHA3	SE209310	"	Kitwood Beck
CHA4	SE209311	"	"
GIL1	SE223387	Charles Pit	Gill Beck
GIL2	SE222386	"	"
GIL3	SE220379	"	"
GIL4	SE219378	"	"
LIM1	SE309819	Limb Lane	Ranwood Beck
LIM2	SE319819	"	"
LIM3	SK315819	"	"
LIM4	SK317819	"	"
SHE1	SK208103	Alexandra Drift/Dearne	Birkhouse Beck
SHE2	SK213102	"	Shepley Dike
SHE3	SK207107	"	"
SHE4	SK206108	"	"
UGH1	SK252902	Limb Lane	Ughill Brook
UGH2	SK256902	"	"
UGH3	SK260902	"	"
UGH4	SK263902	"	"

Appendix X. Yorkshire low impact sites. Mean values of environmental variables (± 2 SE) for each TWINSPAN group at Level 3. Standard errors for percentage values were calculated after arcsine transformation, and shown following back-transformation. (t)/(f)=total/filtered μg l^{-1}. Original Data Source: Environment Agency.

Twinspan site Group (in order of increasing BMWP)

	15	14	11	13	12	10	9	8
GQA Class	E	C	C	C	B	B	A	A
BMWP	29.5	59	63	68	78	91	104	127
No. Taxa (SE)	8 (2.06)	13.5 (5.06)	13.7 (2.66)	15.5 (-)	16 (4.56)	19 (0.82)	19.1 (3.22)	21 (-)
ASPT	3.803	4.37	4.7	4.37	5.106	4.85	5.41	6.165
Cu (t) (SE)	6.95 (1.7)	4.88 (1.24)	3.94 (1.0)	8.54 (-)	4.3 (2.22)	2.55 (0.16)	2.21 (1.0)	1.49 (-)
Sulphate(mg l^{-1}) (SE)	283 (107.1)	177 (30.8)	78 (41.1)	254 (-)	68 (50.68)	96 (99.02)	67 (25.86)	26 (-)
Conductivity (mS cm^{-1}) (SE)	1592 (225.2)	1061 (414)	736 (292.8)	1147 (-)	631 (223.6)	494 (225.2)	720 (217.4)	201 (-)
Chloride (mg l^{-1}) (SE)	133 (80.84)	70 (60.8)	74 (33)	131 (-)	54 (17.68)	52 (15.18)	39 (18.36)	23 (-)
Mn (t) (SE)	1518 (424)	373 (288.8)	379 (279.8)	968 (-)	265 (262.6)	500 (814)	293 (196.2)	57.6 (-)
Dissolved Oxygen (%) (SE)	53 (5.2)	72 (5.6)	67 (2.4)	93 (-)	69 (2.0)	86 (1.2)	87 (0.8)	72 (-)
Fe (f) (SE)	7903 (6036)	1971 (2706)	3804 (5626)	585 (-)	1196 (1568)	389 (200)	1468 (1608)	80 (-)
As(t) (SE)	1.85 (1.4)	1.225 (0.46)	1.87 (1.18)	1.7 (-)	1.4 (0.74)	1.35 (0.42)	1.13 (0.22)	1 (-)
Width (m) (SE)	1.41 (0.64)	1.53 (0.28)	1.58 (0.44)	0.94 (-)	1.56 (0.76)	3.13 (0.25)	1.96 (0.82)	1.75 (-)
Pb (t) (SE)	1.23 (0.30)	1.59 (0.30)	1.633 (0.68)	3.68 (-)	1.29 (0.36)	2.88 (1.22)	1.25 (0.38)	1 (-)
Altitude (m) (SE)	144 (11.08)	256 (331.2)	165 (10.0)	110 (-)	167 (32.4)	110 (0)	140 (201.2)	225 (-)
Distance from Source (km) (SE)	4.11 (4.54)	2.025 (0.94)	8.3 (7.56)	0.9 (-)	2.79 (3.26)	5.875 (0.26)	1.143 (0.48)	1.35 (-)

Appendix XI. Proportional representation of taxa for each TWINSPAN site-group for Yorkshire Low Impact sites (mean percentage per site ± 2 SE). Standard errors were calculated after arcsine transformation, and shown following back-transformation. Original Data Source: Environment Agency.

TWINSPAN Gp Taxonomic Gp	15	14	11	13	12	10	9	8
Ephemeroptera	1.16	3.2	5.8	5.3	6.3	9.3	9.1	14.1
(SE)	(1.9)	(1.4)	(0.05)	(-)	(1.34)	(0.3)	(0.4)	(-)
Plecoptera	2.8	4.0	7.3	10.6	13.2	6	13.3	10.7
(SE)	(2.3)	(0.01)	(0.4)	(-)	(1.9)	(0.1)	(0.09)	(-)
Trichoptera	1.9	42.0	19.1	15.9	21.5	22.0	21.1	28.6
(SE)	(3.3)	(0.7)	(0.1)	(-)	(0.2)	(12.9)	(0.13)	(-)
Mollusca	24.0	18.2	22.6	18.6	17.8	22.1	17.7	10.7
(SE)	(0.9)	(0.4)	(0.1)	(-)	(0.1)	(0.01)	(0.3)	(-)
Crustacea	5.2	6.3	5.8	5.3	7.0	8.5	5.3	3.6
(SE)	(2.3)	(2.9)	(0.04)	(-)	(0.16)	(0.01)	(0.06)	(-)
Coleoptera	13.9	11.4	8.6	15.9	10.5	7.2	12.1	14.3
(SE)	(0.2)	(0.01)	(3.1)	(-)	(1.52)	(0.29)	(0.13)	(-)
Neuroptera	0	0	0	2.5	0	0	0.07	0
(SE)	(0)	(0)	(0)	(-)	(0)	(0)	(0.28)	(-)
Hirudinea	0	3.1	0.6	5.3	0	4.2	0.1	0
(SE)	(0)	(1.4)	(1.7)	(-)	(0)	(0)	(0.41)	(-)
Diptera	29.5	18.7	17.4	15.9	16.1	11.6	12.8	10.7
(SE)	(1.0)	(0.3)	(0.14)	(-)	(0.2)	(0.1)	(0.06)	(-)
Oligochaeta	11.0	7.0	4.6	5.3	4.7	4.2	4.3	3.6
(SE)	(0.2)	(0.4)	(0.04)	(-)	(7.47)	(0.01)	(0.02)	(-)

Appendix XII. Yorkshire low impact data. Classification of family and site groups by TWINSPAN. The number codes show the percentage of occurrence of each taxa between the sites in each group. Abundance Codes: 1=1-19%; 2=20-39%; 3=40-59%; 4=60-79%; 5=80-99%; 6=100%. (Original Data Source: Environment Agency).

FAMILY Group SITE Group

		8	9	10	11	12	13	14	15
15	Sialidae		3				3		
	Chloroperlidae		5			2			
	Ephemeridae		1						
	Perlodidae	6	5						
	Ephemerellidae	6	4	3					
	Philopotamidae	6							
	Odontoceridae	6	1						
	Rhyacophilidae	6	5	6					
	Sericostomatidae		4	4	2				
	Leptophlebiidae		2	2					
	Heptageniidae		4	3					
14	Lepidostomatidae		2					2	
	Scirtidae	6	4		2	2	6		
13	Hydropsychidae		5	6			6		
	Glossosomatidae			2	2	2			
	Leptoceridae			2					
	Planariidae	6	3	6	4	4			
	Leuctridae	6	6	6	4	4		2	
12	Ancylidae		3	6	4	4		2	
	Elmidae	6	3	6	3	3	6		
	Planorbidae			2			6		
11	Asellidae			6				4	
	Psychomyiidae				2	2			
	Polycentropididae		5	6	5	5	6	4	1
	Simuliidae	6	6	6	5	5	6	6	3
	Nemouridae		6	3	3	3	6	6	
	Baetidae	6	6	6	5	5	6		2
	Hydrophilidae	6	6	3	5	5		6	2
	Limnephlebiidae	6	6	6	6	6	6	6	2
10	Lymnaeidae		6	4	4	4	6	4	5
	Oligochaeta	6	6	6	6	6	6	6	6
	Tipulidae	6	6	6	6	6	6	6	6
	Chironomidae	6	6	6	6	6	6	6	6
	Hydrobiidae		3	6	6	6	6	4	2
	Sphaeriidae	6	6	6	6	6	6	6	3
	Gammaridae		5	6	6	6	6	4	4
	Erpobdellidae			6	2		6	4	
	Glossiphoniidae			6	2		3	3	4
	Valvatidae				2				1
9	Dytiscidae	6	3	2	2	2	3	6	4
	Beraeidae		1					3	
8	Corixidae					2		2	2
	Goeridae					2			
	Philopotamidae					3			

Appendix XIIa. Rescaling of Macroinvertebrate Abundance Scores.

The abundance scores as held for information only in the appendix databases, were calculated by totalling and recoding as for Furse *et al.* (1984) shown in Table I.

Table I. Rescaling of macrofauna abundance scales

Abundance	Original code total for 2 seasons	Code Assigned
1 - 9	1	1
10 - 99	2	2
100 - 999	3-4	3
1000 - 9999	5-6	4
>=10,000	>=7	5

Appendix XIII (1). Yorkshire Low Impact Site Database. Source: Environment Agency.

	BMWP	NO.TAXA	ASPT	As(t)	Pb(t)	Pb(f)	Cu(t)	Cu(f)	Zn(t)	Zn(f)	Al(t)	Al(f)	Fe(t)	Fe(f)	Ni(t)	Ni(f)	Mn(t)	Mn(f)
HUN1	106	20	5.305	1.6	3.69	1	2.45	1.82	5.6	5	107	18.3	364	111	2.5	1.97	52.5	22.8
HUN2	87	19	4.68	1.8	1.06	1	2.4	2.07	54.4	5	974	154	17700	389	39.9	39.5	1720	64.9
HUN3	82	18	4.68	1	3.29	1	2.6	2.06	9	5	129	19	1019	487	3.8	3.14	105.4	69.8
HUN4	88	19	4.74	1	3.46	1.36	2.75	2.78	6	5	132	37.5	1208	570	3.8	2.77	121.3	102.1
CLA1	127	21	6.165	1	1	1	1.49	1.36	19.7	18.2	150	87.9	195	80	5.8	5.54	57.6	48.4
CLA2	87	15	5.795	1	1	1	1	1	5	5	21	10	9430	4160	1	1	517	402.7
CLA3	107	22	5.95	1	1	1	1.47	1.29	10.2	9	96.8	32.7	1075	587	3.8	3.75	130.6	117.6
CLA4	149	25	6.075	1	1.04	1	1.62	1.65	9	8	69.2	32.8	499	197	3.6	3.17	74	61.6
ALE1	59	15	3.98	1	3.03	1	6.73	3.84	16.5	10.9	121	34.7	320	91	8.2	13.5	210.3	198
ALE2	22	6	3.665	1	1.3	1	6.27	6.57	31.5	12.9	234.2	81.5	10084	1414	36.9	18.81	1970.7	292
ALE3	36	10	3.49	1	1.63	1	7.62	3.1	28	20.6	196.2	54.9	6530	6123	24.7	24.5	1418.7	898
ALE4	37	10	3.69	1	1.19	1	9.54	3.17	19	15.1	227.5	33.6	1725	569	19.7	18.45	1153	1098.5
CHA1	16	5	3.2	4.3	2.08	1	9.83	6.86	8.9	8.9	84.6	18	791	442	1.8	1.57	602.6	473.3
CHA2	35	9	3.89	1.5	1	1	8.92	3.5	5	5	21.9	22.7	13300	8175	24.9	22	1703	814.3
CHA3	66	15	4.4	2	4.09	1	8.24	7.36	7.5	7.5	79.5	12.1	6100	996	17.3	16.23	1302	752
CHA4	70	16	4.345	1.4	3.26	1	8.83	6.91	5	5	245.8	23.9	1810	174	7.4	6.08	633	206
GIL1	63	13	4.78	1	1	1	8.27	2.44	5	5	87.4	15.1	170	442	1.2	1	35.7	19.2
GIL2	38	9	4.42	2.9	1	1	3.5	1.77	5.3	5.3	956.8	10	10127	4320	4.3	3.79	712.3	476.5
GIL3	73	16	4.68	1.9	1.14	1	4.39	3.07	5	5	172.3	10	8470	5813	3.6	3.46	604.3	581.7
GIL4	79	17	4.655	1	1.18	1	4.16	3.91	5	5	46.7	14.6	460	49	1.7	1.63	48.6	28.3
LIM1	84	17	5.035	1	1.65	1	4.54	4.23	7.8	6.2	208	115.3	225	133	5.5	5.12	33.6	27.8
LIM2	53	11	4.965	1	1.74	1	4.28	3.88	26	17.7	316	141	2060	1800	7.6	7.38	178	170
LIM3	125	22	5.61	1.8	1.41	1	4.24	3.85	14.2	13.7	285	81.3	7360	4925	12	14.15	698	681.5
LIM4	134	23	5.925	1.1	2.32	1.29	3.85	3.6	5	5	13.9	10	214	30	3	1	42.1	37.4
SHE1	99	19	5.345	1.1	1.8	1	3.65	2.82	10.2	5.3	258.9	198.1	1115	496	5.9	5.26	411.6	378.7
SHE2	25	6	4.165	3.9	1	1	5	1.04	14	7.1	87	81.5	16400	15900	23.6	23.9	979	962
SHE3	69	15	4.545	3	1	1	2.95	2.41	15.6	11.1	231.7	85	10447	9358	16.8	15.89	648	609.5
SHE4	67	15	4.59	1.6	2.16	1	4.58	3.24	8.7	5	345	54.5	2643	254	5.4	3.96	311	262.9
UGH1	77	14	5.5	1	1	1	1	1	12.4	11.8	119.9	76.2	147	89	9.13	8.91	85.8	78.8
UGH2	43	9	5	1	1	1	4.23	1.73	20.5	19.1	574.3	150.9	2640	1930	32.07	24.55	628	641
UGH3	116	20	5.945	1	1	1	2.3	1.26	14.8	13	258.5	63.1	1391	781	19	14.9	448.7	421.3
UGH4	132	22	6.125	1	1	1	1.46	1.37	8.4	7.5	133.5	47.8	542	95	8.59	7.85	184	152

Appendix XIII (2). Yorkshire Low Impact Site Database. Source: Environment Agency.

	TEMP	pH	DO %	Conductivity	Alkalinity	SSolids	Sulphate	Chloride	Bedrock%	Boulders%	Cobbles%	Pebbles%	Gravel %	Sand %	Silt %	Ochre%	Width
HUN1	9.9	7.6	91	372	63	5	41.4	43.7	0	15	40	30	10	5	0	50	3
HUN2	9	6.1	67	820	10	14	244	75.2	0	15	60	15	10	0	0	85	3.5
HUN3	9.3	7.5	91	320	60	6	54	45.2	0	10	55	10	15	12.5	2.5	42.5	3
HUN4	10.8	7.7	91	465	71	7	43.4	45.8	0	12.5	60	20	15	0	0	16.5	3
CLA1	9.3	7	90	201	10	3	26.2	22.9	0	22.5	37.5	22.5	12.5	2.5	0	0	1.75
CLA2	10.4	7.2	56	566	162	32	45.3	51.6	0	25	40	20	15	2.5	0	97.5	2.5
CLA3	8.8	7.1	87	315	66	13	30.3	34	0	10	45	30	10	5	0	55	3
CLA4	9.7	7.6	95	320	81	5	29.5	35.9	0	5	15	45	20	10	5	0	1.25
ALE1	7.1	7.3	85	1008	38	8	220.3	156.4	0	0	12.5	27.5	27.5	2.5	20	40	1.125
ALE2	9.5	6.7	66	1590	35	5	234.7	88.7	0	0	15	70	5	5	5	100	1.5
ALE3	7.2	7	81	1788	41	1	352.7	191.9	0	12.5	32.5	22.5	17.5	7.5	7.5	47.5	2
ALE4	9.2	7.5	90	1097	37	9	351	109.3	0	15	30	35	15	5	0	35	2
CHA1	14.1	7.5	51	848	173	21	66.9	113.6	0	0	0	10	80	5	5	0	0.4
CHA2	10.6	6.5	74	1863	188	12	387.7	210	0	0	15	40	30	5	10	100	0.5
CHA3	10.5	7.2	91	1521	180	28	313.7	128.9	0	0	7.5	15	55	12.5	10	90	1
CHA4	10.9	7.7	95	773	133	23	194	132.8	0	0	10	40	40	5	5	50	0.875
GIL1	12.6	7.7	71	541	112	46	37.7	37.7	0	15	30	15	30	7.5	2.5	0	0.75
GIL2	11.6	7	34	973	224	21	167.6	53.4	0	30	30	20	20	0	0	100	1.25
GIL3	12.8	7.3	87	834	204	34	148.3	52.8	5	15	35	15	10	7.5	2.5	57.5	1.625
GIL4	10.9	8.1	90	743	130	3	165	58.6	0	0	10	35	40	15	0	0	1.625
LIM1	9.4	7.1	82	554	31	3	43.4	60.2	5	5	32.5	25	27.5	10	0	2.5	1.25
LIM2	6.6	6.7	68	480	20	5	51.4	69.7	5	32.5	32.5	10	7.5	7.5	5	100	2
LIM3	8.9	7	85	674	24	12	86.7	67.6	0	5	45	15	10	12.5	12.5	100	2.25
LIM4	11	7.4	93	736	21	5	102.2	69.1	0	15	50	10	10	10	5	85	1.25
SHE1	9.9	6.6	63	772	50	15	63.1	83.4	0	0	15	45	30	10	0	0	1.625
SHE2	10	5.7	2	1128	48	2	155.5	42.2	0	10	30	35	20	5	0	50	1.625
SHE3	7.9	6.3	47	987	45	10	118.4	47.3	0	5	25	10	10	0	0	100	1.25
SHE4	8.9	7.4	83	740	72	24	64.1	104	0	15	35	30	15	5	0	0	1.5
UGH1	7.9	6.6	88	555	10	2	22.2	14.3	0	0	12.5	30	25	12.5	20	0	0.75
UGH2	7.6	6.4	26	1660	113	7	175.3	13.5	0	27.5	40	25	7.5	0	0	100	1.75
UGH3	7.7	7.3	90	1043	62	5	102.7	15.2	5	25	22.5	35	12.5	0	0	85	1.75
UGH4	8.1	7.1	92	1143	58	4	82.4	17.2	0	10	25	40	25	0	0	5	4

Appendix XIII (3). Yorkshire Low Impact Site Database. Source: Environment Agency.

	Depth	Altitude	DistSource	Flow	Colour	Substrate	Slope	Heptageniidae	Leptophlebiidae	Ephemerellidae	Ephemeridae	Leuctridae	Perlodidae	Chloroperlidae	Beraeidae	Odontoceridae	Leptoceridae
HUN1	12.15	110	5.6	2.8	1	-5.463	45	3	0	1	0	3	0	0	0	0	1
HUN2	23.35	110	5.8	2.9	1	-6.625	45	1	0	0	0	2	0	0	0	0	0
HUN3	20.83	110	5.9	2.95	1	-5.4	45	0	0	0	0	2	0	0	0	0	0
HUN4	17.5	110	6.2	3.1	1	-6.756	45	0	1	1	0	2	0	0	0	0	0
CLA1	7.15	225	1.35	0.675	1	-5.738	100	1	0	1	1	3	2	0	0	1	1
CLA2	10.8	190	1.9	0.95	1	-6.125	64	0	0	1	0	4	1	1	0	0	1
CLA3	9	190	1.9	0.95	1	-5.463	64	3	0	1	1	4	1	1	0	0	0
CLA4	8.5	190	2	1	1	-3.063	50	2	0	2	2	4	1	0	0	0	2
ALE1	9.85	140	2.25	1.125	1.5	-1.106	33	0	0	0	0	0	0	0	0	0	0
ALE2	11.35	140	2.4	1.2	1	-3.1	33	0	0	0	0	0	0	0	0	0	0
ALE3	13.35	140	2.8	1.4	1	-4.038	33	0	0	0	0	0	0	0	0	0	0
ALE4	9.45	130	3.2	1.6	1	-5.013	25	0	0	0	0	0	0	0	0	0	0
CHA1	5.2	155	0.18	0.087	2	-2.425	29	0	0	0	0	0	0	0	0	0	0
CHA2	8	135	0.5	0.25	1	-2.538	62	0	0	0	0	0	0	0	0	0	0
CHA3	14.35	115	0.8	0.4	1	-1.806	67	0	0	0	0	0	0	0	0	0	0
CHA4	15	105	1	0.5	2	-2.875	50	0	0	0	0	0	0	0	0	0	0
GIL1	4.15	130	1.25	0.625	1	-4.6	52	0	0	0	0	0	0	0	0	0	0
GIL2	8	125	1.3	0.65	1	-5.95	100	0	0	0	0	0	0	0	0	0	0
GIL3	7.5	72	2.5	1.25	1	-4.338	44	0	0	0	0	0	0	0	1	0	0
GIL4	8.7	62	2.7	1.35	1	-2.913	50	0	0	0	0	0	0	0	2	0	0
LIM1	6	200	0.25	0.125	1	-4.413	92	0	0	0	0	3	1	0	0	0	0
LIM2	8.6	175	0.8	0.4	1	-5.056	45	0	0	0	0	3	0	0	0	0	0
LIM3	5.7	160	0.81	0.405	1	-3.438	1500	2	1	0	0	4	1	1	0	0	0
LIM4	6.5	140	0.84	0.42	0.5	-5.088	667	2	1	1	1	4	1	0	0	1	0
SHE1	16.85	190	9.25	4.625	1.5	-3.4	34	0	0	0	0	3	0	0	0	0	0
SHE2	20	160	10.75	5.375	1	-4.788	20	0	0	0	0	0	0	0	0	0	0
SHE3	35.65	160	11.3	5.65	2	-2.975	20	0	0	0	0	3	0	0	0	0	0
SHE4	25.15	160	12.8	6.4	2	-5.238	20	0	0	0	0	2	0	0	0	0	0
UGH1	13.35	850	0.25	0.125	1	-0.906	400	0	0	0	0	3	0	0	0	0	0
UGH2	14.25	750	0.65	0.325	1	-6.288	500	0	0	0	0	0	0	0	0	0	0
UGH3	9.9	500	1	0.5	1	-5.225	7.4	0	0	0	0	3	0	0	0	0	0
UGH4	10.5	500	1.2	3	1	-4.825	7.4	0	0	0	0	2	0	0	0	0	0

Appendix XIII (4). Yorkshire Low Impact Site Database. Source: Environment Agency.

	Goeridae	Lepidostomatidae	Sericostomatidae	Psychomyiidae	Philopotamidae	Nemouridae	Rhyacophilidae	Glossosomatidae	Polycentropodidae	Limnephilidae	Ancylidae	Gammaridae	Corixidae	Dytiscidae	Hydrophilidae	Scirtidae	Elmidae
HUN1	0	0	3	0	0	0	3	2	2	1	1	4	0	0	1	0	3
HUN2	0	0	1	0	0	0	2	0	3	1	1	3	0	0	0	0	2
HUN3	0	0	1	0	0	1	1	0	3	2	1	3	0	0	0	0	2
HUN4	0	0	0	0	0	2	1	0	3	1	1	3	0	1	1	0	2
CLA1	0	0	1	0	1	2	3	0	1	2	1	2	0	2	3	3	1
CLA2	0	0	1	1	0	2	2	0	1	2	0	2	0	0	2	3	1
CLA3	0	0	1	0	0	2	3	0	1	1	2	3	0	1	3	1	2
CLA4	0	0	1	0	0	3	2	0	1	1	2	4	0	2	3	2	2
ALE1	0	0	0	0	0	1	0	0	1	1	0	3	1	3	1	0	0
ALE2	0	0	0	0	0	0	0	0	0	0	0	3	0	2	0	0	0
ALE3	0	0	0	0	0	0	0	0	0	0	0	3	1	1	1	0	0
ALE4	0	0	0	0	0	0	0	0	0	0	0	4	0	3	1	0	0
CHA1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
CHA2	0	0	0	0	0	0	0	0	0	1	0	2	1	1	0	0	0
CHA3	0	0	0	0	0	3	0	0	2	2	0	5	0	0	2	2	2
CHA4	0	0	0	0	0	1	0	0	2	2	0	5	0	1	0	1	1
GIL1	0	0	0	0	1	2	0	0	3	2	0	4	0	0	2	0	2
GIL2	0	0	0	0	1	0	0	0	2	1	0	3	0	0	0	0	0
GIL3	0	1	0	0	0	3	0	0	2	2	0	3	0	2	1	0	0
GIL4	0	0	0	0	0	3	0	0	3	2	0	3	0	2	2	0	0
LIM1	0	0	0	0	0	2	0	1	3	3	3	3	0	0	1	1	0
LIM2	0	0	0	0	0	1	0	1	2	0	1	2	0	0	0	0	0
LIM3	0	0	3	0	0	3	1	0	2	2	2	4	0	0	2	2	0
LIM4	0	1	3	0	0	3	2	0	3	3	1	3	0	1	1	2	0
SHE1	3	0	0	1	0	2	0	3	0	3	3	3	1	0	3	3	4
SHE2	0	0	0	0	0	1	0	0	1	1	0	0	0	2	0	0	0
SHE3	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1
SHE4	0	0	0	0	0	1	1	0	1	1	0	3	0	0	0	1	1
UGH1	3	0	0	1	0	2	0	3	0	3	3	3	1	0	3	3	4
UGH2	0	0	0	0	0	1	0	0	1	1	0	0	0	2	0	0	0
UGH3	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	1	1
UGH4	0	0	0	0	0	1	1	0	1	1	0	3	0	0	0	1	1

Appendix XIII (5). Yorkshire Low Impact Site Database. Source: Environment Agency.

	Hydropsychidae	Tipulidae	Simuliidae	Planariidae	Baetidae	Sialidae	Hydrobiidae	Lymnaeidae	Planorbidae	Sphaeriidae	Valvatidae	Glossiphoniidae	Erpobdellidae	Asellidae	Chironomidae	Oligochaeta
HUN1	**3**	**1**	**3**	**1**	**3**	**0**	**3**	**1**	**1**	**3**	**0**	**3**	**1**	**2**	**4**	**3**
HUN2	2	3	3	1	3	0	3	1	0	3	0	3	2	3	4	4
HUN3	**2**	**1**	**3**	**1**	**3**	**0**	**3**	**1**	**0**	**2**	**0**	**3**	**1**	**3**	**4**	**4**
HUN4	3	0	3	1	3	0	3	1	0	2	0	2	3	3	4	4
CLA1	**2**	**3**	**4**	**2**	**3**	**0**	**0**	**1**	**0**	**2**	**0**	**0**	**0**	**0**	**4**	**3**
CLA2	2	4	3	1	2	0	1	1	0	3	0	0	0	0	4	4
CLA3	**1**	**4**	**3**	**1**	**3**	**0**	**3**	**1**	**0**	**3**	**0**	**0**	**0**	**0**	**4**	**3**
CLA4	2	3	2	1	3	0	3	2	0	3	0	0	0	0	3	3
ALE1	0	2	2	0	3	0	4	4	0	2	0	3	2	2	4	4
ALE2	0	3	1	0	1	0	0	1	0	0	0	1	0	0	3	3
ALE3	**0**	**3**	**0**	**0**	**0**	**0**	**1**	**3**	**0**	**3**	**1**	**2**	**0**	**0**	**4**	**3**
ALE4	0	2	1	0	1	0	3	4	0	3	0	0	0	0	4	3
CHA1	**0**	**3**	**2**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**5**	**4**
CHA2	0	3	1	0	0	0	0	1	0	3	0	2	0	0	3	3
CHA3	**1**	**3**	**2**	**0**	**3**	**0**	**4**	**2**	**1**	**4**	**0**	**0**	**1**	**0**	**3**	**3**
CHA4	3	2	2	0	3	1	4	1	1	4	0	1	1	0	3	3
GIL1	**0**	**3**	**1**	**2**	**1**	**0**	**4**	**1**	**0**	**3**	**0**	**0**	**0**	**0**	**3**	**0**
GIL2	0	3	0	1	0	0	1	0	0	3	0	0	0	0	3	3
GIL3	**0**	**1**	**3**	**0**	**3**	**0**	**3**	**3**	**0**	**2**	**0**	**0**	**1**	**2**	**4**	**3**
GIL4	0	1	4	0	4	0	4	3	0	3	0	1	2	1	4	3
LIM1	**1**	**3**	**3**	**2**	**3**	**0**	**4**	**1**	**0**	**3**	**0**	**0**	**0**	**1**	**4**	**4**
LIM2	1	2	2	0	1	0	1	0	0	2	0	0	0	0	3	3
LIM3	**2**	**3**	**2**	**3**	**2**	**0**	**3**	**4**	**1**	**3**	**0**	**0**	**0**	**1**	**4**	**3**
LIM4	2	2	3	0	2	1	3	3	0	3	0	0	0	1	3	3
SHE1	**0**	**3**	**3**	**3**	**3**	**0**	**5**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**3**	**4**
SHE2	0	3	0	0	0	0	0	0	0	0	0	1	0	0	3	3
SHE3	**1**	**3**	**2**	**1**	**2**	**0**	**2**	**2**	**1**	**2**	**1**	**0**	**0**	**0**	**4**	**3**
SHE4	1	3	3	0	3	0	2	2	1	1	0	1	1	0	4	3
UGH1	**0**	**3**	**3**	**3**	**3**	**0**	**5**	**0**	**0**	**3**	**0**	**0**	**0**	**0**	**3**	**4**
UGH2	0	3	0	0	0	0	0	0	0	0	0	1	0	0	4	4
UGH3	**1**	**3**	**2**	**1**	**2**	**0**	**2**	**2**	**1**	**2**	**1**	**0**	**0**	**0**	**4**	**3**
UGH4	1	3	3	0	3	0	2	2	1	1	0	1	1	0	4	3

Appendix XIV. Site Descriptions for Yorkshire High Impact Sites (Source: Environment Agency)

Site Code	Grid Reference	Mine/Catchment	Watercourse	EA Impact Rank
BUL1	SE215029	Bullhouse	River Don	1
BUL2	SE218028	"	"	
BUL3	SE221030	"	"	
BUL4	SE235034	"	"	
BUL5	SE247037	"	"	
FEN1	SK364753	Fender	Barlow Brook	2
FEN2	SK366751	"	"	
FEN3	SK371749	"	"	
FEN4	SK373749	"	"	
CLF1	SD906237	Clough Foot	Midgelden Brook	3
CLF2	SD924233	"	"	
CLF3	SD924233	"	"	
CLF4	SD967258	"	"	
JAC1	SE165074	Jackson Bridge	New Mill Dike	6
JAC2	SE163078	"	"	
JAC3	-	"	"	
LOX1	SK321894	Loxley	River Loxley	4
LOX2	SK325894	"	"	
LOX3	-	"	"	
UNS1	SK373774	Unstone	River Drone	5
UNS2	SK374773	"	"	
UNS3	SK376765	"	"	
CRA2	SD892260	Craggs Moor	Beaters Clough	7
CRA3	SD914261	"	River Calder	
CRA4	SD926254	"	"	
SUM1	SK365780	Summerley	River Drone	5
SUM2	SK368778	Minewater	"	
SUM3	SK371775	"	"	
		"		
MOR1	SE103324	Morton Clay	Pinch Beck	8
MOR2	SE105324	"	"	
MOR3	SE109324	"	"	

Appendix XV. Yorkshire high impact sites. Mean values (± 2 SE) of environmental variables for each TWINSPAN group at Level 3. Standard errors for percentage values were calculated after arcsine transformation, and given following back-transformation. (t)/(f)=total/filtered μg l^{-1}. Original Data Source: Environment Agency.

	Twinspan site Group (in order of increasing BMWP)						
	7	12	13	9	10	11	8
GQA Class	E	E	E	B	B	B	A
ASPT	3.17	2.96	3.2	5.4	5.03	4.96	5.5
BMWP	19	20	22	79	79	86	108
No. Taxa	6	7	7	15	16	17	20
(SE)	(-)	(2.8)	(1.4)	(1.4)	(5.08)	(2.8)	(-)
As (t)	1.8	1.58	1.72	1	1	1.11	1
(SE)	(-)	(0.6)	(0.2)	(0)	(0)	(0)	(-)
Conductivity (mS cm^{-1})	738	777	770	257	606	407	263
(SE)	(-)	(39.2)	(39.8)	(17.92)	(152.8)	(155.8)	(-)
Chloride (mg l^{-1})	31	69	51	24	37	41	18
(SE)	(-)	(54.6)	(14.4)	(8.34)	(14.14)	(10.48)	(-)
Dissolved Oxygen (%)	69	72	72	92	85	92	95
(SE)	(-)	(2.4)	(0.2)	(0.1)	(0.2)	(0.4)	(-)
Alkalinity (mg l^{-1})	195	114	129	13	64	53	38
(SE)	(-)	(57.4)	(40.8)	(6.6)	(34.06)	(35.8)	(-)
Suspended Solids (mg l^{-1})	9	16	13	8	11	7	1
(SE)	(-)	(6.2)	(4.6)	(0.6)	(8.6)	(3.0)	(-)
Sulphate (mg l^{-1})	150	179	158	50	5.7	72	55
(SE)	(-)	(56.2)	(12.6)	(0.6)	(8.6)	(3.0)	(-)
pH	7.5	7.03	7.34	7.33	7.33	7.53	7.65
Fe (f)	928	5254	691	454	2320	465	326
(SE)	(-)	(5398)	(53.8)	(466)	(3852)	(338)	(-)
Cobbles (%)	65	41	30	57	47	57	43
(SE)	(-)	(0.6)	(2.6)	(0.8)	(0.4)	(0.2)	(-)
Zn (t)	15	31.05	30	15	26.35	15.28	16.5
(SE)	(-)	(13.6)	(16.04)	(0)	(32.22)	(0.54)	(-)
Zn (f)	15	26.53	26.2	15	24.75	13.75	15.5
(SE)	(-)	(11.2)	(13.6)	(0)	(31.6)	(2.5)	(-)
Colour	1	1.75	2.2	1.7	1.5	1.25	1
Ochre (%)	100	85	35	25	40	3.8	0
(SE)	(-)	(30)	(0.4)	(50)	(31.6)	(7.6)	(-)

Appendix XV continued.

Cu (t) (SE)	1 (-)	3.58 (3.0)	4.64 (2.6)	1.7 (0.6)	2.58 (1.4)	1.72 (1.02)	2.7 (-)
Cu (f) (SE)	1 (-)	2.7 (2.10)	3.7 (2.08)	1.4 (0.4)	1.88 (0.64)	1.28 (0.34)	2.25 (-)
Fe (t) (SE)	2760 (-)	7678 (6920)	2827 (1698)	921 (614)	6894 (7688)	1130 (0.68)	619 (-)
Pb (t) (SE)	1.87 (-)	1.74 (0.54)	3.04 (2.22)	1 (0)	0.395 (0.06)	2.74 (1.0)	1 (-)
Pb (f) (SE)	1 (-)	1.07 (0.18)	1.17 (0.16)	1 (0)	1 (0.28)	1.47 (0.5)	1 (-)
Distance from source (km) (SE)	7.1 (-)	3.9 (0.5)	3.5 (2.16)	8.3 (4.8)	2.7 (2.14)	7.9 (2.8)	4.5 (-)

Appendix XVI. Proportional representation of taxa for each TWINSPAN site-group for Yorkshire High Impact sites (mean percentage per site ± 2 SE). Standard errors were calculated after arcsine transformation, and shown following back-transformation. Original Data Source: Environment Agency.

TWINSPAN Gp Taxonomic Gp	7	12	13	9	10	11	8
Ephemeroptera (SE)	0 (-)	7.8 (3.67)	0 (0)	15.7 (0.16)	5.6 (1.51)	9.3 (0.19)	7.5 (-)
Plecoptera (SE)	0 (-)	0 (0)	2.7 (4.12)	8.9 (0.72)	12.0 (0.3)	8.0 (0.06)	13.4 (-)
Trichoptera (SE)	0 (-)	14.9 (7.75)	27.1 (1.38)	18.1 (0.3)	11.9 (1.46)	16.4 (0.30)	19.6 (-)
Mollusca (SE)	33.3 (-)	14.9 (7.75)	24.7 (0.93)	11.1 (0.51)	3.9 (1.8)	19.9 (0.35)	20.2 (-)
Crustacea (SE)	0 (-)	3.1 (4.2)	5.3 (3.73)	13.7 (0.03)	5.4 (1.4)	7.0 (0.7)	5.21 (-)
Coleoptera (SE)	16.7 (-)	0 (0)	0 (0)	3.01 (3.03)	10.2 (1.16)	5.0 (0.6)	8.2 (-)
Neuroptera (SE)	0 (-)	0 (0)	0 (0)	0 (0)	3.8 (1.18)	1.8 (1.1)	0 (-)
Hirudinea (SE)	0 (-)	5.8 (2.9)	11.6 (3.2)	0.8 (3.23)	1.02 (0.81)	3.7 (3.6)	0 (-)
Diptera (SE)	16.7 (-)	17.9 (1.58)	15.9 (0.26)	6.8 (0.01)	7.7 (0.3)	6.7 (0.08)	5.2 (-)
Oligochaeta (SE)	16.7 (-)	17.9 (1.58)	15.9 (0.26)	6.8 (0.01)	7.7 (0.3)	6.7 (0.08)	5.2 (-)

Appendix XVII. Yorkshire high impact data. Classification of family and site groups by TWINSPAN. The number codes show the percentage of occurrence of each taxa between the sites in each group. Abundance Codes: 1=1-19%; 2=20-39%; 3=40-59%; 4=60-79%; 5=80-99%; 6=100%. (Original Data Source: Environment Agency).

FAMILY Group SITE Group

		8	9	10	11	12	13	7
8	Leptoceridae			1	4			
	Beraeidae				1			
	Hydropsychidae	5	2	3	5			
	Hydroptilidae			1	3			
	Sialidae			4	3			
	Polycentropodidae	3		5	2			
	Hydrophilidae			3				
	Dytiscidae	3		5	4			
9	Leuctridae	3	6	6	6			
	Gammaridae	6	6	4	4			
	Heptageniidae	3	2	2	2			
	Planorbidae	3			2			
	Ephemeridae		6	2	2			
	Rhyacophilidae	6	6	2	4			
	Beraeidae	3						
	Sericostomatidae	3	4	1				
	Perlodidae	6		1	1			
10	Baetidae	6	6	4	6	6		
	Tipulidae	6	6	6	6	3		6
	Ancylidae	6		1	4	2		
	Sphaeriidae		4	4	5		3	
11	Planariidae	6	4		2		2	
	Elmidae	6	4	2	1			
6	Nemouridae	6	2	4	2		3	
	Limnephilidae		2	3	1		3	
	Simuliidae	6		4	4	4	2	
	Glossiphoniidae		2		2		2	
14	Lymnaeidae	6	2	3	4	3	3	6
15	Asellidae		6	4	4	3	6	
	Hydrobiidae	6		3	6	4	6	6
	Chironomidae	6	6	6	6	6	6	
	Oligochaeta	6	6	5	6	6	6	
	Erpobdellidae			3	2	4	5	

Appendix XVIII (1). Yorkshire High Impact Site Database. Source: Environment Agency.

	BMWP	NO. TAXA	ASPT	As(t)	Pb(t)	Pb(f)	Cu(t)	Cu(f)	Zn(t)	Zn(f)	Al(t)	Al(f)	Fe(t)	Fe(f)	Ni(t)	Ni(f)	Temperature
BUL1	117	22	5.32	1	1.13	1	1.11	1	15	15	81.2	49.7	553	400	1.31	3.316	14.1
BUL2	47	10	4.7	1	1.46	1	1.02	1	15	15	111	41.4	3030	1830	5.39	4.77	13.6
BUL3	69	16	4.31	1	1	1	1	1	15	15	82.4	35.6	2030	1200	5.39	5.3	13.2
BUL4	98	18	5.44	1	1	1	1	1	15	15	56.2	24.2	1170	610	4.07	3.65	13.2
BUL5	82	16	5.13	1.4	1.38	1	1.92	1.35	15	15	75.5	39.6	967	451	3.6	2.86	13.2
FEN1	76	16	4.75	1.5	7.54	3.86	1	1.07	15	15	34.8	10	267	139	1.32	1.22	12.2
FEN2	16	6	2.67	2.4	2.88	1.09	1	1	15	15	23.5	10	5250	4880	5.7	5.41	10.9
FEN3	19	6	3.17	1.8	1.95	1	1	1	15	15	20.6	10	2760	702	5.29	5.13	10.7
FEN4	16	5	3.2	1.6	1.89	1	1.44	1	21	15	16.9	10	2690	622	5.96	5.82	11.7
CLF1	61	11	5.55	1	1	1	2.84	2.34	5	13.03	42.2	15.5	318	190	2.92	2.71	14
CLF2	8	3	2.67	1	1	1	1.66	1.1	33.2	29.1	200	40	18000	15600	26.9	25.8	12.3
CLF3	60	11	5.45	1	1	1	2.8	1.41	22.8	5	452	14.8	14900	168	11.2	7.77	13.1
CLF4	104	21	4.95	1	8.91	1.91	5.24	2.39	17.2	5	179	30.6	2010	258	4.93	3.29	13.9
JAC1	99	20	4.95	1	1.07	1	1	1	15	15	10	10	900	425	2.08	1.88	14.1
JAC2	55	11	5	1	1	1	1.32	1.18	15	15	10	10	3710	433	10.7	10	11.9
JAC3	62	14	4.43	1	1	1	1.48	1.45	15	15	10	10	139	30	3.49	3.2	12.1
LOX1	91	16	5.69	1	1	1	1.11	1	15	15	69.7	10	332	147	3.13	2.75	14.8
LOX2	76	14	5.43	1	1.32	1	2.19	1.54	15	15	121	47.9	1390	971	5.31	5.19	14.5
LOX3	71	14	5.07	1	1.36	1	1.78	1.65	15	15	115	38.6	1040	364	4.78	4.37	13.9
UNS1	17	6	2.83	1.7	2.05	1.25	7.55	5.91	50	43	73.2	44.1	2940	1320	6.97	6.65	15.1
UNS2	21	7	3	1.6	1.9	1.18	7.41	5.47	48	41	75.5	28.9	3860	2190	6.61	6.1	14.7
UNS3	35	10	3.5	1.3	1.5	1	4.25	3.09	28	21	74.4	10	3600	830	7.23	6.7	12.7
CRA2	27	6	4.5	1	3.16	1	7.16	3.76	138	135	1140	94.3	31000	28800	91.9	90.9	14.7
CRA3	91	17	5.35	1	1	1	1.76	1.44	18	16	129	41.4	766	391	9.28	8.15	12.5
CRA4	124	22	5.64	1	1	1	3.63	3.06	15	15	79.6	40.9	471	277	4.48	4.14	12.3
SUM1	26	9	2.89	2.1	1.88	1.45	5.5	5.39	15	15	11.8	10	157	99	2.57	2.52	15
SUM2	22	7	3.14	1.7	1	1	1.73	1.47	15	15	11.6	10	5530	3130	6.3	6.15	11.8
SUM3	31	8	3.88	1.5	2.08	1.16	7	4.94	49	43	127	33	2820	1140	6.37	6.37	15.1
MOR1	126	24	5.25	1	1	1	2.5	2.3	5	5	10	10	106	59	1	1	12.3
MOR2	83	18	4.61	1	1	1	1.47	1.24	5	5	82.7	10	2740	1530	5.67	1.76	11.9
MOR3	149	27	5.52	1	1	1	1.52	1.77	5	5	29.8	10	348	77	2.84	2.52	10.6

Appendix XVIII (2). Yorkshire High Impact Site Database. Source: Environment Agency.

	pH	DO (%)	Conductivity	Alkalinity	SSolids	Sulphate	Chloride	Boulders %	Cobbles %	Pebbles %	Gravel %	Sand %	Silt %	Ochre %	Width	Depth	Colour
BUL1	7.5	97	217	18	6	18.6	35.5	5	70	15	5	5	0	0	7	11.6	1
BUL2	6.9	96	350	18	10	54.9	36.7	5	60	20	10	5	0	100	7	11.6	2
BUL3	6.9	96	312	21	7	57.5	37.7	15	65	10	5	5	0	100	7	13.3	1
BUL4	7.3	96	321	25	8	57.4	38.4	10	60	20	5	5	0	0	7	11.6	1
BUL5	7.5	97	339	33	10	52.4	43.6	15	40	30	10	5	0	0	7	13.3	2
FEN1	8.4	103	469	160	3	36.1	28.7	5	55	15	10	10	5	0	2.5	10	1
FEN2	7	74	724	195	11	145	30	10	55	15	5	5	10	100	4.5	10	1
FEN3	7.5	87	738	195	9	150	30.6	10	65	15	0	0	10	100	3	13.3	1
FEN4	7.5	87	735	192	11	153	32.2	10	60	15	0	0	15	0	4	18.3	3
CLF1	7.3	80	466	19	1	41.7	24	0	40	30	20	10	0	0	1	6.7	1
CLF2	7.1	85	816	60	24	261	16.7	5	40	30	15	10	0	100	1	8.3	1
CLF3	7.7	88	444	31	36	120	16.8	10	50	30	5	5	0	100	2	15	3
CLF4	7.7	85	257	58	15	47.2	20.2	0	50	30	10	10	0	0	8.5	11.7	1
JAC1	7.7	84	383	18	2	54.6	53.2	10	60	10	10	10	0	0	2.5	11.6	1
JAC2	7.5	81	975	95	9	334	67.3	15	65	10	5	5	0	0	2.5	5	2
JAC3	7.2	87	919	92	5	253	68.4	10	60	15	10	5	0	0	2.5	13.3	1
LOX1	7.4	96	241	10	7	44.2	18.3	10	65	15	5	5	0	0	8	28.3	2
LOX2	7.1	91	257	10	8	52.2	21.2	10	45	25	10	10	0	100	9	16.6	1
LOX3	7.5	92	272	20	8	53.4	32	10	60	20	5	5	0	0	6	28	2
UNS1	7	73	790	85	14	148	76.1	5	35	20	20	20	0	100	3	15	3
UNS2	6.8	46	793	94	11	141	99.7	5	30	30	25	15	0	100	2	13.3	2
UNS3	7.2	76	775	108	16	167	130	5	40	25	15	15	0	0	5	13.3	3
CRA2	6.4	62	583	10	22	247	9.6	5	35	30	20	10	0	50	1	6.6	2
CRA3	7.6	89	280	38	1	63	17.9	15	40	25	10	10	0	0	4	13.3	1
CRA4	7.7	92	245	38	1	47.2	18.7	0	45	30	15	10	0	0	3	11.6	1
SUM1	8	87	830	152	6	181	52.6	2	50	40	4	4	0	0	2.5	6.66	1
SUM2	7.2	84	718	130	14	161	44.3	2	3	70	10	5	10	100	3	8.3	1
SUM3	7	48	775	85	20	147	51.9	5	15	50	15	15	0	0	2.5	11.6	3
MOR1	7.8	58	532	96	1	76.1	57.8	0	40	30	20	10	0	0	1	6.6	1
MOR2	7.2	58	772	124	10	183	33.6	0	30	50	10	10	0	100	1	10	1
MOR3	7.9	88	766	117	2	186	45.7	0	50	25	15	10	0	0	1.5	10	1

Appendix XVIII (3). Yorkshire High Impact Site Database. Source: Environment Agency.

	Altitude	Slope	DistSource	Substrate	Flow	Heptageniidae	Ephemerellidae	Leuctridae	Perlodidae	Beraeidae	Leptoceridae	Sericostomatidae	Nemouridae	Rhyacophilidae
BUL1	220	28	9	-6.3625	3	1	0	2	0	0	1	0	1	1
BUL2	80	30.4	7.4	2.0875	3	0	0	1	0	0	1	0	0	0
BUL3	90	26	8.4	1.4125	3	0	0	1	0	0	0	0	0	0
BUL4	100	29	2.25	-6.1375	3	1	1	2	0	0	2	0	0	0
BUL5	100	29	2.75	-5.4625	3	0	1	2	0	0	1	0	0	1
FEN1	220	28	9.25	-5.2625	3	0	0	1	0	0	1	0	0	0
FEN2	300	67	1.5	2.4125	3	0	0	0	0	0	0	0	0	0
FEN3	90	26	8.4	1.7	2	0	0	0	0	0	0	0	0	0
FEN4	180	49	0.75	-5.9125	2	0	0	0	0	0	0	0	0	0
CLF1	220	28	9.75	-4.525	3	0	1	2	0	0	0	0	1	0
CLF2	300	67	1.6	3.25	3	0	0	0	0	0	0	0	0	0
CLF3	100	30	3	2.3125	3	0	0	1	1	0	0	0	0	0
CLF4	170	50	0.95	-4.975	3	0	0	1	1	1	0	0	0	1
JAC1	220	28	11.5	-5.875	2	0	1	2	0	0	0	0	1	1
JAC2	190	71	3.5	-6.5875	3	0	0	2	0	0	0	0	1	0
JAC3	100	30	3.25	-6.1375	3	0	0	2	0	0	0	0	0	1
LOX1	220	28	12.25	-6.3625	3	1	2	2	0	0	0	2	0	2
LOX2	150	67	9	2.8	3	0	1	1	0	0	0	1	0	1
LOX3	140	53	4	-6.1375	3	0	1	2	0	0	0	0	1	1
UNS1	80	30.4	6.25	4	3	0	0	0	0	0	0	0	0	0
UNS2	180	52	2.6	3.8	3	0	0	0	0	0	0	0	0	0
UNS3	250	100	1.75	-4.4875	3	0	0	0	0	0	0	0	0	0
CRA2	180	52	3.1	-0.525	2	0	0	1	0	0	0	0	0	0
CRA3	220	15	3.75	-5.2	3	0	0	1	1	0	0	0	1	1
CRA4	150	50	1.35	-4.75	3	2	0	0	1	1	0	1	1	2
SUM1	80	30.4	6.5	-5.38	2	0	0	0	0	0	0	0	0	0
SUM2	180	52	3.1	5.1125	3	0	0	0	0	0	0	0	1	0
SUM3	170	33	5.25	-3.3625	3	0	0	0	0	0	0	0	1	0
MOR1	80	30.4	7.1	-4.525	2	2	2	3	0	0	0	0	2	2
MOR2	90	26	8	3.925	2	0	0	3	0	0	0	0	1	0
MOR3	110	42	1.9	-4.975	3	1	2	3	0	0	1	1	1	2

Appendix XVIII (4). Yorkshire High Impact Site Database. Source: Environment Agency.

	Polycentropodidae	Limnephilidae	Ancylidae	Hydroptilidae	Gammaridae	Dytiscidae	Hydrophilidae	Elmidae	Hydropsychidae	Tipulidae	Simuliidae	Planariidae	Baetidae	Sialidae	Hydrobiidae
BUL1	1	0	2	1	2	1	0	0	1	2	3	1	2	1	2
BUL2	0	0	0	0	0	0	0	0	0	2	2	0	1	1	1
BUL3	1	0	0	0	2	1	0	0	1	2	2	0	1	1	3
BUL4	1	0	1	1	0	2	0	0	1	2	1	0	3	0	1
BUL5	0	0	0	0	1	1	0	0	1	2	1	0	2	0	3
FEN1	0	0	2	1	0	1	0	0	2	2	0	0	2	0	3
FEN2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3
FEN3	0	0	0	0	0	0	0	1	0	1	0	0	0	0	2
FEN4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2
CLF1	1	0	0	0	0	2	1	0	0	2	1	0	2	0	0
CLF2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
CLF3	1	0	0	0	1	1	0	0	1	1	1	0	2	0	0
CLF4	0	0	2	1	1	0	0	1	1	2	2	0	4	1	2
JAC1	0	1	2	0	1	1	0	0	1	1	0	1	1	2	1
JAC2	1	1	0	0	1	0	0	0	0	1	0	0	0	1	0
JAC3	0	0	0	0	2	2	1	0	0	2	3	0	2	0	2
LOX1	0	1	0	0	3	0	0	1	0	2	0	2	2	0	1
LOX2	0	1	0	0	2	0	0	0	0	2	0	1	1	0	0
LOX3	0	0	0	0	1	0	0	1	2	1	0	0	2	0	1
UNS1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
UNS2	0	0	0	0	0	0	0	0	0	0	3	0	1	0	2
UNS3	0	0	1	0	0	0	0	0	0	1	5	0	1	0	2
CRA2	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0
CRA3	1	0	1	0	1	1	0	1	0	1	1	1	2	0	1
CRA4	1	1	1	0	1	0	0	1	2	2	1	1	2	0	1
SUM1	0	0	0	0	0	0	0	0	0	0	0	2	0	0	2
SUM2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
SUM3	0	1	0	0	0	0	0	0	0	0	3	0	0	0	3
MOR1	1	1	3	0	2	3	2	1	2	1	1	0	3	1	3
MOR2	1	1	0	0	1	1	1	1	0	2	0	0	3	1	2
MOR3	1	2	0	1	2	3	1	1	2	2	1	0	3	1	3

Appendix XVIII (5). Yorkshire High Impact Site Database. Source: Environment Agency.

	Lymnaeidae	Planorbidae	Sphaeriidae	Glossiphoniidae	Erpobdellidae	Asellidae	Chironomidae	Oligochaeta
BUL1	**1**	**0**	**1**	**0**	**0**	**2**	**3**	**2**
BUL2	0	0	0	0	0	1	2	3
BUL3	**1**	**0**	**2**	**0**	**1**	**1**	**3**	**3**
BUL4	0	0	1	0	1	1	3	3
BUL5	**1**	**0**	**1**	**0**	**2**	**0**	**3**	**3**
FEN1	4	1	1	1	0	0	3	3
FEN2	**3**	**0**	**0**	**0**	**1**	**0**	**2**	**3**
FEN3	2	0	0	0	0	0	2	2
FEN4	**2**	**0**	**0**	**0**	**0**	**0**	**2**	**3**
CLF1	0	0	0	0	0	0	3	2
CLF2	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**
CLF3	0	0	0	0	0	0	2	2
CLF4	**2**	**0**	**2**	**1**	**3**	**1**	**3**	**3**
JAC1	3	2	2	0	0	1	3	2
JAC2	**0**	**0**	**1**	**0**	**0**	**1**	**2**	**1**
JAC3	2	0	1	0	0	1	2	2
LOX1	**0**	**0**	**2**	**0**	**0**	**2**	**2**	**3**
LOX2	0	0	1	1	0	1	2	2
LOX3	**2**	**0**	**0**	**0**	**0**	**1**	**2**	**2**
UNS1	0	0	0	0	1	3	3	4
UNS2	**0**	**0**	**0**	**0**	**1**	**3**	**3**	**4**
UNS3	2	0	0	0	1	3	3	4
CRA2	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**1**
CRA3	2	0	0	0	0	0	2	2
CRA4	**2**	1	**1**	**0**	**0**	**0**	**3**	**2**
SUM1	1	0	1	2	1	3	3	2
SUM2	**0**	**0**	1	**0**	1	**0**	**2**	**3**
SUM3	0	0	0	0	1	3	3	4
MOR1	**3**	**0**	**2**	**0**	1	**3**	**3**	**3**
MOR2	2	0	2	0	2	1	2	2
MOR3	**2**	**0**	1	**2**	1	**2**	**3**	**3**

Appendix XIX. Site Descriptions for Northumbria (Source: Environment Agency)

Site Code	Grid Reference	Mine/Catchment	Watercourse
ARN1	NZ061241	Arn Gill	Arn Gill
ARN2	NZ065243	"	"
ARN3	NZ071243	"	"
ARN4	NZ073244	"	"
WOO1	NZ043248	Woolly Hill	Hindon Beck
WOO2	NZ044248	"	"
WOO3	NZ053247	"	"
WOO4	NZ056248	"	"
HAG1	NZ212495	Hagwood	Wardles Burn
HAG2	NZ213495	"	"
HAG3	NZ218493	"	"
HAG4	NZ219493	"	"
BLA1	NY742478	Blaghill Burn	Blaghill Burn
BLA2	NY741476	"	"
BLA3	NY740473	"	"
BLA4	NY470472	"	"
HAL1	NY996678	Halton	Stagshawbank Burn
HAL2	NY996677	"	"
HAL3	NY002671	"	"
HAL4	NZ005669	"	"
NET1	NZ094911	Netherwitton	River Font
NET2	NZ096911	"	"
NET3	NZ097910	"	"
CHI1	NZ097970	Chirm	Maglin Burn
CHI2	NZ098971	"	"
CHI3	NZ099973	"	"
CHI4	NZ100974	"	"
FOR1	NT953370	Ford Hill	Cannon Burn
FOR2	NT953369	"	"
FOR3	NT944366	"	"
ALL1	NT974476	Allerdeanmill	Allerdeanmill Burn
ALL2	NT976475	"	"
ALL3	NT986473	"	"
ALL4	NT996467	"	"
ALL5	NT998465	"	"
BYR1	NY664649	Byron's Drift	Tipalt Burn
BYR2	NY667646	"	"
BYR3	NY670644	"	"

Appendix XX. Northumbria sites. Mean values (± 2 SE) of environmental variables for each TWINSPAN group at Level 4. Standard errors for percentage values were calculated after arcsine transformation, and shown following back-transformation. (t)/(f)=total/filtered μg l^{-1}. Original Data Source: Environment Agency.

Twinspan site Group (in order of increasing BMWP)

	8	21	20	12	11	19	13	18	7
GQA Class	C	C	B	A	A	A	A	A	A
APST	5.03	4.71	5.27	5.01	5.53	5.94	5.95	6.6	6.18
BMWP	52	68	78	97	118	128	131	140	169
Substrate (phi units)	-6.9	1.77	.845	1.97	0.7	-5.22	-3.805	-5.01	-6.5
Silt (%) (SE)	1.5 (-)	50 (3.64)	36 (3.10)	46 (11.06)	40 (5.1)	10 (1.7)	10 (-)	5 (-)	1.25 (0.92)
Boulders (%) (SE)	73 (-)	17 (3.6)	12 (5.1)	18 (11.1)	11 (5.1)	48 (1.7)	60 (-)	46 (-)	61 (0.94)
No. Taxa (SE)	10 (-)	14 (2.4)	15 (3.4)	19 (2.2)	21 (2.6)	21 (1.94)	22 (-)	21 (-)	29 (0.96)
Width (m) (SE)	3 (-)	1.7 (0.52)	1.5 (0.44)	1.2 (0.48)	1.3 (0.08)	2.4 (1.4)	6 (-)	1.6 (-)	5 (0.4)
Chloride (mg l^{-1}) (SE)	20.5 (-)	40 (7.4)	23 (8.12)	50.5 (10.7)	37.5 (9.34)	31 (16.10)	22.9 (-)	10 (-)	21 (7.6)
Al (f) (SE)	217 (-)	37 (19.34)	82 (123)	37 (53.96)	14 (5.6)	106 (123)	125 (-)	125 (-)	45 (18.6)
Altitude (m) (SE)	30 (-)	51 (31.92)	33 (9.74)	51 (19.54)	31 (8.22)	43 (20.46)	30 (-)	72 (-)	18 (0.50)
Flow (units)	2	1.95	2.1	1.4	1.3	1.6	1	1.25	1
Cobbles (%) (SE)	20 (-)	13 (1.0)	21 (1.2)	7.5 (2)	27 (0.4)	31 (1.0)	8 (-)	29 (-)	40 (18)
Ochre (%) (SE)	90 (-)	60 (8)	50 (12)	4 (8)	32 (44)	6 (6)	23 (-)	0 (-)	80 (14)
Zn (t) (SE)	25 (-)	10.7 (9.56)	11.6 (7.6)	6.5 (1.8)	6.4 (1.98)	96 (101.4)	7.7 (-)	19.4 (-)	6.2 (1.42)
Distance from Source (km) (SE)	1.7 (-)	1.3 (0.42)	1.8 (0.22)	0.9 (0.56)	1.2 (0.22)	1.05 (0.94)	3.8 (-)	0.8 (-)	2.7 (0.68)

Appendix XX continued.

Pb (t) (SE)	1 (-)	1 (0)	1 (0)	1 (0)	1 (0)	4.1 (3.96)	1 (-)	1.5 (-)	1 (0)
Alkalinity (mg l^{-1}) (SE)	10 (-)	174 (54.6)	68 (75.6)	138 (51.4)	72 (46.6)	82 (29.2)	106 (-)	36 (-)	126 (60.8)
Slope (m km^{-1}) (SE)	3.4 (-)	2.7 (0.84)	3.56 (0.44)	1.85 (1.10)	2.5 (0.46)	2.09 (1.9)	7.5 (-)	1.6 (-)	5.3 (1.34)

Appendix XXI. Proportional representation of taxa for each TWINSPAN site-group for Northumbria sites (mean percentage per site ± 2 SE). Standard errors for percentage values were calculated after arcsine transformation, and shown following back-transformation. Original Data Source: Environment Agency.

TWINSPAN Gp Taxonomic Gp	8	21	20	12	11	19	13	18	7
Ephemeroptera	6.7	5.0	6.9	9.0	10.9	12.5	13.6	17.3	11.5
(SE)	(-)	(0.97)	(1.81)	(1.00)	(1.42)	(0.17)	(-)	(-)	(0.34)
Plecoptera	20.0	6.1	7.5	5.7	10.4	14.0	1.9	15.4	13.3
(SE)	(-)	(0.68)	(0.48)	(2.78)	(0.15)	(0.06)	(-)	(-)	(0.84)
Trichoptera	13.3	14.4	19.3	14.0	16.5	20.0	22.7	26.9	26.2
(SE)	(-)	(0.8)	(0.33)	(0.21)	(1.29)	(0.08)	(-)	(-)	(0.12)
Mollusca	6.7	12.4	11.28	14.4	15.1	12.3	9.1	5.8	10.7
(SE)	(-)	(0.69)	(0.42)	(0.14)	(0.19)	(0.02)	(-)	(-)	(2.53)
Crustacea	6.7	7.0	3.8	5.5	4.1	3.3	4.5	3.9	3.3
(SE)	(-)	(0.08)	(1.02)	(0.34)	(0.06)	(0.42)	(-)	(-)	(0.76)
Coleoptera	20.0	19.3	19.8	14.5	13.6	14.5	18.2	15.4	8.2
(SE)	(-)	(0.1)	(0.10)	(1.64)	(0.01)	(0.1)	(-)	(-)	(0.07)
Neuroptera	6.7	3.3	2.09	1.02	4.1	2.91	0	1.9	1.9
(SE)	(-)	(1.6)	(1.48)	(1.36)	(0.06)	(0.33)	(-)	(-)	(0.87)
Hirudinea	0	1.9	0.97	5.5	4.2	0.8	4.6	0	5.8
(SE)	(-)	(0.6)	(1.5)	(0.33)	(0.06)	(0.3)	(-)	(-)	(0.23)
Diptera	13.3	14.1	14.6	12.1	12.5	11.1	13.6	11.5	10.0
(SE)	(-)	(0.06)	(0.02)	(0.13)	(0.19)	(0.01)	(-)	(-)	(2.4)
Oligochaeta	6.7	6.7	5.8	4.44	4.4	3.9	4.5	3.9	3.3
(SE)	(-)	(0.05)	(0.06)	(0.02)	(0.06)	(0.01)	(-)	(-)	(0.6)

Appendix XXII. Northumbria data. Classification of family and site groups TWINSPAN. The number codes show the percentage of occurrence of each taxa between the sites in each group. Abundance Codes: 1=1-19%; 2=20-39%; 3=40-59%; 4=60-79%; 5=80-99%; 6=100%. (Original Data Source: Environment Agency).

FAMILY Group SITE Group

		8	18	19	20	21	11	12	13	7
16	Gerridae						2			
	Coenagriidae						4			
17	Philopotamidae		3	2						
	Chloroperlidae		6	3	3					
	Siphlonuridae	6	6	3	3					
	Hydroptilidae			4			4			2
	Gyrinidae		3	4		1				2
9	Scirtidae	6	6	4	2	5	2	3		
	Beraeidae		6		5	1	2			2
	Planorbidae			3	3	1	2			2
20	Corixidae					2		2		
	Hydrobiidae			3	2	4	2	4	6	6
	Lymnaeidae			3	4	5	4	4	6	4
	Dytiscidae	6	3		6	4	6	6	6	6
21	Gammaridae	6	3	5	5	6	6	6	6	6
	Sialidae		3	3	4	3	6	3		4
	Baetidae		6	5	4	3	4	6	6	6
	Simuliidae		6	6	4	3	6	4	6	6
	Elmidae	6	6	6	5	3	4	6	6	6
	Ephemerellidae		3	1	2		4	2	6	
	Asellidae			1		1		2		
	Leuctridae	6	6	6	4	2	6	3	6	3
	Chironomidae	6	6	6	6	6	6	6	6	6
	Limnephilidae	6	6	5	6	5	6	6		6
	Tipulidae		6	6	6	6	6	6	6	6
	Oligochaeta	6	6	6	6	6	6	6	6	6
	Sphaeriidae		6	6	6	5	6	6		4
	Hydrophilidae		6	6	6	5	6	6	6	2
	Nemouridae		6	6	2	5	4	3	6	6
22	Odontoceridae		6	1	4				6	3
	Leptophlebiidae	6	6	3		1	6	3		4
	Rhyacophilidae		6	6	2	2	2	3	6	4
23	Heptageniidae		6	6			2	3		6
	Perlodidae		6	3						4
	Goeridae		6				2			2
	Polycentropodidae		6	3	2	1		3		3
	Hydropsychidae			4		1			6	6
24	Sericostomatidae		3	2	2	1	2	3	6	6
	Glossiphonidae			1	2	2	6	6	6	6
	Ephemeridae						2		6	2
25	Haliplidae				2	1		4	6	2
	Notonectidae							2		
26	Piscicolidae								6	
	Psychomyiidae							2		3
	Lepidostomatidae									6
	Leptoceridae								6	6
	Perlidae									2
	Planorbidae						2			4
	Erpobdellidae					1				4
	Caenidae			1						4
27	Taeniopterygidae									6
7	Capniidae			1						2
	Ancylidae		3	5				4	3	6

Appendix XXIII (1). Northumbria Site Database. Source: Environment Agency.

	BMWP	No. TAXA	ASPT	As(t)	Pb(t)	Pb(f)	Cu(t)	Cu(f)	Zn(t)	Zn(f)	Al(t)	Al(f)	Fe(t)	Fe(f)	Ni(t)	Ni(f)	Mn(t)	Mn(f)	Temperature	pH	DO %	Conductivity
ARN1	**101**	**20**	**5.025**	**1**	**1**	**1**	**1**	**1**	**9.2**	**9.1**	**54.4**	**21.9**	**476**	**185**	**3.46**	**3.15**	**108.2**	**62**	**10**	**6.5**	**81**	**165**
ARN2	104	19	5.485	1	1	1	1	1	5	5	27.6	10	2970	1520	1.97	2.05	366.5	304.5	9.3	6.4	72	216
ARN3	**105**	**20**	**5.41**	**1**	**1**	**1**	**1**	**1**	**5**	**5**	**23.9**	**10**	**1270**	**127**	**1.9**	**1.7**	**272**	**207**	**4.1**	**6.9**	**86**	**185**
ARN4	107	20	5.34	1	1	1	1	1	5	5	32.7	10	854	86	1.8	1.4	83.1	170	3.9	7.4	98	185
WOO1	**139**	**21**	**6.49**	**1**	**1**	**1**	**1.24**	**1**	**27**	**53.3**	**242.7**	**211.4**	**375**	**417**	**3.53**	**3.5**	**339**	**327.5**	**10.2**	**6**	**101**	**84**
WOO2	90	16	5.605	1	1	1	1.36	1	25.8	22.4	222.8	272.5	1413	1021	5.01	4.66	385.5	375.5	9	5.6	83	125
WOO3	**54**	**11**	**4.84**	**1**	**1**	**1**	**1**	**1**	**11.7**	**28.8**	**84.4**	**59.4**	**391**	**185**	**3.4**	**77.05**	**226**	**218**	**3.9**	**7.1**	**103**	**130**
WOO4	85	16	5.45	1	1	1	1	1	10.7	10.6	88.1	57	400	163	3.3	223	223	192	3.8	7	104	140
HAG1	**124**	**20**	**6.17**	**1**	**1**	**1**	**1.39**	**1**	**5**	**60.8**	**10**	**10**	**114**	**53**	**1.23**	**1**	**27.7**	**22.1**	**6.6**	**6.4**	**81**	**510**
HAG2	56	11	5.05	4.6	1	1	3.94	3.72	58.2	118.2	49.4	49.4	37933	34300	70.55	71.1	4445	4385	9.8	5.4	3	1694
HAG3	**133**	**24**	**5.645**	**1**	**1**	**1**	**2.1**	**2.24**	**10.7**	**36.6**	**14.3**	**14.3**	**4988**	**2853**	**18.3**	**30.1**	**725**	**182**	**6.9**	**6.4**	**89**	**1016**
HAG4	109	20	5.46	1	1	1	1.6	1.6	5.3	112	15.5	15.5	744	215	3.5	3.4	166	140	3	7.7	104	1073
BLA1	**141**	**21**	**6.7**	**1**	**2**	**1**	**1**	**1**	**11.7**	**8.9**	**54**	**38.6**	**170**	**122**	**1.9**	**1.5**	**64.1**	**49.2**	**10.3**	**7.3**	**91**	**174**
BLA2	118	19	6.185	1	15.2	1	9.19	6.93	353.2	349.5	1993.3	462.2	12063	4577	91.9	83.3	2115	2045	10.4	6.5	87	301
BLA3	**124**	**20**	**6.025**	**1**	**4.01**	**1**	**3.17**	**2.2**	**101.1**	**73.3**	**593**	**132.8**	**2590**	**566**	**27.5**	**25.6**	**631**	**602**	**9.1**	**7.1**	**98**	**301**
BLA4	125	21	5.915	1	5.6	1.9	2	1.5	194	131	423	64.4	1340	232	16.8	16.3	402	352	5.2	7.8	96	243
HAL1	**98**	**20**	**4.98**	**1**	**1**	**1**	**1.77**	**1.1**	**8.7**	**7.3**	**40.9**	**10**	**261**	**60**	**4.2**	**4.08**	**65.3**	**12.7**	**6.8**	**7.2**	**60**	**461**
HAL2	60	14	4.26	1	1	1	1.9	1.3	5	5	10	10	3983	3843	4.95	3.5	321	84.4	9.7	6.7	21	707
HAL3	**149**	**25**	**6.08**	**1**	**1**	**1**	**1**	**1**	**5**	**5**	**10**	**10**	**2417**	**1857**	**4.48**	**2.23**	**191**	**183.5**	**8.2**	**6.9**	**58**	**620**
HAL4	121	22	5.63	1	1	1	1.8	1.4	7.2	5	45.7	10	418	53	2.7	2.2	93.6	57	4.6	7.9	86	604
NET1	**151**	**29**	**6.875**	**1**	**1**	**1**	**1.38**	**1.18**	**5**	**5**	**121.8**	**45.3**	**490**	**351**	**1**	**1**	**21.2**	**13.1**	**8.1**	**7**	**93**	**341**
NET2	131	22	5.95	1.3	1	1	1.61	1.25	7.7	6.6	305.7	124.7	24467	23433	10.5	1	1160	1140	9.3	5.4	7	621
NET3	**160**	**26**	**6.15**	**1**	**1**	**1**	**1.48**	**1.15**	**5**	**5**	**135.3**	**44.7**	**1390**	**872**	**1.1**	**1**	**66.4**	**41.6**	**8**	**6.5**	**87**	**382**
CHI1	96	18	5.255	1	1	1	2.11	1.95	5	5	226.1	117.9	367	338	1.3	1.1	15.7	10	3.3	7.3	93	320
CHI2	**52**	**10**	**5.025**	**2.1**	**1**	**1**	**3.58**	**3.48**	**25**	**5**	**297**	**217**	**83600**	**78700**	**37.6**	**1.65**	**4060**	**171.5**	**1.7**	**5.2**	**71**	**1318**
CHI3	35	8	4.59	1	1	1	2.16	1.86	5	5	222	111.3	2230	1835	2.7	2.19	179.3	180	3.3	7	93	355
CHI4	**60**	**14**	**4.44**	**1**	**1**	**1**	**1.5**	**1.4**	**5**	**5**	**199**	**48.3**	**1670**	**1040**	**2.6**	**1.65**	**163**	**163**	**3.3**	**7.5**	**93**	**335**
FOR1	71	17	4.18	1	1	1	1.685	1.91	5	5	84.3	10	408	69	2.16	1.8	157	127.3	5.1	7.8	93	727
FOR2	**50**	**12**	**4.165**	**1**	**1**	**1**	**2.55**	**2.38**	**16.6**	**9.85**	**119.3**	**32.4**	**17233**	**14567**	**8.52**	**7.43**	**1100**	**1071.7**	**9.3**	**6.7**	**57**	**1679**
FOR3	48	12	4.125	1	1	1	1.78	1.3	5	5	146.3	10	3747	30	3.07	2.115	341	271.7	7.5	8	92	1099
ALL1	**105**	**21**	**4.85**	**1**	**1**	**1**	**1.45**	**1.45**	**5**	**5**	**42.9**	**10**	**397**	**162**	**3.35**	**2.9**	**143.4**	**121.2**	**7.3**	**7.2**	**73**	**955**
ALL2	67	14	4.79	1	1	1	1.5	1	5	5	62.6	60.7	28833	29200	12.4	12.3	982	1012.5	8.7	6.4	30	745
ALL3	**55**	**11**	**5.065**	**1**	**1**	**1**	**1**	**1**	**5**	**5**	**26.7**	**10**	**3753**	**3197**	**1**	**1**	**275**	**266**	**10.8**	**6.4**	**2**	**539**
ALL4	66	14	4.88	1	1	1	1	1	8.4	5	85.3	10	3460	2040	8.35	8.7	510	485	7.1	7.1	85	914
ALL5	**96**	**18**	**5.28**	**1**	**1**	**1**	**1.65**	**1.5**	**5**	**5.9**	**79.9**	**53.4**	**3393**	**2900**	**8.8**	**10.1**	**630.7**	**598**	**7.6**	**6.8**	**59**	**1098**
BYR1	166	28	5.93	1	1	1	1.6	1.23	5	5	96.3	68.7	374	300	1.2	1.3	24.6	19.7	7.6	7.4	115	350
BYR2	**180**	**30**	**6**	**1**	**1**	**1**	**2.4**	**1.9**	**7.8**	**5.6**	**39.4**	**23.2**	**10353**	**8285**	**12.1**	**11**	**869.5**	**784**	**11.4**	**6.3**	**63**	**678**
BYR3	177	30	5.9	1	1	1	1.65	1.6	7	5	86.8	43.8	6570	3211	7.3	10.6	461.5	450	5.6	7.4	93	303

Appendix XXIII (2). Northumbria Site Database. Source: Environment Agency.

Alkalinity		Ssolids	Sulphate	Chloride	Bedrock %	Boulders %	Cobbles %	Pebbles %	Gravel %	Sand %	Silt %	Ochre %	Width (m)	Depth	Altitude	Slope	DistSource	Flow	Colour	Substrate	Siphlonuriidae
26	**ARN1**	**3**	**9.1**	**33.9**	**0**	**27.5**	**32.5**	**0**	**0**	**25**	**15**	**0**	**1.2**	**19**	**270**	**32**	**2.5**	**1.25**	**1**	**-2.95**	**0**
54	ARN2	6	18.7	28.3	0	0	20	0	0	20	60	100	1.25	31.5	260	20	3	1.5	2	3.65	0
40	**ARN3**	**2**	**19.8**	**28**	**0**	**0**	**10**	**0**	**0**	**40**	**50**	**0**	**2**	**22.5**	**260**	**20**	**3.7**	**1.85**	**1**	**4.025**	**1**
39	ARN4	2	26.4	27.7	0	35	10	0	0	15	40	0	1	14	260	20	4	2	2	0.0125	0
10	**WOO1**	**2**	**12.2**	**11**	**0**	**40**	**30**	**0**	**0**	**25**	**5**	**0**	**1**	**39**	**340**	**34**	**2.5**	**1.25**	**1.5**	**-4.525**	**2**
32	WOO2	3	15.9	11	0	35	40	0	0	17.5	7.5	50	1.375	22.5	340	34	2.7	1.35	1.5	-4.8625	2
25	**WOO3**	**3**	**15.5**	**19.4**	**0**	**17.5**	**27.5**	**0**	**0**	**40**	**15**	**50**	**1.25**	**22.5**	**310**	**30**	**3.7**	**1.85**	**2.5**	**-1.4875**	**0**
24	WOO4	6	15.5	19.8	0	2.5	27.5	0	0	47.5	22.5	50	0.875	28	310	30	4	2	3	0.425	0
110	**HAG1**	**2**	**63**	**48.9**	**0**	**40**	**40**	**0**	**0**	**20**	**10**	**0**	**0.975**	**10.5**	**135**	**57**	**0.65**	**0.325**	**1**	**-5**	**0**
287	HAG2	27	782.3	58.9	0	30	25	0	0	27.5	17.5	50	1.375	17.5	135	57	0.75	0.375	1	-2.3125	0
150	**HAG3**	**20**	**350.7**	**52.9**	**0**	**25**	**52.5**	**0**	**0**	**2.5**	**20**	**40**	**1.625**	**13**	**130**	**12.5**	**1.15**	**0.575**	**1.5**	**-4.35625**	**1**
69	HAG4	2	73.1	55	0	12.5	52.5	0	0	10	25	0	1.375	15	125	20	1.4	0.7	1.5	-2.8375	0
62	**BLA1**	**2**	**14**	**9**	**0**	**52.5**	**27.5**	**0**	**0**	**15**	**5**	**0**	**2.125**	**8.5**	**396**	**109**	**0.7**	**0.35**	**1**	**-5.5**	**1**
52	BLA2	36	155.8	7.3	5	60	10	0	0	5	5	75	2	10	381	75	0.9	0.45	2.5	-4.925	1
50	**BLA3**	**8**	**90**	**8.6**	**0**	**72.5**	**20**	**0**	**0**	**5**	**2.5**	**0**	**2.25**	**9.5**	**350**	**78**	**1.3**	**0.65**	**2**	**-6.86875**	**1**
46	BLA4	4	52.9	14.2	0	60	29	0	0	6	5	0	2	11.5	345	25	1.5	0.75	2	-6.3775	0
140	**HAL1**	**2**	**36.6**	**53.9**	**0**	**2.5**	**12.5**	**0**	**0**	**45**	**40**	**0**	**0.75**	**9.5**	**150**	**73**	**1.3**	**0.65**	**1**	**2.9375**	**0**
290	HAL2	3	95.4	24.6	0	1	12.5	0	0	22.5	64	100	0.75	15	140	200	1.35	0.675	1	4.52375	0
136	**HAL3**	**7**	**78**	**50.3**	**0**	**5**	**27.5**	**0**	**0**	**22.5**	**45**	**5**	**1.375**	**11**	**120**	**40**	**1.85**	**0.925**	**1**	**1.53125**	**0**
165	HAL4	3	61.5	54.6	0	0	5	0	0	45	50	0	1	23	120	40	2.05	1.025	1	4.5125	0
99	**NET1**	**3**	**33.1**	**30.7**	**0**	**70**	**25**	**0**	**0**	**5**	**0**	**0**	**5.5**	**26**	**106**	**17**	**7.3**	**3.65**	**1**	**-7.2625**	**0**
106	NET2	7	175.3	22.9	0	60	2	0	0	10	10	15	6	10	100	30	7.5	3.75	1	-3.805	0
96	**NET3**	**3**	**38.9**	**30.3**	**0**	**67.5**	**20**	**0**	**0**	**7.5**	**5**	**0**	**6.75**	**17.5**	**100**	**30**	**7.75**	**3.875**	**1**	**-6.23125**	**0**
65	CHI1	3	18.8	34.8	0	70	25	0	0	5	0	0	1.875	14.5	105	30	3.3	1.65	2.5	-7.2625	0
10	**CHI2**	**5**	**538**	**20.5**	**0**	**72.5**	**20**	**0**	**0**	**6**	**1.5**	**100**	**3**	**7**	**100**	**30**	**3.4**	**1.7**	**2**	**-6.92875**	**0**
59	CHI3	6	42.8	35.9	0	50	35	0	0	10	5	100	3.5	9	100	50	3.6	1.8	3	-5.9875	0
64	**CHI4**	**5**	**43.6**	**31.5**	**0**	**60**	**27.5**	**0**	**0**	**9**	**3.5**	**5**	**2.25**	**13.5**	**100**	**50**	**3.9**	**1.95**	**3**	**-6.32125**	**0**
181	FOR1	7	131.3	58.8	0	0	0	0	0	5	95	50	1.125	5	49	61	0.75	0.375	1	7.7	0
240	**FOR2**	**13**	**457.7**	**25**	**0**	**0**	**7.5**	**0**	**0**	**27.5**	**65**	**100**	**1.125**	**10.5**	**48**	**20**	**0.8**	**0.4**	**2**	**5.16875**	**0**
180	FOR3	25	226.7	39.9	0	0	0	0	0	2.5	97.5	100	1	11	47	1	1.9	0.95	2.5	7.85	0
213	**ALL1**	**5**	**195.7**	**43.9**	**0**	**0**	**0**	**0**	**0**	**20**	**80**	**0**	**1.5**	**27**	**40**	**13.3**	**1.5**	**0.75**	**2**	**6.8**	**0**
119	ALL2	14	211.3	47.3	0	0	5	1	1	15	80	95	1	12	40	50	3.5	1.75	2	6.2475	0
219	**ALL3**	**8**	**29.1**	**34.8**	**0**	**2.5**	**7.5**	**0**	**0**	**5**	**85**	**100**	**2**	**22**	**40**	**50**	**3.7**	**1.85**	**2.5**	**6.125**	**0**
195	ALL4	7	200.7	44.6	0	5	40	0	0	20	35	60	2	16	40	50	4	2	1.5	-0.2875	0
229	**ALL5**	**7**	**212.7**	**59.6**	**0**	**3.5**	**16.5**	**0**	**0**	**17.5**	**62.5**	**3.75**	**2.75**	**17.5**	**40**	**50**	**4.3**	**2.15**	**1.5**	**3.8**	**0**
107	BYR1	3	43.9	22.3	0	75	20	0	0	4	1	0	4.5	30	122	18	4.3	2.15	1	-7.2025	0
216	**BYR2**	**22**	**135.3**	**12.5**	**0**	**50**	**15**	**0**	**0**	**4**	**1**	**50**	**5.5**	**20**	**122**	**18**	**4.7**	**2.35**	**1**	**-4.8775**	**0**
82	BYR3	3	42.9	19.4	0	50	40	0	0	7	3	80	5	25	122	18	5	2.5	1	-6.595	0

Appendix XXIII (3). Northumbria Site Database. Source: Environment Agency.

Heptageniidae		Leptophlebiidae	Ephemerellidae	Ephemeridae	Taeniopterygidae	Leuctridae	Capniidae	Perlodidae	Perlidae	Chloroperlidae	Beraeidae	Odontoceridae	Leptoceridae	Goeridae	Lepidostomatidae	Sericostomatidae	Psychomyiidae
0	ARN1	4	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0
0	ARN2	3	0	0	0	3	0	0	0	0	3	0	0	0	0	0	0
0	ARN3	0	0	0	0	2	0	0	0	0	1	2	0	0	0	0	0
0	ARN4	0	0	0	0	2	0	1	0	0	0	0	0	0	0	1	0
3	WOO1	1	1	0	0	4	0	1	0	1	1	3	0	1	0	2	0
0	WOO2	0	0	0	0	3	0	0	0	1	2	1	0	0	0	0	0
0	WOO3	0	2	0	0	1	0	0	0	0	1	0	0	0	0	0	0
0	WOO4	0	2	0	0	0	0	0	0	1	1	1	0	0	0	2	0
3	HAG1	2	0	0	0	3	1	1	0	1	0	0	0	0	0	0	0
0	HAG2	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
2	HAG3	1	0	0	0	3	1	1	0	0	0	0	0	0	0	0	0
1	HAG4	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0
3	BLA1	1	0	0	0	3	0	3	0	1	1	1	0	2	0	0	0
3	BLA2	0	0	0	0	2	0	2	0	2	0	0	0	0	0	0	0
2	BLA3	1	0	0	0	3	0	3	0	2	0	0	0	0	0	0	0
2	BLA4	0	0	0	0	4	1	2	0	2	0	0	0	0	0	1	0
0	HAL1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
0	HAL2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
3	HAL3	1	1	1	0	3	1	1	0	1	0	0	0	1	0	3	0
1	HAL4	0	1	1	0	2	1	0	0	0	0	0	0	0	0	3	2
4	NET1	0	4	3	1	3	1	2	2	0	0	2	2	1	1	3	0
0	NET2	0	2	1	0	2	0	0	0	0	0	1	1	0	0	1	0
3	NET3	2	0	1	0	2	0	1	0	0	0	1	0	0	0	1	0
2	CHI1	1	0	0	0	0	2	2	0	0	0	0	0	0	0	0	0
1	CHI2	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0
0	CHI3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	CHI4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	FOR1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	FOR2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	FOR3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	ALL1	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0
0	ALL2	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
0	ALL3	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
0	ALL4	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0
0	ALL5	1	0	0	0	2	0	0	0	0	0	1	0	0	0	2	0
2	BYR1	1	0	0	1	0	0	1	0	0	0	0	1	0	3	2	0
1	BYR2	0	0	0	1	1	0	1	0	0	0	1	1	0	1	2	1
1	BYR3	1	0	0	1	0	0	0	0	0	1	0	1	0	1	1	1

Appendix XXIII (4). Northumbria Site Database. Source: Environment Agency.

Philopotamidae		Caenidae	Nemouridae	Rhyacophilidae	Polycentropodidae	Limnephilidae	Ancylidae	Hydroptilidae	Gammaridae	Coenagriidae	Gerridae	Notonectidae	Corixidae	Haliplidae	Dytiscidae	Gyrinidae	Hydrophilidae	Scirtidae
0	**ARN1**	0	2	0	0	3	0	1	3	3	0	0	1	0	3	0	3	0
0	ARN2	0	3	0	3	2	0	1	3	1	1	0	0	0	2	0	2	0
0	**ARN3**	0	0	3	2	3	0	0	3	0	0	0	0	1	3	0	2	1
0	ARN4	0	1	3	1	3	0	0	3	0	0	0	1	0	3	0	3	1
0	**WOO1**	0	3	1	2	2	0	0	0	0	0	0	0	0	2	0	2	3
0	WOO2	0	2	0	2	3	0	0	0	0	0	0	0	0	2	0	1	2
0	**WOO3**	0	0	0	0	3	0	0	3	0	0	0	0	0	1	0	1	0
0	WOO4	0	0	1	0	3	0	0	3	0	0	0	0	0	1	1	2	0
3	**HAG1**	0	3	3	2	2	2	0	3	0	0	0	0	0	0	0	3	3
0	HAG2	0	1	0	1	1	0	0	3	0	0	0	0	0	0	2	2	3
0	**HAG3**	0	4	3	2	3	3	1	4	0	0	0	0	0	1	2	3	2
0	HAG4	0	3	2	1	2	2	0	3	0	0	0	0	0	2	0	2	1
1	**BLA1**	0	2	2	3	3	3	0	4	0	0	0	0	0	0	1	3	2
1	BLA2	0	1	2	2	2	1	1	3	0	0	0	0	0	0	2	3	0
0	**BLA3**	0	2	2	2	2	1	1	2	0	0	0	0	0	0	2	2	1
0	BLA4	0	4	3	1	3	0	1	0	0	0	0	0	0	0	2	3	2
0	**HAL1**	0	4	2	3	3	1	0	3	0	0	0	0	1	4	0	3	2
0	HAL2	0	3	0	1	2	0	0	2	0	0	0	0	0	3	0	1	1
0	**HAL3**	0	2	3	1	3	0	1	4	0	0	0	0	0	2	0	2	3
0	HAL4	0	2	2	1	3	1	0	4	0	0	0	0	1	3	0	1	2
0	**NET1**	1	3	3	1	3	3	1	3	0	0	0	0	0	2	1	0	0
0	NET2	0	1	1	0	0	0	0	2	0	0	0	0	1	1	0	1	0
0	**NET3**	1	1	2	0	0	1	1	2	0	0	0	0	0	0	1	1	0
0	CHI1	0	1	0	3	3	3	0	3	0	0	0	0	1	3	0	3	1
0	**CHI2**	0	0	0	1	1	0	0	2	0	0	0	0	0	2	0	0	1
0	CHI3	0	0	0	1	1	0	0	1	0	0	0	0	0	2	0	2	0
0	**CHI4**	0	2	0	2	2	0	0	1	0	0	0	0	0	2	0	1	1
0	FOR1	0	0	1	0	2	0	0	4	0	0	1	3	0	2	0	1	3
0	**FOR2**	0	1	0	0	2	0	0	4	0	0	0	1	0	0	0	1	3
0	FOR3	0	1	0	0	2	0	0	4	0	0	0	0	0	0	0	0	1
0	**ALL1**	1	3	1	0	4	0	1	3	0	0	0	3	2	3	0	3	2
0	ALL2	0	0	2	0	3	0	0	2	0	0	0	0	0	2	0	2	2
0	**ALL3**	0	1	0	0	2	0	0	2	0	0	0	0	0	2	0	1	0
0	ALL4	0	1	1	1	1	0	0	4	0	0	0	0	0	1	0	1	1
0	**ALL5**	0	3	2	1	2	0	0	4	0	0	0	0	0	1	0	1	2
0	BYR1	1	1	1	0	1	2	0	3	0	0	0	0	0	1	0	0	0
0	**BYR2**	0	2	1	0	1	1	0	3	0	0	0	0	1	1	0	0	0
0	BYR3	1	2	0	1	1	1	0	2	0	0	0	0	0	1	0	1	0

Appendix XXIII (5). Northumbria Site Database. Source: Environment Agency.

Elmidae		Hydropsychidae	Tipulidae	Simuliidae	Planariidae	Baetidae	Sialidae	Hydrobiidae	Lymnaeidae	Physidae	Planorbidae	Sphaeriidae	Glossiphoniidae	Erpobdellidae	Asellidae	Chironomidae	Oligochaeta
3	**ARN1**	**0**	**3**	**3**	**0**	**2**	**3**	**0**	**2**	**0**	**3**	**3**	**3**	**0**	**0**	**3**	**3**
1	ARN2	0	3	3	0	0	3	0	2	0	0	3	2	0	0	3	3
2	**ARN3**	**0**	**4**	**3**	**0**	**1**	**2**	**0**	**3**	**0**	**0**	**3**	**3**	**0**	**0**	**4**	**4**
2	ARN4	1	3	4	0	3	1	0	1	0	0	3	1	0	0	3	3
3	**WOO1**	**0**	**3**	**4**	**0**	**4**	**2**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**3**	**3**
3	WOO2	0	3	1	0	2	2	0	0	0	0	2	0	0	0	3	3
3	**WOO3**	**0**	**3**	**0**	**1**	**1**	**0**	**0**	**2**	**0**	**0**	**2**	**0**	**0**	**0**	**3**	**3**
3	WOO4	0	3	2	1	2	0	3	0	0	0	3	0	0	0	3	3
3	**HAG1**	**2**	**3**	**1**	**0**	**1**	**1**	**4**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**3**	**3**
0	HAG2	1	3	0	0	0	1	3	0	0	0	0	0	0	0	3	3
3	**HAG3**	**1**	**3**	**2**	**0**	**2**	**2**	**4**	**2**	**1**	**0**	**2**	**0**	**0**	**0**	**4**	**4**
1	HAG4	2	2	0	0	2	1	0	3	0	0	1	0	0	0	2	2
3	**BLA1**	**0**	**2**	**1**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**2**	**2**
3	BLA2	1	3	1	1	3	1	0	2	0	0	1	0	0	0	3	1
3	**BLA3**	**1**	**3**	**2**	**0**	**2**	**2**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**1**	**3**	**2**
2	BLA4	2	3	3	3	3	1	0	1	0	0	2	0	0	0	3	3
2	**HAL1**	**0**	**4**	**4**	**0**	**2**	**1**	**3**	**3**	**0**	**0**	**1**	**3**	**0**	**0**	**3**	**3**
0	HAL2	0	3	0	0	2	0	2	2	0	0	3	2	0	0	3	2
2	**HAL3**	**1**	**3**	**4**	**2**	**3**	**1**	**4**	**0**	**0**	**0**	**2**	**2**	**0**	**0**	**3**	**2**
3	HAL4	0	3	3	0	3	1	4	0	0	0	2	2	0	0	3	2
3	**NET1**	**3**	**3**	**2**	**1**	**3**	**0**	**3**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**3**	**3**
2	NET2	2	2	1	0	2	0	2	1	0	0	0	1	0	0	2	2
2	**NET3**	**3**	**2**	**1**	**1**	**2**	**0**	**2**	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**1**	**2**
3	CHI1	0	3	0	0	3	0	0	3	0	0	2	2	0	0	2	3
1	**CHI2**	**0**	**1**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**2**
0	CHI3	0	1	0	0	0	3	0	0	0	0	0	0	0	0	3	3
2	**CHI4**	**0**	**2**	**1**	**0**	**0**	**3**	**0**	**2**	**0**	**0**	**2**	**1**	**0**	**0**	**2**	**2**
0	FOR1	0	3	3	1	3	0	4	3	0	0	3	2	2	3	3	3
1	**FOR2**	**0**	**3**	**0**	**0**	**3**	**0**	**0**	**1**	**0**	**0**	**3**	**1**	**0**	**2**	**2**	**2**
2	FOR3	1	3	0	0	2	0	2	1	0	0	3	0	1	0	3	3
1	**ALL1**	**0**	**3**	**2**	**0**	**0**	**2**	**4**	**4**	**1**	**0**	**3**	**1**	**2**	**1**	**4**	**3**
3	ALL2	0	3	0	0	0	0	3	3	0	0	4	0	0	0	3	1
0	**ALL3**	**0**	**2**	**0**	**0**	**0**	**3**	**0**	**3**	**0**	**0**	**4**	**1**	**0**	**0**	**3**	**1**
0	ALL4	0	2	0	0	2	0	3	2	0	0	3	0	0	0	3	3
0	**ALL5**	**0**	**2**	**3**	**0**	**3**	**1**	**3**	**2**	**0**	**0**	**3**	**0**	**0**	**0**	**3**	**2**
2	BYR1	3	3	2	0	2	1	3	3	0	1	0	1	3	0	2	2
2	**BYR2**	**1**	**2**	**1**	**0**	**2**	**1**	**2**	**3**	**0**	**1**	**2**	**1**	**3**	**0**	**1**	**2**
2	BYR3	1	1	1	0	1	2	2	2	0	1	2	2	2	0	2	2

Appendix XXIV. Site Descriptions for Wales Data (Source: Environment Agency)

Site Code	Grid Reference	Catchment / Receiving Water	EA Impact Rank
IU 1D	SO27060312 SO27020308	Afon Llwyd. Aber Sychan	24
2U 2D	SO27620183 SO27640178	Afon Llwyd	9
3U 3D	SO28610057 SO28680056	Afon Llywd	18
4U 4D	ST21219762 ST21229744	Ebbw	16
5U 5D	ST17629737 ST17589733	Ebbw. Sirhowy. Adit	17
6U 6D	ST18039565 ST18029559	Ebbw. Sirhowy	14
7U 7D	ST15509632 ST15449626	Rhymney at Fleur-de-Lis	15
8U 8D	SN97120283 SN97210280	Taff / Cynon.Dare Tributary	29
9U 9D	SO23450956 SO24450896	Afon Llwyd	6
10U 10D	ST05349534 ST05379529	Taff. River Clydach	20
11U 11D	ST04189454 ST04220449	Taff. Yffrwd (Tributary of Clydach)	10
12U 12D	ST05919063 ST05959059	Taff. River Rhondda	30
13U 13D	SS89398766 SS89358756	Ogmore. Tributary of River Llynfi	19
14U 14D	SS85588459 SS85578456	Afon. Cynffig.Nant Craig yr Aber	2
15U 15D	SN88960073 SN88830062	Afan/Pelenna.Afon Corrwg	11
16U 16D	SN88150080 SN88130074	Afan/Pelenna. Afon Corrwg Fechan	13
17U 17D	SS89219735 SS89179724	Afan/Pelenna. Nant Gwynfi	3
18U 18D	SS87199637 SS87199629	Afan. R.Afon	31

Appendix XXIV Continued. Site descriptions for Wales Data (Source: Environment Agency)

Site Code	Grid Reference	Receiving Water	EA Impact Rank
19U 19D	SS86389765 SS86389757	Afan. Afon Corrwg	25
20U 20D	SS81739241 SS81689218	Afan/Pelenna.Ffrwd Wyllt	32
21U 21D	SS78778983 SS78698975	Afan/Pelenna.Ffrwd Wyllt	26
22U 22D	SS81599755 SS81599749	Afan/Pelenna. Nant Blaen Pelenna	21
23U 23D	SS81619728 SS81629717	Afan/Pelenna.Nant Blaen Pelenna	4
24U 24D	SS79929732 SS79909614	Afan/Pelenna.Tributary of Cwm Gwenffrvd	5
25U 25D	SS84429736 SS84779731	Afan/Pelenna.Tributary of Nant Cregan	27
26U 26D	SS87899582 SS87719580	Afan/Pelenna. Nant Y Fedw	28
27U 27D	SN63280963 SN63230966	Loughor. R. Cathan	12
28U 28D	SN57000200 SN57030197	Loughor. Morlais	1
29U 29D	SS58739434 SS59319369	Gower. Tributary of Clyne	8
30U 30D	SN70061021 SN70201118	Tawe. Nant Melyn	23
31U 31D	SN70020536 SN70010530	Tawe.Tributary of Lower Clydach River	7
32U 32D	SJ30656570 SJ30686572	Dee. Broughton Brook	22

Appendix XXV. Welsh sites. Mean values of environmental variables (± 2 SE) for each TWINSPAN group at Level 4. Standard errors for percentage values were calculated after arcsine transformation, and given following back-transformation. (t)/(f)=total/filtered mg l^{-1}. Original Data Source: Environment Agency.

TWINSPAN Group (in order of increasing ASPT)

	30	31	8	29	18	19	23	28	13	10	25	24	22
GQA Class	E	E	D	E	B	B	A	C	A	A	A	A	A
ASPT	3.5	3.8	4.63	5.23	5.3	5.35	5.5	5.62	5.8	6	6.33	6.44	6.48
Discharge (units)	.7	0	4	0	3.4	3.3	0	0.5	0	1.25	0.06	0.33	1.6
Alkalinity (mg l^{-1}) /SE	66.6 /57.0	11.9 /-	64.5 /23.6	24.5 /16.2	103 /19.7	99.1 /42.8	21 /-	18.3 /9.8	94 /-	44.4 /24.4	28.6 /8.6	22.3 /14.8	26.6 /12.8
Slope (m km^{-1}) (SE)	25.1 /15.1	36 /-	11 /14.0	37 /8.66	7.97 /1.2	11.8 /11.6	0.8 /-	32.3 /5.4	10 /-	22.3 /20.4	52.5 /28.2	66 /59.2	27.6 /18.2
Width (m) (SE)	2.8 /2.8	2.4 /-	9.98 /9.2	4.72 /2.4	8.18 /1.2	7.25 /2.2	13 /-	4.3 /0.6	4 /-	4.63 /1.9	3.58 /1.8	1.4 /0.84	5.14 /1.10
Conductivity (mS cm^{-1}) (SE)	337 /141	687 /-	250 /320	340 /312	599 /326	393 /125	74 /-	119 /49.6	230 /-	210 /82	129 /25.8	277 /272	144 /25
Sulphate (t) (mg l^{-1}) (SE)	71.2 /35	191 /-	52 /23.6	90.5 /94.6	190 /146	96 /39.6	5.4 /-	24.8 /6.88	6.9 /-	40 /23	28.4 /9.44	113 /172	20.7 /7.6
Depth (cm) (SE)	9.7 /1.44	11 /-	14.7 /8.8	17.8 /6.4	23.8 /4.8	27.7 /7.2	6 /-	17.5 /1.4	5 /-	17.2 /7.68	14.9 /3.24	9.7 /1.76	18 /5.9
Field pH	7.37	5.81	8.07	7.28	8.2	7.6	6.27	7.45	6.9	7.8	7.6	7.55	7.7
Fe (t) (SE)	2.8 /4.4	21.2 /-	0.5 /0.14	0.38 /5.2	3.26 /5.2	0.68 /0.66	0.35 /-	0.36 /0.30	0.17 /-	0.57 /0.48	0.24 /0.12	0.78 /1.28	0.15 /0.1
pH	7.2	5.2	7.91	7.2	7.6	7.8	7	6.9	7.8	7.22	7.4	7.2	7.04
Distance from Source (km) (SE)	11.4 /18.7	12.0 /-	15.8 /28.6	2.3 /0.50	17.2 /3.16	12.6 /6.16	3 /-	2.7 /0.42	1 /-	7.13 /4.56	2.9/ 1.24	0.9 /0.72	3.9 /0.26
Al (t) (SE)	0.19 /0.2	0.04 /-	0.04 /0.00	0.15 /0.14	0.05 /0.02	0.05 /0.02	0.01 /-	0.20 /0.12	0.04 /-	0.06 /0.04	0.09 /0.04	0.11 /0.10	0.06 /0.04
Altitude (m) (SE)	190 /145	418 /-	71/ 4.00	164/ 0.50	124/ 28.2	137/ 26.2	160 /-	193 /61	74 /-	81.3 /67.8	190 /25.8	173 /189	93 /57.4
No. Taxa (SE)	3 /1.34	6 /-	10 /1.76	6 /2.18	14.6 /2.76	15 /3.16	20 /-	8.83 /0.14	17 /-	18 /0.96	17 /2.02	18 /7.4	19 /2.02

Appendix XXV continued.

TWINSPAN Group (in order of increasing ASPT)

	30	31	8	29	18	19	23	28	13	10	25	24	22
BMWP	13	21	48	30	79.5	79	111	51	99	110	107	114	122
Temperature (°C) (SE)	7.2 /0.70	5.2 /-	15.1 /3.2	7.2 /0.28	13.0 /1.2	12.4 /0.8	7.0 /-	6.9 /1.6	13.7 /-	13.3 /1.4	7.4 /0.6	7.2 /2.8	13.0 /0.70

Appendix XXVI. Proportional representation of taxa for each TWINSPAN site-group for Welsh sites (mean percentage per site ± 2 SE). Standard errors for percentage values were calculated after arcsine transformation, and shown following back-transformation. Original Data Source: Environment Agency.

TWINSPAN Gp Taxonomic Gp	30	31	8	29	18	19	23	28
Ephemeroptera (SE)	0 (0)	8.4 (-)	21.9 (2.99)	20.4 (4.55)	23.0 (0.16)	21.6 (0.2)	5.0 (-)	13.5 (0.17)
Plecoptera (SE)	16.7 (14.4)	0 (-)	4.1 (4.16)	19.0 (3.83)	7.8 (0.5)	2.0 (1.56)	20.0 (-)	24.0 (0.79)
Trichoptera (SE)	0 (0)	10 (-)	9.8 (0.08)	15.6 (0.39)	14.8 (0.9)	15.6 (0.39)	21.4 (-)	17.2 (3.01)
Mollusca (SE)	0 (0)	0 (-)	15.7 (0.5)	0.3 (1.14)	4.0 (0.9)	10.5 (2.17)	10.0 (-)	0 (0)
Crustacea (SE)	0 (0)	0 (-)	9.7 (0.08)	0 (0)	10.1 0.6	8.3 (1.7)	5.0 (-)	0 (0)
Coleoptera (SE)	0 (0)	26.7 (-)	4.4 (4.45)	0 (0)	3.1 (1.04)	2.3 (1.96)	10.0 (-)	1.5 (2.49)
Hirudinea (SE)	0 (0)	0 (-)	0 (0)	0 (0)	6.4 (0.51)	1.6 (1.31)	0 (-)	0 (0)
Diptera (SE)	50.0 (0)	36.7 (-)	18.7 (1.98)	16.2 (3.02)	11.2 (0.18)	16.5 (0.45)	15.0 (-)	24.8 (0.59)
Oligochaeta (SE)	32.9 (3.02)	18.4 (-)	9.8 (0.07)	8.1 (3.57)	7.7 (0.2)	6.7 (0.04)	5.0 (-)	12.0 (0.13)

Appendix XXVI. Continued.

TWINSPAN Gp Taxonomic Gp	13	10	25	24	22
Ephemeroptera	11.8	15.9	17.3	21.0	20.6
(SE)	(-)	(0.12)	(0.26)	(0.06)	(0.14)
Plecoptera	11.8	6.4	20.16	12.3	7.5
(SE)	(-)	(0.24)	(0.12)	(0.94)	(0.32)
Trichoptera	17.7	28.9	23.6	21.0	32.0
(SE)	(-)	(0.39)	(0.2)	(1.58)	(0.3)
Mollusca	11.8	6.9	1.1	0.45	1.2
(SE)	(-)	(0.33)	(0.69)	(1.8)	(1.56)
Crustacea	5.9	5.6	0.03	0	3.3
(SE)	(-)	(0.01)	(0.12)	(0)	(0.7)
Coleoptera	5.9	6.9	6.13	17.4	3.5
(SE)	(-)	(0.33)	(0.4)	(2.46)	(0.75)
Hirudinea	0	1.3	0	0	0
(SE)	(-)	(1.79)	(0)	(0)	(0)
Diptera	17.7	13.06	17.8	15.6	16.3
(SE)	(-)	(0.44)	(0.08)	(0.41)	(0.06)
Oligochaeta	5.9	5.6	14.5	6.0	5.5
(SE)	(-)	(0.01)	(0.03)	(0.23)	(0.02)

Appendix XXVII. Wales data. Classification of family and site groups by TWINSPAN. The number codes show the percentage of occurrence of each taxa between the sites in each group. Abundance Codes: 1=1-19%; 2=20-39%; 3=40-59%; 4=60-79%; 5=80-99%; 6=100%. (Original Data Source: Environment Agency).

FAMILY Group SITE Group

		8	18	19	10	22	23	24	25	13	28	29	30	31
15	Curculionidae			1		4			1					
	Goeridae				2	5	6		1					
	Gerridae						6							
	Odontoceridae					2								
29	Gammaridae		5	5	6	5	6		1	6				
	Hydroptilidae		3	1	1									
	Hygrobiidae		1									1		
	Asellidae	6	5	3										
	Erpobdellidae		5	2	2									
	Glossiphoniidae		1	1										
28	Lymnaeidae	4		4					1					
	Hydrobiidae	6	4	3	4	3	6		1	6				
	Ancylidae		1	4				2	1					
	Caenidae	4	5	1		5		2	2					
13	Planariidae		2	2	6	4	6		2	6				
	Mesovelidae					2	6			6				
12	Hydropsychidae		5	3	6	6	6	2	5					
	Ephemerelliae	4	6	6	6	6		6	5			2		
	Limnephilidae		2	2	3	4	6	4	2					
	Heptageniidae		4	6	4	6		4	4		1			
23	Cordulegasteridae				2	3	6	4	2					
	Sphaeriidae			1	2	2	6		2					
	Planorbiidae				2				1					
	Sericostomatidae	2			6	5		4	2	6	1			
	Lepidostomatidae				3	2			1		1			
22	Tipulidae	4		5	3	6	6	6	5	6	2	2	2	
	Dytiscidae		2	2	3			4	1	6				6
	Elminthidae	2	4	3	4	6	6	6	4		3			
	Rhyacophilidae	4	5	6	6	6	6	6	6	6	5			
	Baetidae	6	5	6	6	6	6	6	6		5	5		3
10	Oligochaeta	6	6	6	6	6	6	6	6	6	6	4	6	6
	Simuliidae	2	4	4	6	6	6	4	5	6	6	1	2	6
	Chironomidae	6	6	6	6	6	6	6	6	6	6	5	6	6
	Leuctridae	2	5	3	6	6	6	6	6	6	6	4	4	
9	Hydrophilidae			1			6	4	1					3
	Polycentropidae		2	3	3	6		6	6	6	4	4		3
	Chloroperlidae				2	3	6	2	4	6	3			
17	Nemouridae	2			2			2	5	6	4	4		
16	Philopotamidae					3			3					
	Perlodidae		2					4	3					
	Helodidae	2							2					
	Gyrinidae							2						
	Dryopidae							4	1					
	Ephemeridae							4						
	Perlidae								1					

Appendix XXVIII (1). Wales 1993 Single Season Site Database. Source: Environment Agency.

	NO. TAXA	BMWP	ASPT	Width	Depth	Bou/Cobb %	Pebbles/Gravel %	Sand %	Silt/Clay %	Altitude	Dist Source	Discharge	Alkalinity	Slope	Flow	Clarity
1U	16	91	5.6	8	30	80	10	10	0	170	10	3	56	9	2	1
1D1	19	116	6.11	7	28	85	10	5	0	170	10	3	76	9	2	1
2U	20	114	5.7	6	35	95	4	1	0	130	12	3	72	8.3	2	1
2D1	12	63	5.25	8	32	90	8	2	0	130	12	3	79	8.3	2	1
3U	21	111	5.29	10.2	31	95	4	1	0	120	13	3	102.5	10	2	1
3D1	17	96	5.6	9.5	30	95	4	1	0	120	13	3	111	10	2	1
4U	13	65	5	8	34	95	3	2	0	110	21	5	89	5.9	1.5	1
4D1	10	49	4.9	7.3	30	95	2	3	0	110	21	5	109	5.9	1.5	1
5U	19	106	5.6	8	20	75	15	10	0	150	21	4	109	5.7	2	1
5D1	15	81	5.4	7.7	18	80	15	5	0	150	21	4	79	5.7	1	1
6U	15	83	5.5	10	25	85	10	5	0	130	23	4	81	8	2	1
6D1	12	61	5	9	23	85	10	5	0	130	23	4	115	8	2	1
7U	16	87	5.4	9.6	22	85	10	5	0	125	18.5	4	112	10	2	0
7D1	11	55	5	9.8	27	85	10	5	0	125	18.5	4	135	10	1	1
8U	22	146	6.6	2.7	15	95	5	0	0	230	1.75	0	38	65	2	1
8D1	19	115	6	1.9	17	50	50	0	0	230	1.75	0	45	65	2	1
9U	25	166	6.72	2	10	30	65	5	0	362	1.5	1	37.05	40	2	1
9D1	2	3	1.5	2	8	60	35	5	0	320	30	1	72	32.3	2	1
10U	17	113	6.6	4.3	13	85	14	1	0	160	5.5	0	45.02	16	3	1
10D1	12	70	5.8	4.1	10	80	9	1	0	160	5.5	0	40.1	16	3	1
11U	14	89	6.3	1.3	5	85	14	1	0	232	2	0	7.7	30	3	1
11D1	6	34	5.6	1.4	8	85	10	5	0	229	2	0	28.35	30	3	1
12U	12	60	5	14.1	18	95	4	1	0	69	23	6	77	4	3	1
12D1	9	42	4.6	15	20	95	4	1	0	69	23	6	75.5	4	3	1
13U	22	136	6.2	2.1	12	40	55	5	0	85	1	0	18.5	33	2	1
13D1	13	88	6.7	1.6	11	35	55	10	0	85	1	0	14.5	33	2	1
14U	17	100	5.8	2.9	16	70	25	5	0	80	3	1	16.5	25	2	1
14D1	7	39	5.6	3.2	20	75	20	5	0	75	3	1	43	25	2	1
15U	10	56	5.6	7.5	20	90	9	1	0	31	3	0	25.8	50	1	1
15D1	5	27	5.4	6.9	25	90	8	2	0	31	3	0	24.95	50	1	1
16U	6	35	5.8	4.2	24	90	9	1	0	310	2	0	2.8	33	1	1
16D1	2	11	5.5	7.4	22	90	9	1	0	310	2	0	6.3	33	1	1
17U	11	73	6.6	5.2	18	95	4	1	0	290	2	0	16.9	40	1	1
17D1	8	41	5.1	4.6	18	95	4	1	0	280	2	0	20.5	40	1	1
18U	15	98	6.5	6.5	28	80	10	10	0	190	5.5	4	23.4	40	2	1
18D1	16	92	5.75	8	29	80	10	10	0	190	5.5	4	27.1	40	2	1
19U	15	98	6.5	11.1	28	80	15	5	0	180	7	4	35.3	16.6	1	1
19D1	15	98	6.5	10.7	25	80	15	5	0	180	7	4	62.4	16.6	1	1
20U	19	127	6.7	0.6	8	40	55	5	0	124	0.4	0	18.4	36	2	1
20D1	19	125	6.6	3.3	10	30	65	5	0	124	0.4	0	29.3	36	2	1
21U	20	136	6.8	5.3	16	60	35	5	0	40	5	2	9.7	6	2	1
21D1	21	127	6	5.9	20	80	15	5	0	40	5	2	21.4	6	2	1
22U	11	63	5.7	4.3	13	90	8	2	0	180	3	1	13.6	33	1	1
22D1	7	34	4.8	4.6	18	90	9	1	0	180	3	1	12.27	33	1	1
23U	10	58	5.8	3.4	15	80	15	5	0	180	3.25	1	7.6	33	2	1
23D1	4	18	4.5	4.2	16	80	15	5	0	180	3.25	1	14.8	33	2	1
24U	10	58	5.8	4.8	16	75	20	5	0	155	3	0	9.3	23	2	1

Appendix XXVIII (2). Wales 1993 Single Season Site Database. Source: Environment Agency.

	NO. TAXA	BMWP	ASPT	Width	Depth	Bou/Cobb %	Pebbles/Gravel %	Sand %	Silt/Clay %	Altitude	Dist Source	Discharge	Alkalinity	Slope	Flow	Clarity
24D1	**5**	**20**	**4**	**4.3**	**14**	**80**	**15**	**5**	**0**	**135**	**3**	**0**	**5.14**	**23**	**2**	**1**
25U	23	145	6.2	2.2	16	90	5	5	0	210	1	0	14.6	171	1	1
25D1	**14**	**93**	**6.6**	**1.9**	**13**	**85**	**10**	**5**	**0**	**190**	**1**	**0**	**22.5**	**171**	**1**	**1**
26U	17	111	6.5	1.5	15	90	5	5	0	250	2	0	9.5	33	2	1
26D1	**14**	**91**	**6.5**	**1.4**	**18**	**95**	**4**	**1**	**0**	**250**	**2**	**0**	**29.1**	**33**	**1**	**1**
27U	19	123	6.5	4.7	16	65	30	5	0	70	3.5	0	49	50	2	1
27D1	**18**	**108**	**6**	**5.2**	**15**	**70**	**25**	**5**	**0**	**70**	**3.5**	**0**	**43**	**50**	**2**	**1**
28U	19	117	6.1	3.4	10	10	90	0	0	5	12	1	42	5	2	1
28D1	**5**	**21**	**4.2**	**4.2**	**8**	**10**	**90**	**0**	**0**	**5**	**12**	**1**	**159**	**5**	**2**	**1**
29U	17	99	5.8	4	5	0	90	10	0	74	1	0	94	10	2	1
29D1	**4**	**18**	**4.5**	**2.1**	**5**	**0**	**90**	**10**	**0**	**69**	**1**	**0**	**113**	**10**	**2**	**1**
30U	20	111	5.5	1.3	6	0	40	60	0	160	3	0	21	0.8	2	1
30D1	**16**	**95**	**5.9**	**2**	**10**	**50**	**40**	**10**	**0**	**160**	**3**	**0**	**188**	**0.8**	**2**	**1**
31U	15	89	5.9	0.6	8	30	50	20	0	72.5	0.25	0	15.3	125	2	1
31D1	**6**	**22**	**3.6**	**0.5**	**8**	**30**	**50**	**20**	**0**	**700**	**0.25**	**0**	**18.7**	**12.5**	**2**	**1**
32U	10	43	4.3	0.85	6	0	60	35	5	75	1.5	0	40.95	25	2	1
32D1	**4**	**14**	**3.5**	**0.9**	**8**	**0**	**95**	**5**	**0**	**70**	**1.5**	**0**	**58.71**	**25**	**2**	**1**

Appendix XXVIII (3). Wales 1993 Single Season Site Database. Source: Environment Agency.

	Sewage Litter	Temperature	pH	Field pH	DO (mg/l)	DO %	Conductivity	Fe(f)	Fe(t)	Al(f)	Al(t)	SO4 (f)	SO4(t)	Alkalinity	SSolids	Substrate	Heptageniidae
1U	2	10.7	7.88	8.31	14.2	128	224	0.098	0.248	0.061	0.152	40.9	41.1	56	6	0	2
1D1	2	11.2	7.86	8.11	14.2	130	312	0.234	0.676	0.055	0.115	70.9	71.4	76	5	0	2
2U	2	10.8	7.83	7.98	11.5	104	300	0.142	0.426	0.044	0.102	59.3	59.3	72	4	0	1
2D1	2	10.9	7.78	7.75	10.1	91.6	364	0.21	0.463	0.021	0.091	87	87	79	3	0	2
3U	1	11.6	7.8	8.2	12.6	116	318	0.099	0.5	0.019	0.087	81.9	81.9	102.5	3	0	1
3D1	3	11.4	7.8	8.21	12	110	383	0.101	0.443	0.015	0.075	80.8	80.8	111	4	0	1
4U	2	12.1	8.5	8.42	11.4	106	415	0.145	0.22	0.029	0.063	95.1	95.1	89	3	0	2
4D1	2	12.7	8.1	7.57	9.4	88.8	484	3.33	0.562	0.025	0.029	144.3	144.8	109	5	0	1
5U	1	13.6	8.1	8.05	10.8	104	277	0.086	0.1	0.018	0.037	50	50.9	109	3	0	2
5D1	1	13.5	7.9	7.87	11.1	107	277	3.33	0.065	0.019	0.023	52.6	52.6	79	3	0	1
6U	2	14.2	7.9	7.71	10.4	102	377	0.132	0.174	0.021	0.044	71.2	71.6	81	3	0	1
6D1	2	13.3	7.5	10.6	10.6	102	654	3.33	1.16	0.016	0.016	213.7	230	115	7	0	0
7U	2	16.4	7	8.25	10.7	110	457	0.205	0.205	0.0205	0.028	117	117	112	5	0	3
7D1	2	11	6.9	7.12	8.3	75.4	1799	3.33	3.39	0.016	0.016	736	736	210	7	0	0
8U	1	11.2	7.7	7.5	11.6	106	153	0.003	0.003	0.004	0.009	51.9	51.9	38	3	0	2
8D1	1	11.6	7.6	7.2	11.1	102	157	0.19	0.24	0.011	0.013	29.9	29.9	45	3	0	2
9U	1	12.4	7.6	8.41	9.8	92	176	0.15	0.185	0.066	0.123	35.8	37.1	38.5	3	0	2
9D1	1	12.6	7.6	7.89	11.5	108	354	0.27	0.341	0.048	0.098	88.12	90.2	66	3	0	0
10U	1	11.8	7.7	7.71	12.2	113	185	0.101	0.127	0.048	0.072	37.4	37.8	45.02	3	0	1
10D1	1	11.4	7.32	7.08	12.5	115	198	0.252	0.288	0.094	0.143	48.9	49	40.1	3	0	0
11U	1	11.8	7.7	7.71	12.2	113	74	0.213	0.262	0.138	0.162	14.4	14.5	7.7	3	0	0
11D1	1	11.4	7.32	7.08	12.5	115	267	1.13	2.03	0.004	0.009	27.8	27.8	37.5	6	0	0
12U	2	16.8	7.92	8.51	11.1	115	91	0.319	0.499	0.01	0.039	40.9	41	77	3	0	0
12D1	2	16.5	7.9	8.34	12.1	124	88	0.372	0.626	0.011	0.048	39.9	39.9	75.5	5	0	0
13U	1	12.4	7.2	7.84	12.7	119	67	0.31	0.59	0.041	0.13	13.7	13.7	18.5	6	0	0
13D1	1	12.4	7	7.75	11.5	108	109	1.07	2.07	0.039	0.18	17.5	17.5	14.5	13	0	0
14U	1	13.6	7	7.06	11.3	109	112	0.039	0.06	0.032	0.053	17.6	17.7	23.4	3	0	0
14D1	1	12.9	6.4	6.73	10.2	96.8	164	0.049	0.078	0.032	0.039	18.2	18.4	27.1	3	0	0
15U	1	10.9	7.6	7.99	15	136	94	0.071	0.099	0.071	0.082	11.6	11.6	25.8	3	0	0
15D1	1	11.7	7.6	7.65	13	120	551	16.5	16.7	0.05	0.05	242	242	39.6	10	0	0
16U	1	10.8	6.1	7.46	13.8	125	46	0.063	0.1	0.19	0.38	9.9	10	2.8	3	0	0
16D1	1	11.1	6.7	6.82	11.4	104	62	0.42	0.51	0.18	0.37	13.8	14	6.3	4	0	0
17U	1	10.9	7.24	7.91	12.1	110	76	0.056	0.104	0.053	0.088	10	10.1	16.9	3	0	1
17D1	1	10.8	7.02	7.27	12	109	95	1.023	1.032	0.071	0.074	27.3	27.5	20.5	4	0	0
18U	1	13.2	7.4	7.91	10.2	97.5	112	0.039	0.06	0.032	0.053	17.6	17.7	23.4	3	0	2
18D1	1	13.3	7.4	7.92	10.7	102	164	0.049	0.078	0.032	0.039	18.2	18.4	27.1	3	0	3
19U	1	12.6	7.5	7.99	13.4	126	129	0.065	0.102	0.045	0.078	22.4	22.7	35.3	3	0	2
19D1	1	12.7	7.7	7.86	13.4	127	149	0.309	0.503	0.044	0.052	29.5	29.5	62.4	28	0	1
20U	1	12.2	7.3	8.1	15.5	145	123	0.023	0.077	0.08	0.095	17.2	17.4	18.4	3	0	3
20D1	1	11.9	7.5	7.83	15.4	143	132	0.184	0.235	0.074	0.076	18.3	18.7	29.3	21	0	1
21U	1	13	7	7.69	14.1	134	142	0.028	0.046	0.011	0.025	18.9	9.7	4		0	2
21D1	1	12.8	7.1	7.75	12.7	120	148	0.189	0.298	0.043	0.113	24.8	25.4	21.4	3	0	1
22U	1	12.7	6.8	7.88	15.9	150	87	0.105	0.154	0.222	0.308	20	20.1	7.6	3	0	0
22D1	1	11.1	7	7.79	16.9	154	117	0.154	0.661	0.155	0.41	28.4	28.8	14.8	12	0	0
23U	1	13.7	7.1	7.97	14.9	144	114	0.133	0.216	0.192	0.402	27.3	27.9	13.6	3	0	0
23D1	1	13.6	6.9	6.51	16.5	159	207	0.732	0.841	0.171	0.41	35	36.3	12.27	3	0	0
24U	1	15.9	6.9	7	13.8	140	150	0.045	0.088	0.103	0.211	36	36	9.3	3	0	0

Appendix XXVIII (4). Wales 1993 Single Season Site Database. Source: Environment Agency.

	Sewage Litter	Temperature	pH	Field pH	DO (Mg/l)	DO %	Conductivity	Fe(f)	Fe(t)	Al(f)	Al(t)	SO4 (f)	SO4(t)	Alkalinity	SSolids	Substrate	Heptageniidae
24D1	1	12.1	4.31	5.24	13.8	129	764	32.05	38.45	0.045	0.028	340	108.9	12.1	14	0	0
25U	1	15.4	7.2	7.37	13.9	139	93	0.026	0.026	0.019	0.019	11	14	14.6	3	0	1
25D1	1	12.9	7.2	6.92	15.1	143	200	3.33	0.566	0.062	0.07	66	68	22.5	3	0	1
26U	1	13.9	7	7.8	11.3	110	54	0.123	0.024	0.012	0.014	4.7	4.7	9.5	3	0	1
26D1	1	12.9	7.3	7.68	11.5	109	132	0.447	0.447	0.012	0.094	24.3	24.8	29.1	35	0	1
27U	1	14.1	6.2	7.41	10.9	106	188	0.048	0.12	0.017	0.022	32	32	49	12	0	2
27D1	1	13.9	6.7	7.82	10.2	99	210	1.2	1.2	0.024	0.042	42	42	43	6	0	1
28U	1	14.6	7.3	8.27	11.3	111	204	0.275	0.345	0.035	0.035	29.5	29.5	42	3	0	1
28D1	1	14.8	7	7.8	7.9	78.2	1239	22.57	26.42	0.029	0.029	456	456	159	74	0	0
29U	1	13.7	7.8	6.9	9.3	89.9	230	0.172	0.173	0.012	0.036	6	6.9	94	3	0	0
29D1	1	12.5	7	7.7	7.8	73.4	449	5.47	7.17	0.057	0.074	87	87	113	19	0	0
30U	1	14.3	7	6.27	9.5	93	74	0.211	0.345	0.004	0.01	5.3	5.4	21	3	0	0
30D1	1	13.8	7.1	5.94	7.8	75.5	615	2.137	2.282	0.004	0.009	145	151	188	5	0	1
31U	1	16.5	6.9	6.48	9	92.4	546	0.037	0.094	0.009	0.022	284	284	15.3	6	0	2
31D1	1	15.1	6.1	6.37	8.9	88.7	610	2.389	4.068	0.004	0.044	274	274	18.7	34	0	0
32U	1	11.9	7.9	7.35	10.05	93.3	570	0.39	0.4	0.046	0.046	75.8	75.8	40.95	3	0	0
32D1	1	11	7.8	6.66	6.39	58.1	1018	3.33	3.1	0.01	0.01	237.8	237.8	58.71	20	0	0

Appendix XXVIII (5). Wales 1993 Single Season Site Database. Source: Environment Agency.

	Ephemerellidae	Ephemeridae	Leptophlebiidae	Perlidae	Perlodidae	Chloroperlidae	Leuctridae	Sericostomatidae	Goeridae	Odontoceridae	Lepidostomatidae	Cordulegasteridae	Philopotamidae	Caenida
1U	4	0	0	0	0	0	1	0	0	0	0	0	0	1
1D1	3	0	0	0	0	0	1	1	0	0	1	0	0	0
2U	3	0	0	0	1	0	1	0	0	0	0	0	0	1
2D1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
3U	4	0	0	0	0	0	1	0	0	0	0	0	0	0
3D1	3	0	0	0	1	0	1	0	0	0	0	0	0	0
4U	4	0	0	0	0	0	0	0	0	0	0	0	0	0
4D1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
5U	3	0	0	0	0	0	3	0	0	0	0	0	0	3
5D1	3	0	0	0	0	0	2	0	0	0	0	0	0	2
6U	2	0	0	0	0	0	1	0	0	0	0	0	0	2
6D1	2	0	0	0	0	0	1	0	0	0	0	0	0	1
7U	3	0	0	0	0	0	1	0	0	0	0	0	0	2
7D1	3	0	0	0	0	0	1	0	0	0	0	0	0	1
8U	3	0	0	0	2	1	2	1	0	0	1	0	1	0
8D1	3	0	0	0	2	2	2	0	0	0	0	0	0	0
9U	1	1	0	0	2	2	2	1	0	0	0	1	0	2
9D1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10U	3	0	0	0	1	0	2	1	0	0	0	0	1	1
10D1	3	0	0	0	0	0	1	0	0	0	0	0	0	1
11U	0	0	0	0	1	1	1	0	0	0	0	1	2	0
11D1	1	0	0	0	0	0	2	0	0	0	0	0	0	0
12U	3	0	0	0	0	0	1	0	0	0	0	0	0	1
12D1	2	0	0	0	0	0	0	0	0	0	0	0	0	1
13U	2	0	0	0	1	2	2	1	0	0	0	0	1	0
13D1	1	1	0	0	1	0	1	1	0	0	0	0	0	0
14U	1	0	0	0	0	0	2	2	0	0	0	1	0	0
14D1	0	0	0	0	0	0	1	0	0	0	1	0	0	0
15U	1	0	0	0	0	0	1	0	0	0	0	0	0	0
15D1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
16U	0	0	0	0	0	0	2	0	0	0	0	0	0	0
16D1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17U	0	0	0	0	0	1	2	1	0	0	0	0	0	0
17D1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
18U	3	0	0	0	0	1	2	1	0	0	0	0	0	3
18D1	3	0	0	0	0	0	2	0	0	0	0	0	0	3
19U	2	0	0	0	1	1	2	0	0	0	0	0	0	1
19D1	3	0	0	0	0	1	2	0	0	0	0	0	0	1
20U	2	0	0	0	0	0	1	1	0	0	1	1	2	0
20D1	2	0	0	0	0	1	1	0	1	0	0	1	2	1
21U	3	0	0	0	0	0	3	2	1	1	1	0	0	2
21D1	3	0	0	0	0	0	3	2	1	0	0	0	0	2
22U	0	0	0	0	0	1	1	0	0	0	0	0	0	0
22D1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
23U	0	0	0	0	0	1	1	0	0	0	0	0	0	0
23D1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
24U	0	0	0	0	0	1	1	0	0	0	0	0	0	0

Appendix XXVIII (6). Wales 1993 Single Season Site Database. Source: Environment Agency.

	Ephemerellidae	Ephemeridae	Leptophlebiidae	Perlidae	Perlodidae	Chloroperlidae	Leuctridae	Sericostomatidae	Goeridae	Odontoceridae	Lepidostomatidae	Cordulegasteridae	Philopotamidae	Caenidae
24D1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
25U	3	0	0	0	1	1	2	0	1	0	0	1	2	0
25D1	**3**	**0**	**0**	**0**	**1**	**1**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
26U	2	0	0	1	0	2	2	0	0	0	0	0	1	0
26D1	**2**	**0**	**0**	**0**	**0**	**1**	**2**	**0**	**0**	**0**	**0**	**0**	**1**	**0**
27U	3	0	0	0	0	0	2	1	1	0	0	1	1	0
27D1	**2**	**0**	**0**	**0**	**0**	**0**	**2**	**1**	**0**	**0**	**1**	**0**	**0**	**0**
28U	3	0	0	0	0	2	2	1	1	0	0	0	0	0
28D1	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
29U	0	0	1	0	0	2	3	1	0	0	0	0	1	0
29D1	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
30U	0	0	0	0	0	1	2	0	1	0	0	1	0	0
30D1	**1**	**0**	**0**	**0**	**0**	**1**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
31U	2	0	0	0	0	0	2	0	0	0	0	1	0	0
31D1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
32U	0	0	0	0	0	0	0	1	0	0	0	0	0	0
32D1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Appendix XXVIII (7). Wales 1998 Single Season Site Database. Source: Environment Agency.

	Nemouridae	Rhyacophilidae	Polycentropodidae	Limnephilidae	Ancylidae	Hydroptilidae	Gammaridae	Mesovelidae	Gerridae	Hygrobiidae	Dytiscidae	Gyrinidae	Hydrophilidae	Helodidae	Dryopidae	Elminthida
1U	0	2	1	0	0	1	2	0	0	0	0	0	0	0	0	0
1D1	0	2	1	1	0	1	1	0	0	0	1	0	0	0	0	0
2U	0	2	0	1	0	1	2	0	0	0	1	0	0	0	0	1
2D1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1
3U	0	2	1	1	1	1	2	0	0	0	0	0	0	0	0	0
3D1	0	2	0	1	0	0	1	0	0	0	0	0	0	0	0	0
4U	0	2	1	0	1	0	1	0	0	0	0	0	0	0	0	0
4D1	0	2	0	0	1	0	2	0	0	0	1	0	0	0	0	0
5U	0	2	0	1	1	1	1	0	0	0	1	0	0	0	0	1
5D1	0	2	0	0	0	0	1	0	0	0	0	0	0	0	0	1
6U	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	1
6D1	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	1
7U	0	1	0	0	0	1	2	0	0	0	1	0	0	0	0	0
7D1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8U	2	2	1	1	0	0	0	0	0	0	0	0	0	1	0	1
8D1	2	2	1	0	1	0	0	0	0	0	0	0	0	1	0	1
9U	2	2	1	1	1	0	0	0	0	0	1	1	1	0	1	2
9D1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10U	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	1
10D1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
11U	2	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0
11D1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
12U	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
12D1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
13U	1	2	1	1	0	0	0	0	0	0	1	0	1	1	0	1
13D1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1
14U	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	1
14D1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15U	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
15D1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
16U	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
16D1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
17U	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
17D1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
18U	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	2
18D1	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	2
19U	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1
19D1	0	2	2	0	0	0	0	0	0	0	0	0	0	0	0	1
20U	1	1	1	1	0	0	3	0	0	0	0	0	0	0	0	1
20D1	0	2	1	0	0	0	2	0	0	0	0	0	0	0	0	1
21U	0	2	1	1	0	0	1	0	0	0	0	0	0	0	0	2
21D1	0	2	1	1	0	0	1	1	0	0	0	0	0	0	0	2
22U	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
22D1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
23U	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1
23D1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24U	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix XXVIII (8). Wales 1993 Single Season Site Database. Source: Environment Agency.

	Nemouridae	Rhyacophilidae	Polycentropidae	Limnephilidae	Ancylidae	Hydroptilidae	Gammaridae	Mesovelidae	Gerridae	Hygrobiidae	Dytiscidae	Gyrinidae	Hydrophilidae	Helodidae	Dryopidae	Elminthidae
24D1	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**
25U	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	2
25D1	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
26U	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2
26D1	**1**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
27U	0	2	1	1	0	0	2	0	0	0	0	0	0	0	0	0
27D1	**1**	**1**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
28U	0	2	0	0	0	1	3	0	0	0	0	0	0	0	0	2
28D1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
29U	0	0	2	0	0	0	4	1	0	0	1	0	0	0	0	0
29D1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
30U	0	1	0	1	0	0	1	1	1	0	0	0	2	0	0	2
30D1	**0**	**1**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**0**
31U	0	1	1	0	0	0	0	0	0	0	1	0	2	0	1	1
31D1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**0**
32U	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
32D1	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Appendix XXVIII (9). Wales 1993 Single Season Site Database. Source: Environment Agency.

	Hydropsychidae	Tipulidae	Simuliidae	Planariidae	Baetidae	Valvatidae	Hydrobiidae	Lymnaeidae	Planorbiidae	Sphaeriidae	Glossiphonidae	Erpobdellidae	Asellidae	Chironomidae	Oligochaeta
1U	2	0	2	1	3	0	1	0	0	0	0	0	1	3	3
1D1	2	0	1	1	2	0	1	0	0	0	0	1	0	4	4
2U	2	1	0	1	2	0	1	0	0	0	0	1	1	4	4
2D1	1	1	1	0	2	0	0	0	0	0	0	0	1	3	4
3U	1	1	3	1	3	0	2	1	0	1	0	1	1	4	4
3D1	1	1	1	1	3	0	1	0	0	0	0	1	1	4	4
4U	0	0	0	0	3	0	2	1	0	0	0	1	2	2	3
4D1	0	1	0	0	3	0	3	0	0	0	1	0	2	3	3
5U	3	0	1	0	3	0	1	0	0	0	0	1	1	3	3
5D1	2	0	1	0	3	0	1	0	0	0	0	1	1	3	3
6U	1	0	0	0	3	0	1	0	0	0	0	1	1	3	4
6D1	2	0	0	0	3	0	0	0	0	0	0	1	1	3	3
7U	1	0	0	0	3	0	0	0	0	0	0	1	2	3	3
7D1	1	0	0	0	3	0	2	0	0	0	1	0	1	3	3
8U	1	1	2	1	3	0	1	0	0	0	0	0	0	3	3
8D1	2	1	3	0	3	0	0	0	1	1	0	0	0	3	3
9U	2	1	1	0	3	0	0	0	0	0	0	0	0	3	3
9D1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
10U	2	1	3	0	3	0	0	0	0	0	0	0	0	3	3
10D1	2	0	3	0	2	0	0	0	0	0	0	0	0	3	2
11U	0	1	3	0	3	0	0	0	0	0	0	0	0	2	2
11D1	0	0	0	0	1	0	0	0	0	0	0	0	0	2	2
12U	0	1	0	0	3	0	1	1	0	0	0	0	1	3	3
12D1	0	0	0	0	3	0	1	0	0	0	0	0	1	3	3
13U	1	2	2	1	2	0	0	0	0	1	0	0	0	3	3
13D1	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1
14U	1	0	2	1	1	0	0	0	1	0	0	0	0	2	2
14D1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	3
15U	0	0	1	0	1	0	1	0	0	0	0	0	0	1	1
15D1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
16U	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0
16D1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
17U	0	0	1	0	3	0	0	0	0	0	0	0	0	2	2
17D1	0	1	1	0	1	0	0	0	0	0	0	0	0	1	1
18U	1	1	1	0	3	0	0	0	0	0	0	0	0	2	3
18D1	1	2	1	1	3	0	0	1	0	0	0	0	0	3	3
19U	0	1	1	0	3	0	0	0	0	0	0	0	0	1	1
19D1	0	1	1	1	3	0	0	0	0	0	0	0	0	1	1
20U	2	1	1	0	2	0	0	0	0	0	0	0	0	2	2
20D1	1	1	1	1	3	0	0	0	0	0	0	0	0	2	2
21U	2	2	2	2	3	0	0	0	0	0	0	0	0	3	3
21D1	2	2	2	2	3	0	1	0	0	1	0	0	0	2	3
22U	1	1	2	0	1	0	0	0	0	0	0	0	0	1	1
22D1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1
23U	0	0	2	0	1	0	0	0	0	0	0	0	0	1	1
23D1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1
24U	0	1	1	0	1	0	0	0	0	0	0	0	0	2	2

Appendix XXVIII (10). Wales 1993 Single Season Site Database. Source: Environment Agency.

	Hydropsychidae	Tipulidae	Simuliidae	Planariidae	Baetidae	Valvatidae	Hydrobiidae	Lymnaeidae	Planorbiidae	Sphaeriidae	Glossiphonidae	Erpobdellidae	Asellidae	Chironomidae	Oligochaeta
24D1	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**2**
25U	2	1	1	1	3	0	0	1	0	1	0	0	0	2	2
25D1	**1**	**1**	**0**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**1**
26U	2	1	2	1	2	0	0	0	0	0	0	0	0	3	3
26D1	**1**	**1**	**2**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**3**	**2**
27U	2	1	3	0	3	0	2	0	0	0	0	0	0	2	2
27D1	**1**	**1**	**2**	**1**	**2**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**2**	**2**
28U	1	1	1	1	2	0	1	0	0	0	0	2	0	3	3
28D1	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**3**	**3**
29U	0	2	3	1	2	0	4	0	1	0	0	0	0	3	2
29D1	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**
30U	1	3	2	1	2	0	2	0	0	1	0	0	0	2	2
30D1	**0**	**2**	**2**	**0**	**3**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**3**	**2**
31U	0	2	2	0	2	0	0	0	0	0	0	0	0	2	1
31D1	**0**	**0**	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**
32U	0	1	3	0	1	0	1	1	0	0	0	0	1	3	3
32D1	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**2**

Appendix XXIX. Results of Tukey's Test result : paired comparison, and one way ANOVA of all upstream sites in Yorkshire, Northumbria and Wales. Significant differences within regions are highlighted in bold.

1. Tukey Test. Significant differences only

NARN1/NHAG1	**NBLA1/NALL1**	NALL1/YGIL1	**W7U/W20U**
NARN1/NHAL1	NBLA1/YALE1	NALL1/YLIM1	**W7U/W24U**
NARN1/NFOR1	NBLA1/YCHAL1	NALL1-W1U - W34U	**W7U/W25U**
NARN1/NALL1	NBLA1/YSHEL1	NBYR1/YALE1	**W7U/W26U**
NARN1/YALE1	NBLA1/YSHEP1	NBYR1/W12U	**W7U/W28U**
NARN1/YCHAL1	NBLA1/YUGH1	NBYR1/W13U	**W7U/W32U**
NARN1/YGIL1	**NHAL1/NNET1**	NBYR1/W16U	**W9U/W11U**
NARN1/YLIM1	**NHAL1/NALL1**	NBYR1/W17U	**W9U/W12U**
NARN1/YSHE1	NHAL1/W11U	NBYR1/W18U	**W9U/W13U**
NARN1/YSHEP1	NHAL1/W12U	NBYR1/W19U	**W9U/W16U**
NARN1/YUGH1	NHAL1/W13U	NBYR1/W24U	**W9U/W17U**
NARN1/W9U	NHAL1/W16U	NBYR1/W25U	**W9U/W18U**
NARN1/W22U	NHAL1/W17U	NBYR1/W26U	**W9U/W20U**
NARN1/W33U	NHAL1/W18U	NBYR1/W28U	**W9U/W21U**
NW001/NHAG1	NHAL1/W19U	NBYR1/W32U	**W9U/W19U**
NW001/NHAL1	NHAL1/W20U	**YHUN1/YALE1**	**W9U/W23U**
NW001/NFOR1	NHAL1/W21U	**YHUN1/YCHA1**	**W9U/W24U**
NW001/NALL1	NHAL1/W23U	**YHUN1/YUGH1**	**W9U/W25U**
NW001/YALE1	NHAL1/W24U	YHUN1/W12U	**W9U/W26U**
NW001/YCHA1	NHAL1/W25U	YHUN1/W18U	**W9U/W28U**
NW001/YGIL1	NHAL1/W26U	YHUN1/W24U	**W9U/W30U**
NW001/YLIM1	NHAL1/W28U	**YCOL1/YALE1**	**W9U/W32U**
NW001/YSHEL1	NHAL1/W32U	**YCOL1/YCHA1**	**W12U/W33U**
NW001/YSHEP1	**NNET1/NFOR1**	**YCOL1/YSHE1**	**W12U/W34U**
NW001/YUGH1	**NNET1/NALL1**	**YCOL1/YSHEP1**	**W13U/W22U**
N2001/W7U	NNET1/YALE1	**YCOL1/YUGH1**	**W13U/W33U**
NW001/W9U	NNET1/YCHA1	YALE1/W1U - W34U	**W13U/W34U**
NW001/W22U	NNET1/YGIL1	YCHA1/W1U	**W15U/W18U**
NW001/W33U	NNET1/YLIM1	YCHA1/W2U	**W16U/W22U**
NHAG1/NNET1	NNET1/YSHE1	YUGH1/W1U - W34U	**W16U/W33U**
NHAG1/NALL1	NNET1/YSHEP1	**W2U/W18U**	**W16U/W34U**
NHAG1/W11U	NNET1/YUGH1	**W3U/W18U**	**W17U/W22U**
NHAG1/W12U	NNET1/W9U	**W4U/W12U**	**W17U/W33U**
NHAG1/W13U	NNET1/W22U	**W4U/W18U**	**W17U/W34U**
NHAG1/W16U	NNET1/W32U	**W4U/19U**	**W18U/W22U**
NHAG1/W17U	**NCHI1/NFOR1**	**W4U/W24U**	**W18U/W33U**
NHAG1/W18U	**NCHI1/NALL1**	**W4U/W25U**	**W18U/W34U**
NHAG1/W19U	NCHI1/YALE1	**W4U/W28U**	**W19U/W22U**
NHAG1/W20U	NCHI1/YCHA1	**W4U/W34U**	**W19U/W33U**
NHAG1/W21U	NCHI1/YSHE1	**W5U/W18U**	**W19U/W34U**
NHAG1/W23U	NCHI1/YSHEP1	**W6U/W12U**	**W20U/W22U**
NHAG1/W24U	NCHI1/YUGH1	**W6U/W18U**	**W20U/W33U**
NHAG1/W25U	NFOR1/YHUN1	**W7U/W12U**	**W21U/W22U**
NHAG1/W26U	NFOR1/YCLO1	**W7U/W13U**	**W21U/W33U**
NHAG1/W28U	NFOR1/WIU - W32U	**W7U/W16U**	**W22U/W23U**
NHAG1/W30U	NALL1/NBYR1	W7U/W17U	**W22U/W24U**

Appendix XXIX. All sites, Tukey test and ANOVA results cont.

NHAG1/W32U	NALL1/YHUN1	**W7U/W18U**	**W22U/W25U**
NBLA1/NFOR1	NALL1/YCLO1	**W7U/W19U**	**W22U/W26U**
YCHA1/W3U	YGIL1/W20U	YSHE1/W17U	YSHEP1/W28U
YCHA1/W4U	YGIL1/W21U	YSHE1/W18U	YSHEP1/W29U
YCHA1/W5U	YGIL1/W23U	YSHE1/W19U	YSHEP1/W30U
YCHA1/W6U	YGIL1/W24U	YSHE1/W20U	YSHEP1/W31U
YCHA1/W10U	YGIL1/W25U	YSHE1/W21U	YSHEP1/W32U
YCHA1/W11U	YGIL1/W26U	YSHE1/W23U	YUGH1/W1U
YCHA1/W12U	YGIL1/W28U	YSHE1/W24U	YUGH1/W2U
YCHA1/W13U	YGIL1/W30U	YSHE1/W25U	YUGH1/W3U
YCHA1/W15U	YGIL1/W32U	YSHE1/W26U	YUGH1/W4U
YCHA1/W16U	YLIM1/W12U	YSHE1/W27U	YUGH1/W5U
YCHA1/W17U	YLIM1/W13U	YSHE1/W28U	YUGH1/W6U
YCHA1/W18U	YLIM1/W16U	YSHE1/W29U	YUGH/W10U
YCHA1/W19U	YLIM1/W17U	YSHE1/W30U	YUGH1/W11U
YCHA1/W20U	YLIM1/W18U	YSHE1/W31U	YUGH1/W12U
YCHA1/W21U	YLIM1/W19U	YSHE1/W32U	YUGH1/W13U
YCHA1/W23U	YLIM1/W20U	YSHEP1/W1U	**W28U/W33U**
YCHA1/W24U	YLIM1/W21U	YSHEP1/W2U	**W28U/W34U**
YCHA1/W25U	YLIM1/W23U	YSHEP1/W5U	**W32U/W33U**
YCHA1/W26U	YLIM1/W24U	YSHEP1/W10U	**W32U/W34U**
YCHA1/W27U	YLIM1/W25U	YSHEP1/W11U	**W11U/W22U**
YCHA1/W28U	YLIM1/W26U	YSHEP1/W12U	**W11U/W33U**
YCHA1/W29U	YLIM1/W28U	YSHEP1/W13U	**W12U/W22U**
YCHA1/W30U	YLIM1/W32U	YSHEP1/W16U	**W22U/W28U**
YCHA1/W31U	YSHE1/W1U	YSHEP1/W17U	**W22U/W30U**
YCHA1/W32U	YSHE1/W2U	YSHEP1/W18U	**W22U/W32U**
YCHA1/W34U	YSHE1/W3U	YSHEP1/W19U	**W23U/W33U**
YGIL1/W11U	YSHE1/W5U	YSHEP1/W20U	**W24U/W33U**
YGIL1/W12U	YSHE1/W10U	YSHEP1/W21U	**W24U/W34U**
YGIL1/W13U	YSHE1/W11U	YSHEP1/W23U	**W25U/W33U**
YGIL1/W16U	YSHE1/W12U	YSHEP1/W24U	**W25U/W34U**
YGIL1/W17U	YSHE1/W13U	YSHEP1/W25U	**W26U/W33U**
YGIL1/W18U	YSHE1/W15U	YSHEP1/W26U	**W26U/W34U**
YGIL1/W19U	YSHE1/W16U	YSHEP1/W27U	

Summary results

Total comparisons:1275 (51 column combinations)

Total significant differences: 443 = 35% of site pairs significantly different at p=0.05

2. Analysis of variance:

Source	DF	SS	MS	F	Prob
Between sites	50	4.239E005	8477.84	0.5134	0.9978
Within sites	459	7.579E006	16515.06		
Total	609	8.003E006			

ie: there is no overall difference between sites, although 35% of the comparisons show significant differences. Wales sites have fewer internal differences, so more homogeneous.

Appendix XXX. Polynomial models derived from GMDH for the prediction of biological scores in Yorkshire, Northumbria and Wales. Best formulas and legend descriptions given.

1.Yorkshire High Impact sites

a. BMWP

Y=-1.9*X1-0.81*X2-0.52*X3+3*X1^2+0.42*X3^2+3.8*X1*X2-0.98*X2*X3
X1=2.*As(t)/.6-1.
X2=2.*(Ni(f)-.3)/1.53-1.
X3=2.*(Conductivity-2.34)/.65-1.
Y=2.*(BMWP-.9)/1.27-1.

b. ASPT

Y=0.58-0.87*X2-0.74*X2^2+3.6*X1^3+X1*X2-7.6*X1*X3
X1=2.*As(t)/.6-1.
X2=2.*(Fe(f)-1.5)/2.7-1.
X3=2.*(Sulphate-1.27)/1.25-1.
Y=2.*(ASPT-0.43)/0.33-1.

c.Number of taxa

Y=-0.13+0.26*X2-2.3*X3-0.61*X2^3+2.1*X3^3-0.77*X2*X3+0.22*X2*X5-0.61*X3*X5-2*X3*X5
X1=2.*As(t)/.6-1.
X2=2.*(Zn(f)-.78)/1.27-1.
X3=2.*(Fe(t)-2.06)/2.15-1.
X4=2.*(Temperature-10.6)/4.5-1.
X5=2.*Ochre/100.-1.
Y=2.*(Number of taxa-3.)/24.-1.

2.Yorkshire Low Impact Sites

a.BMWP

Y=0.19-0.78*X1+0.39*X2-0.42*X1^2+0.2*X2^2+0.5*X1*X2
X1=2.*Cu(t)/.99-1.
X2=2.*(DO-2.)/93.-1.
Y=2.*(BMWP-1.2)/0.97-1.

b.ASPT

Y=0.21-0.7*X1-0.33*X2-0.23*X1^2+0.48*X2^2-0.54*X2^3+X1*X2
X1=2.*Cu(t)/.99-1.
X2=2.*(Depth-0.62)/.93-1.
Y=2.*(ASPT-3.2)/2.96-1.

c.Number of taxa

Y=-X1+0.57*X2-0.56*X1^2+0.55*X1^3+0.31*X1*X2
X1=2.*Cu(t)/0.99-1.
X2=2.*(DO(%)-2.)/93.-1.
Y=2.*(Number of taxa-5.)/20.-1.

3. Northumbria sites

a.BMWP

Y=0.37*X6+0.37+0.36*X2-0.48*X1-0.26*X4-0.33*X5+0.53*X4^2-0.13*X6^2+0.79*X4^3-0.89*X6^3-0.41*X4*X5+0.59*X4*X6-0.69*X1^2-0.17*X3^2

X1=2.*As(t)/.66-1.

X2=2.*(SS-.3)/1.26-1.

X3=2.*(Chloride-0.86)/.91-1.

X4=2.*(Depth-.7)/.89-1.

X5=2.*(colour-1.)/2.-1.

X6=2.*Ochre/100.-1.

b.ASPT

Y=-0.23*X5-0.49-X2-0.86*X3+0.51*X2^2-0.16*X5^2+0.74*X2^3-0.7*X2*X3+0.26*X3*X5-0.49*X1^2+0.3*X4^2

X1=2.*Pb(t)/1.18-1.

X2=2.*Cu(t)/.96-1.

X3=2.*Silt/97.5-1.

X4=2.*(Depth-.7)/.89-1.

X5=2.*(Colour-1.)/2.-1.

Y=2.*(ASPT-4.13)/2.75-1.

c. Number of taxa

Y=-0.3-0.33*X1+0.59*X3-0.46*X4-0.12*X4^2-0.49*X3^3-0.3*X5^3-0.41*X3*X4-0.19*X4*X5-0.32*X3*X4*X5

X1=2.*As(t)/.66-1.

X2=2.*Ni(f)/2.35-1.

X3=2.*(Width-0.75)/6.-1.

X4=2.*(Colour-1.)/2.-1.

X5=2.*Ochre/100.-1.

Y=2.*(Number of taxa-8.)/22.-1.

4. Welsh sites

a.BMWP

Y=0.55-0.53*X1-0.15*X1^2

X1=2.*(Fe(t)+2.52)/4.11-1.

Y=2.*(BMWP-.48)/1.74-1.

b.ASPT

Y=0.49-0.29*X1-0.56*X2+0.52*X2^3

X1=2.*(Fe(t)+2.52)/4.11-1.

X2=2.*(SO_4(f)-.67)/2.19-1.

Y=2.*(ASPT-1.5)/5.3-1.

c.Number of taxa

Y=-0.85*X1+0.35*X2-0.23*X2^2

X1=2.*(Fe(t)+2.52)/4.11-1.

X2=2.*(Alkalinity-.45)/1.88-1.

Y=2.*(Number of Taxa-2.)/23.-1.

Appendix XXXI. Test site data for across-region and season tests. AU=Autumn sample, SU=Summer sample. NGRs for sites new to the study are stated.

	BMWP	As(t)	Ni(f)	Conductivity (µs/cm)
NARNSU1	106	0.5	4.1	152
NARNAU1	95	0.5	3.4	210
NARNAU2	104	1.0	2.3	221
NWOOAU1	152	1.0	6.0	64
NHAGSU1	109	1.0	1.0	540
NHAGAU1	138	1.0	1.0	510
NBLASU1	124	1.0	1.0	297
NBLAAU3	132	1.0	23.9	165
NHALSU1	113	1.0	4.9	539
NHALAU1	82	1.0	3.3	342
NHALSU4	129	1.0	2.2	604
NHALAU4	113	1.0	2.2	604
NNETSU1	158	1.0	1.0	280
NNETAU3	160	1.1	1.0	403
NCHIAU1	114	1.0	1.0	284
NFORAU2	51	1.0	7.5	1123
NBYRAU1	166	1.0	1.6	275
YCLOAU1	147	1.0	5.5	201
YALEAU3	23	1.0	15.9	1700
YALEAU4	32	1.0	13.6	1097
YCHAAU2	35	1.1	19.2	1863
YGILAU2	47	1.9	4.7	1050
YUGHSU1	71	1.0	7.6	1285
YUGHSU2	45	1.0	19.7	2700
YHUNAU1	101	1.0	2.9	351
YHUNAU3	77	1.0	3.7	440
YCLOAU3	116	1.0	4.9	315
YALEAU1	44	1.0	13.5	980
YCHAAU4	72	1.5	3.2	773
YGILAU1	81	1.0	1.1	541
YGILAU3	79	1.4	3.3	774
YGILAU4	76	1.0	2.3	743
YLIMSU1	62	1.0	5.4	724
YLIMSU4	113	1.0	1.0	853
YUGHAU1	83	1.0	10.2	242
YUGHAU4	118	1.0	8.8	343
YSHEAU1	104	1.1	2.0	766
YSHEAU4	67	2.1	5.4	750
NHELSU1 (NZ194357)	55	1	1	418
NHELSU3 (NZ191353)	39	1	2.89	888
NLAMSU1 (NY673595)	129	1	1.27	142
NLAMSU2 (NY673596)	50	1	6.14	336
NACOSU1 (NY929659)	105	1	1	349