The comparison of macroinvertebrates and diatoms as two biotic indices of water pollution

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THE COMPARISON OF MACROINVERTEBRATES AND DIATOMS AS TWO BIOTIC INDICES OF WATER POLLUTION

by

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ABSTRACT

The value of macroinvertebrates and diatom communities as monitors of water quality was studied at 25 sites in the North East of England. The aim was to compare and contrast the two methods on their benefits as environmental quality indicators. Few studies have been made on a critical level between the two groups although similar reasons in support of their use have been made.

Invertebrates were collected by a Standard kick sample and community quality was evaluated using a BMWP score. Diatom communities were scraped from stones and a Generic Diatom Index was applied to the result.

Both methods appeared to give good indications of levels of organic pollution, though the correlation between the methods was poor. Multivariate analyses suggested that in both cases physical environmental factors overrode water quality in detecting community composition and that the precise nature of these was different for macroinvertebrates and diatoms. It does seem that diatom methods of water quality monitoring should be further studied as an addition to the solely used invertebrate methods

CHAPTER 1

INTRODUCTION

1.1 General Introduction

The earliest procedures used to monitor water quality in the U.K relied purely upon physical and chemical techniques and biological methods were largely ignored. Although it was subsequently accepted that water pollution was a biological phenomenon and should be measured as such, for a long time there was still a tendency to regard biological and chemical methods as alternatives (James,1979). The main advantage that physical and chemical monitoring was thought to provide was speed, ease and precision of sampling e.g. the measurement of a maximum temperature achieved over a given period, but other physio-chemical parameters are not so easily sampled. Sampling needs to be done at frequent intervals and then may only provide a transient picture (Hellawell,1986). Chemical monitoring of the effects of pollution in rivers is difficult often requiring measurement at the exact time of the pollution incident. There are methods of using automatic sampling apparatus which do give more indicative pictures of conditions, but these are still likely to miss the extremes.

Against this background the use of biological methods appears very attractive as organisms are continuously exposed to all aspects of environmental variants and provide an integrated response to any ecological imbalance. The only problem is to be able to accurately describe and comprehend the distributions of biota that are observed and what is expected to occur. It should then prove possible to undertake methods of pollution control and to manipulate the environment (Hellawell,1986). It is now generally agreed that both chemical and biological methods of water quality assessment are necessary (James,1979), and if the biologist and chemist work together they can discover much more together than alone (Hynes,1960)

1.2 Biological Indicators

The idea of an organism being used as to give an indication of the quality of freshwaters relies on the organism having a specific known tolerance to a particular environmental variable. Once the limits of tolerance of the organism are established then the presence of that organism in a specific habitat can

define given parameters of that habitat. The idea of an indicator can go beyond the idea of presence or absence of an organism in a particular site; the proportions of different organisms are also important. Furthermore the indicator species may also undergo stress and suffer impaired biological functions such as decreased reproduction rate or it may accumulate chemicals into its body tissues so that they reflect the levels found in the environment. These types of indicator are termed bio-sensors and bio-accumulators respectively.

1.2.1 Selection of indicators

The selection of a suitable indicator depends upon what is to be measured. In freshwaters the 'ideal' is an organism that is readily identified taxonomically; is easily sampled qualitatively and quantitatively; is widely distribution; readily accumulate pollutants; has an abundance of autecological data; is economically important as a resource or as a pest; is easily cultured in the laboratory and has low variability in genetic and habitat niche (Hellawell,1978). All of the major taxonomic groups of organisms possess some of the above criteria but to varying degrees and all have been used as indicator species. There are two groups that are utilised more frequently that the others; these are macroinvertebrates and algae.

In the early days of biological monitoring in Britain invertebrates and algae were both used but in later work macroinvertebrates have figured almost exclusively. Hellawell selects macroinvertebrates as good indicators for biological monitoring of rivers because they form a heterogeneous assemblage of animal phyla therefore it is probable that some members will respond to whatever stresses are placed upon them. They are mainly sedentary thereby allowing detection of the precise location of pollutant sources. Most macroinvertebrates have relatively long life histories and are able to colonise virtually all habitats encountered in British rivers. They are easily sampled quantitatively though qualitative sampling is harder. There also exist numerous taxonomic keys to identify all species effectively.

1.3 The History of Biological Monitoring of the UK

Biological methods of assessing water quality have been used in Europe since the beginning of the twentieth century when Kolkwitz and Marsson (1908,1909) proposed the Saprobien system. They recognised four stages in the oxidation of organic matter - Polysaprobic, α mesosaprobic, β mesosaprobic and oligosaprobic. These four zones are related to decreasing pollution and their sequence reflects self-purification (Wilhm, 1975). Each stream zone is also based on the occurrence of organisms that are also classified into four groups according to their pollution tolerance.

Little work was done in Britain before the 1950's and this was not received enthusiastically by water authorities. It was not until the beginning of the 1960's that a few authorities developed biological methods for classifying the pollution conditions of rivers. Of particular note was the Trent Biotic Index, a system introduced by the former Trent River Board in the late 1950's and described later by Woodiwiss (1964). He believed that a coded form of presentation is the only practical way of presenting the biological data alongside the corresponding chemical statistics.

The index was based on two principles (i) the number of species in a community decreases with pollution; based on the theories of community diversity (ii) in a polluted stream there is a progressive loss of certain 'key' species as the degree of pollution increases. The index was only attributable to organic pollution and eleven classes were recognised. The grading of a site was based on the presence of key taxa and the number of indicator groups of macroinvertebrate fauna collected in the sample. The grading classes (O to X) as water quality increases, can be derived several ways by different combinations of community diversity and indicators, except for the extreme classes, X and O, where an abundance of 16+ indicator groups of Plecoptera nymphs leads to class X only and if no organisms except those not requiring oxygen are present in the sample then class O is assigned. The number of indicator groups fall into the categories: 0-1, 2-5, 6-10, 11-15, and16+ and were designed to be reached without the need for lengthy taxonomic techniques. However the index took no account of amounts of the indicator species nor was any standard sampling procedure cited, nonetheless the procedure was simple and an assessment could be achieved in the field if preferred, which is it's strength as an index.

Graham (1965) adapted the Trent index for use in Scotland as the Lothian Index, in this form the index had only six grades and also took no account of community diversity.

The next major step was a modification that took account of the abundance of the key taxa (Chandler,1970). The index was named the Biotic Score and produced five levels of abundance, the score assigned to each taxon being weighted in relation to its abundance. The tolerance of the species to organic pollution is also taken into account. Abundant intolerant species gain high scores, abundant intolerant species low scores. The taxa in this method have to be counted as well as identified therefore the index takes longer to derive than the Trent Index. There is no upper limit to the value of the biotic score, but a lower limit of zero is obtained when no macroinvertebrates are present.

Although there was criticism of this method it was widely used for a number of years and several modifications were made. Hellawell (1978) believed the Biotic Score to have met the requirements of a suitable pollution index.

In 1970 the Department of the Environment introduced their River Pollution Survey distinguishing water quality according to four classes, A, B, C and D, each containing characteristic groups of animals indicative of clean to polluted conditions. The survey was based on the earlier work of Carpenter (1926) and was based only on the appearance or disappearance of individuals from the community. This system was applied in a national survey of all the rivers in England and Wales and it was not successful as it failed to represent all rivers satisfactorily, particularly deep, slow flowing rivers (eg. those found in East Anglia) and because of this the Biological Monitoring Working Party (BMWP) was set up in 1976 to provide a system that could be used to assess the biological condition of a river and could do so for all the rivers in the U.K (Chesters,1980; Armitage et al.,1983). The biological score system as recommended by the BMWP was described as a score for the assessment of the biological quality of rivers suitable for all sites for national survey purposes. At each site a three minute kick sample, was collected and all families present at that site are listed. A score system was derived that assigns a score to each family on the basis of their tolerance to organic pollution. Scores for all families are added to give a total cumulative site score. The number of taxon at each site is also recorded enabling an Average

Score per Taxon (ASPT) to be calculated and used in site assessment. There was no account taken of abundance: families are recorded as either present or absent.

The development of multivariate statistic techniques during the 1960's and 70's permitted new approaches to be developed. This direction was followed by a team from the Freshwater Biological Association, (now the Institute of Freshwater Ecology) who set out to develop procedures to predict the expected fauna at given sites by the use of environmental features (Wright et al.,1984,1985). The project consisted of three phases: Phase 1 (1977-1981) made use of TWINSPAN (Hill,1979b) a cluster analysis technique to classify unpolluted sites, selected on 41 river systems, according to macroinvertebrate fauna and demonstrated that the concept of using environmental features to predict the fauna at unpolluted sites to be viable (Wright et al.,1984, Furse et al.,1984). Phase 2 (1981-1984) enlarged the number of sites to encompass 61 river systems and calculated the probability of finding a given species at any site (Moss et al.,1987).

Finally Phase 3 resulted in a software program, RIVPACS (River InVertebrate Prediction and Classification System) which took prediction methods further by enabling the classification of sites on the basis of their macroinvertebrate fauna and then using multiple dicriminant analysis (MDA) to find the combination of environmental variables which best replicate the site groupings of the classification (Wright et al.,1989). Taxa can be entered at family or species level and permit a site specific prediction based on environmental features which can be used to set a target from which any loss in biological quality due to environmental stress can be measured by the ratio of observed/expected scores (Wright et al.,1989).

This is set to become the standard tool for water quality monitoring in Britain in the 1990's. Whilst it is an improvement on earlier indices in that it measures all the different types of stress to which invertebrates respond it still makes the assumption that all environmental groups of the biota are responding in the same way as macroinvertebrates. Though widely held, this assumption has never been rigorously tested.

1.4 Diversity Indices

Alongside the development of biotic scores another group of techniques based around the concept of ecological diversity were being considered. Diversity indices can also be used to measure stress in the environment and the application of these to biological monitoring is based on the idea that communities suffering stress will show reduced diversity. It is considered that unpolluted environments are characterised by a large number of species with no single species making up the majority. If an environment becomes stressed then species sensitive to that particular stress will be eliminated thereby reducing the richness of the community, whilst certain favoured species will become more abundant (Archibald, 1972).

A great number of diversity indices have been derived to gauge the adverse effects of pollution and environmental disturbance. These include models of community structure such as the lognormal distribution model (Preston, 1948). Species richness (S) is the most widely adopted measure of diversity (Magurran, 1988). The simplest index is the Sequential Comparison index (Cairns et al.,1968) which requires no taxonomic knowledge and the sampler merely distinguishes between individuals on the basis of shape, size or colour, leading to a very unsubtle albeit effective approach (Mason,1989). Indices have also been developed that take no account of any assumed community structure. These work by just relating the numbers of species to the total number of individuals found, or representing the numbers of species within a total community (Hellawell, 1978). Generally it is accepted that environments that are stressed or polluted do shift from the log normal pattern of species abundance and will show an increased dominance of one or two species with a decrease in species richness (Magurran,1988). A review by Archibald (1972) showed that all indices give a similar measure of diversity but should be used with caution as indicators of water quality. Sampling method, area sampled, time of year and level of identification are all found to influence the diversity index of benthic stream macroinvertebrates (Hughes,1978). Hellawell (1986) agreed with the findings of Archibald (1972) and recommended the Sequential Comparison Index (Cairns et al.,1968) and Simpson's Index (1949) as the best measures of diversity.

Hawkes (1979) recommends the use of both diversity indices and biotic scores for a comprehensive surveillance strategy of rivers because each responds to effects induced by different types of pollution. However, diversity indices do need fully quantitative data to be effective (Magurran,1989). Moreover, a substantial amount of ecological information is lost when a diversity index is calculated.

1.5 Biological Monitoring using Diatoms

Against this background it has been thought that there is a need to expand the methods employed by biologists to give a broader picture when monitoring. Diatoms have figured in water quality assessments from the earliest studies (Kolkwitz & Marsson,1909.,Butcher,1947). The case for algae is put by Whitton (1979) who states that algae are better indicators of most chemical features of the environment whereas invertebrates are better indicators of physical features and oxygen concentrations. An important point recognised by Whitton (1979) is that algae have the advantage over invertebrates of a more cosmopolitan distribution enabling data from different parts of the world to be compared directly.

Diatoms are often the most abundant autotrophic organism in rivers colonising all available surfaces (Round,1992). Unlike invertebrates they don't have specific food requirements or specialist habitat niches nor are they governed by streamflow (Round,1992). He proposes the use of epilithic diatom communities as the ideal indicator. The reasons behind this idea are that the epilithic diatom community is easily sampled being visible to the naked eye and easily identified by touch. The epilithon offers the advantage encompassing a single niche and therefore reducing the confusion gained from comparing data from several communities. The diatom community is present throughout the whole of the river throughout the whole of the year and is sensitive to water quality, eutrophication and pollution but relatively insensitive to physical features. The cell cycle is rapid so enabling a quick reaction to perturbations in the environment. The actual sampling methods are judged more efficient by Round (1991) than those for invertebrates and cell counting by microscope is rapid and accurate, the cell numbers per unit area of substratum are very large meaning random counts give an excellent assessment of the flora. In the method of preparation for microscopy permanent slides are made enabling long term storage for future comparisons.

The use of diatoms in France were developed after problems with the monitoring of canalised streams using the standard techniques of the water authorities, macroinvertebrates (Prygiel,1991). The French algologists stated that the use of benthic diatoms allowed them to take eutrophication and salinity into account as well as organic pollution. The system was successful with diatoms providing good indication of the water qualities of the canals. Hellawell (1978) and Mason (1989) also mentioned that algae are useful monitors for eutrophication estimates with attached forms being sensitive to turbidity. They both do not recommend them for use in monitoring heavy organic pollution and indicate they are less sensitive to pesticides and heavy metals than invertebrates. Hellawell (1978) also implies that a high level of taxonomic expertise is needed to identify samples but the same applies for some invertebrate groups, and that it is difficult to distinguish between dead and living cells. However it is thought that analyses of plant material are of more value than that of the water and invertebrates as plants integrate events in the environment over long periods (Whitton, 1975).

1.6 Aims

It is clear from the above that different workers often cite very similar reasons in support of different groups of organisms as routine monitors of pollution. Relatively few workers have made critical comparisons between groups and therefore study of the community composition of two groups, macroinvertebrates and diatoms collected from a range of water qualities, was planned. The null hypothesis is that no differences exist between the estimations of water quality gained using these two groups.

CHAPTER 2
MATERIALS AND METHODS

2.1 Introduction

Samples were collected from 25 sites between May and July 1992. Full details of sites are given in chapter 3 and Appendix A. All samples were collected from within defined 10m 'reaches' (Holmes & Whitton, 1981)

2.2 Physical and Chemical Variables

2.2.1 Field Measurements

Physical and chemical variables recorded in the field included substratum type, average depth and width, current, conductivity, temperature, pH, total alkalinity, dissolved oxygen and bankside shading.

Substratum was estimated by eye as a percentage according to the Wentworth Scale.

Average depth at 3 points within the reach and width were measured in centimetres and metres respectively.

The measurement of current used a calibrated Ott current meter. The impeller was positioned at the point of fastest current in the reach about 10cm below the surface and perpendicular to direction of flow (Patterson,1983).

Conductivity and temperature were measured using a WTW meter (model FC910).

pH was determined using a WTW meter (model pH91).

Total alkalinity was determined by titrating 50ml of water with 0.02M Hydrochloric acid to an end point of pH 4.2. Total alkalinity (meq l^{-1}) was calculated as follows:

$$\text{Total alkalinity} = \frac{\text{vol of HCL added to end point (ml)} \times \text{normality of acid}}{\text{vol of sample (ml)}}$$ (Golterman et al.,1978)

Measurements of oxygen were made using a WTW meter (model OX 191).

The degree of bankside shading was measured on a scale of $1 \rightarrow 5$.

2.2.2 Collection of water samples

Water samples were collected in 250ml iodised, polypropylene bottles, which had been acid washed in 10% H_2SO_4 for at least an hour and rinsed six times in distilled water. In advance of filling they were rinsed twice on site in stream water. Samples were stored in an ice box until return to the laboratory.

2.2.3 Lab Procedures.

Water for Phosphorus analysis was filtered immediately on arrival through Whatman GF/F filters washed with Milli-Q (MQ) water. Phosphorus analysis was performed the same day. Water for Nitrite, Nitrate and Ammonia analyses was deep frozen until needed.

All glassware used for chemical analyses was acid washed in H_2SO_4.

For sites 1→4 Nitrite, Nitrate, Ammonia and BOD_5 analyses were performed in Durham. All other chemical analyses were performed by the NRA, Washington Lab, according to methods approved by the Standing Committee of Analysts.

2.2.4 Phosphorus

Phosphorus was determined using the modification of the method of Murphy & Riley (1962) proposed by Eisenreich et al.(1975). The detection limit was 0.001 mg l^{-1} P.

2.2.5 Nitrite

Nitrite was determined by method of N-1-napthylethylenediamine dihydrochloride (Stainton et al.,1977). The detection limit was 0.005 mg l^{-1} N.

2.2.6 Nitrate

Nitrate was reduced to nitrite by a cadmium-copper couple and analysed as for nitrite (Stainton et al.,1977) . The detection limit was 0.005 mg l^{-1} N.

2.2.7 Ammonia

Ammonia was determined by the Indophenol blue method (Stainton et al., 1977). The detection limit was 0.005 mg l^{-1} N.

2.2.8 Biochemical Oxygen Demand (BOD)

BOD was determined by measuring O_2 concentration in samples before and after incubation in the dark at 20°C for five days. (Golterman et al., 1978). The detection limit was 1.0 mg l^{-1} O.

2.3 Macro invertebrates

2.3.1 Field Procedures

Macroinvertebrates were sampled using a standard three minute kick sample as employed by the NRA. It involves disturbing the area immediately upstream of a vertically held pond net with the feet. Dislodged fauna are caught in the net. All the major habitats present within the sample area are sampled. A pond net with mesh size 900μm was used.

Samples were transported in plastic bottles in an ice box to the laboratory.

2.4.2 Lab Procedures

Samples were initially sorted by eye in flat bottomed white trays and preserved in 70% alcohol. Further examination by binocular microscope identified the specimens to family (except class Oligochaeta) using available taxonomic keys (Cranston, 1982; Croft, 1986; Eddington & Hildrew, 1981; Elliott & Mann, 1979; Elliott, Umpesch & Macan, 1988; Fitter & Manuel, 1986; Friday, 1988; Hynes, 1977 and Macan, 1959). Problematic samples were checked by biologists at the NRA laboratory, Washington.

Counts of all specimens collected enabled a BMWP score (Table 2.3) and an ASPT to be calculated (D.O.E,1983).

Table 2.1 The BMWP score system

Families	Score
Siphlonuridae Heptageniidae Leptophlebiidae Ephemerellidae Potamanthidae Ephemeridae Taeniopterygidae Leuctridae Capniidae Perlodidae Perlidae Chloroperlidae Aphelocheriidae Phryganeidae Molannidae Baraeidae Odonteoceridae Leptoceridae Goeridae Lepidostomatidae Brachycentridae Sericostomatidae	10
Astacidae Lestidae Calopterygidae Gomphidae Cordulegasteridae Aeshnidae Corduliidae Libellulidae Psychomyiidae Philopotamidae	8
Caenidae Nemouridae Rhyacophilidae Polycentropodidae Limnephilidae	7
Neritidae Viviparidae Ancyclidae Hydroptilidae Unionidae Corophiidae Gammaridae Platycnemididae Coenagriidae	6
Mesoveliidae Hydrometridae Gerridae Nepidae Naucoridae Notonectidae Pleidae Corixidae Haliplidae Hygrobiidae Dytiscidae Gyrinidae Hydrophilidae Clambidae Scirtidae Dryopidae Elmidae Hydropsychidae Tipulidae Simuliidae Planariidae	5
Baetidae Sialidae Piscicolidae	4
Valvatidae Hydrobiidae Lymnaeidae Physidae Planorbidae Sphaeriidae Glossiphoniidae Hirudidae Erpobdellidae Asellidae	3
Chironomidae	2
Oligochaeta (whole class)	1

2.4 Diatoms

2.4.1 Field Procedures

Samples of the diatom population were taken from the epilithon. Five cobbles or small boulders, that were fairly free of filamentous algae, were selected, rinsed in stream water and then scraped with a toothbrush to remove the diatom film. Samples were transported in 250ml polythene bottles in an ice

box to the laboratory. The samples were allowed to settle overnight in the refrigerator, the supernatant was decanted and transferred to a boiling tube and allowed to settle again before cleaning took place.

2.4.2 Lab Procedures

Preparation of clean frustules for microscopy involved removing organic material using strong oxidising agents. The method used was that of Carter (unpublished) and involved digestion by the addition of 5ml conc. H_2SO_4, two crystals of Potassium permanganate and 10ml saturated Oxalic acid. The solution was allowed to sediment overnight. It was then centrifuged in distilled water five times to remove all traces of acidity.

Permanent slides were made by spreading drops of sample evenly on cover slips, these were dried on a hot plate at 80°C and then mounted on a slide using Naphrax, a high resolution mountant.
Samples were examined under oil immersion and identified to genus using available keys (Barber & Haworth, 1981) and taxonomic works (Round et al., 1990).

A count of approximately 200 individuals was made and the Generic Diatom Index (Rumeau & Coste, 1988) was applied. The GDI is based on two scores assigned to each diatom genus, S: the resistance to pollution with a score of 1 to 5, where 1 = very resistant/tolerant and 5 = very sensitive , and V: the ecological niche of usefulness as an indicator of the genus with a score of 1 to 3, where 1 = strong, 2 = medium and 3 = weak (Table 2.2).
The GDI is calculated as follows:

$$GDI = \frac{\Sigma S_i A_i V_i}{\Sigma V_i A_i}$$

Where A_i = count (±200 frustules) of genus i, V_i = ecological amplitude and S_i = resistance

Table 2.2 Genera and their scores for calculating the GDI

Taxa	S	V
Achnanthes	5	1
Amphipleura	5	3
Amphora	3	2
Anomoeoneis	5	2
Asterionella	4	1
Attheya	2	3
Caloneis	4	2
Campylodiscus	5	2
Ceratoneis (Hannaea)	5	2
Cocconeis	4	1
Cyclotella	3	1
Cymatopleura	4	2
Cymbella	5	1
Denticula	5	3
Diatoma	4	1
Diploneis	5	1
Epithemia	5	2
Eunotia	5	1
Fragilaria	4	1
Frustulia	5	2
Gomphoneis	4	2
Gomphonema	3	2
Gyrosigma	4	3
Hantzchia	1	3
Melosira	3	1
Meridion	5	2
Navicula orthostichae	2	2
Navicula punctate	1	2
Navicula (others)	3	1
Neidium	4	3
Nitzchia dissipatae	4	2
Nitzchia (others)	1	1
Pinnularia	4	3
Rhizosolenia	2	3
Rhoicosphemia	4	1
Rhopalodia	5	3
Stauroneis	5	2
Stenopterobia	5	3
Stephanodiscus	2	1
Suriella	3	3
Synedra	3	1
Tabellaria	5	1
Tetracyclus	5	3
Thalassiosira	2	3

ASPT was calculated for diatom data by dividing the GDI score by the number of taxa found at each site. Round's system (1992) was also utilised when assessing the data (Table 2.2). This is based on dividing a river into 6 zones, with zone 1 found in the uppermost reaches and zone 5 in lowland reaches. Zone 6 is indicative of saline conditions caused by eg. salt mining. Dominant diatom fauna are associated with each zone as well as physio-chemical parameters.

Table 2.3 Round's System for diatom classification of rivers

Zone	pH	Alkalinity	Dominant diatom genera
1:cleanwater,uppermost reaches	3.6-4.1	none	*Eunotia* *Achnanthes* *Tabellaria* *Pinnularia*
2: nutrient richer, higher pH	5.6-7.1	2.8-5.7	*Hannaea* *Fragelaria* *Achnanthes* *Diatoma* *Meridion*
3: nutrient rich	6.5-7.3	5.0-23.3	*Achnanthes* *Cymbella* *Cocconeis* (lower reaches) *Reimeria* (lower reaches) *Amphora* (lower reaches)
4: Eutrophic, restricted flora	unspecified	unspecified	*Gomphonema* *Amphora* (absent) *Cocconeis* (absent) *Reimeria (absent)*
5: Flora grossly restricted	unspecified	unspecified	*Navicula* small spp. *Nitzchia* small spp. *Gomphonema*
6: Saline effects	unspecified	unspecified	*Synedra*

2.5 Computing and Statistics

2.5.1 Diversity Indices

The index of Simpson (1949) , D, was calculated for both data sets.

$$D = \frac{n_i (n_i - 1)}{N (N - 1)}$$

where n_i = number of individuals of the ith species and N = total number of animals in the sample.

D increases as diversity decreases therefore Simpson's Index in this study is expressed as 1–D (Magurran,1988).

The diversity index was calculated for invertebrates and diatoms at a family and genus level respectively. Simpson's Index was applied because it is thought to be generally unaffected by the level of identification (Pinder, 1987). It is also recommended as very effective on theoretical grounds and most useful for water management purposes (Hellawell, 1977). Simpson's Index has also got a fixed measure of diversity 0→1, enabling comparisons and trends in diversity to be discussed (Archibald, 1972)

2.5.2 Correlation

Comparisons between biotic indices, ASPT, diversity indices and physical and chemical parameter between the two data sets were made by calculating r, the Pearson Product - Moment correlation coefficient

2.5.3 Site Classification

In this study, TWo way INdicator Species ANalysis (TWINSPAN) was used (Hill, 1979b). Two data sets were compiled: invertebrates - all species and diatoms - all species. For each data set classifications were examined at division levels, 1→4, these having two, four, eight and sixteen end groups respectively (except if a group becomes too small for further division).

The levels of abundance used are termed as pseudospecies cut levels and followed a logarithmic scale as used by the NRA, Washington:

Pseudospecies abundance	Original abundance level
1	1
2	10
3	100
4	1000

Basically the program divides sites into groups by repeated dichotamization. The program first constructs a classification of the sites then uses this classification to obtain a classification of the species according to their ecological preferences. The two classifications are then used to obtain an ordered two-way table expressing the species synecological relations as concisely as possible. The program also constructs a key to the site classification by identifying indicator species which are particularly

diagnostic of each division in the classification. These 'differential' species are ones with clear ecological preferences therefore they can be used to identify particular environmental conditions (Hill,1979b).

2.5.4 Ordination

Ordination allows communities to be organised in a system of coordinates so that the most similar appear closest together (Davis, 1986). For the ordination of both data sets, DEtrended CORrespondance ANAlysis (DECORANA) was used (Hill, 1979b). This method is regarded as superior to other ordination methods as it avoids the tendency of rare species to distort the analysis (Hill, 1979a).

The main purpose for using ordination in this study was to correlate the axes scores of the two data sets to assess the similarity of the individual axis strengths. The eigen values of the individual axes may be regarded as a measure of their relative diversity and indicate an important environmental variable.

2.5.5 Computing

Classification, correlation and ordination were carried out on Viglen VI/33 computers. The text of this project was processed using Microsoft *Word* for *Windows*. The graphics were produced using the Cricket graph software package

CHAPTER 3

FIELD SITES

3.1 General Introduction

All sites were situated in the North East England. Twenty five sites were selected for study. These included a wide range of water qualities and both upland and lowland sites. The sites fell into five geographical categories and are found on four river systems.

A. Lowland Durham Coalfield

This is an area of dense population. It is farmed extensively and exploited for industry (Dewdney, 1970). The three sites in the Darlington district are also grouped within this category.

B. Weardale.

This area of has been heavily mined in the past for lead. The landscape is a mixture of limestone and sandstone and the most upland sites comprise moorland that is grazed by grouse and sheep.

C. Alston Moor

This is an area of the north that was formally a very productive lead mining area in the past. The geology of this area is as for Weardale.

D. Allendale

This is also an upland area that was mined heavily and now comprises mainly upland moorland grazing that is divided into two arms, the east and the west. The geology is as for Weardale.

E. North Tynedale

A lowland valley of the R.North Tyne. Anthropogenic influences come from farming activities. The River North Tyne is regulated by Kielder Water.

Water quality categories are those supplied by the NRA, Northumbrian Region. A→D indicates a decreasing level of water quality.

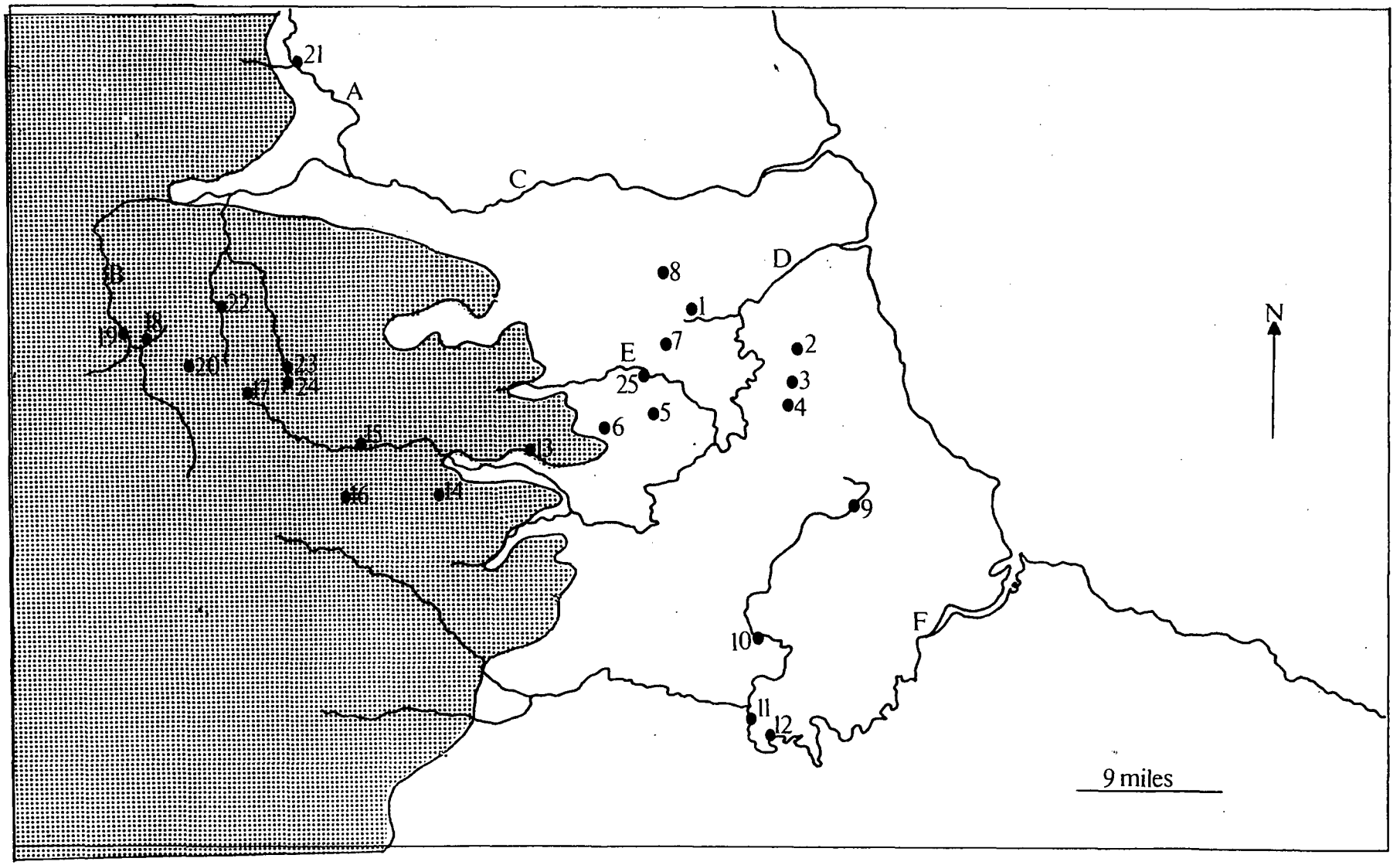
3.2 Sites

Fig.3.1 shows a map of sites.

Fig. 3.1Map of sites

The map shows details of all sites and the main river systems. The overlay shows land over 250m.

Key to main rivers	
A	R.North Tyne
B	R.South Tyne
C	R. Tyne
D	R.Wear
E	R.Browney
F	R.Tees



Land above 250m

Fig 3.1

3.2.1 Site Descriptions

The details of physical and chemical environment of the sites is illustrates in Tables 3.2 and 3.3.

A. Lowland Durham Coalfield - all sites are tributaries of the R.Wear.

1.Twyzell Burn.

The site is a lowland polluted stream near to the town of Chester le Street. The stream was grossly polluted, and sewage fungus was evident throughout.

2.Coalford Burn

A lowland polluted stream, on the Permian limestone, containing a lot of coal waste, at East Rainton near to Durham.

3.Sherburn Hall Beck.

A lowland stream near to Broomside Hall, Durham. The stream bed was coated with Iron oxide.

4.Old Durham Burn.

This stream at Sherburn is heavily organically polluted with a dense growth of *Cladophora* covering the substratum

5.R.Deerness, Ushaw Moor

A relatively clean lowland site to the east of Durham.

6.R.Deerness, East Hedleyhope.

A clean site in the upper part of the catchment.

7.Cong Burn.

This lowland site at Edmondsley had a lot of coal waste and evidence of Iron oxide deposition.

8.R.Team.

A small stream at Beamish, which contains both organic pollution and Zn from a factory at Stanley. It is a tributary of the R.Tyne.

25.R.Browney.

The sample area of this river was fairly clean, despite being downstream of Langley Park sewage treatment works.

9.R.Skerne, Fishburn.

This site was approximately 5 km below Hurworth Burn reservoir. It was a slow flowing ponded stretch with dense growth of *Spirogyra* and macrophytes (eg. *Callitriche*).

10.R.Skerne.

This site at Coatham Mundeville contains heavy organic pollution and abundant *Cladophora* covering the substratum.

11.R.Tees, Blackwell Bridge.

The site sampled was a wide unpolluted reach above the point where the R.Skerne drains into the R.Tees from Darlington.

12.R.Tees, Hurworth.

This site was downstream of the confluence of the R.Skerne from Darlington and was moderately organically polluted.

B.Weardale

13.Houselop Beck.

This site close to Tow Law is an upland site, just below a pig farm that in the past was responsible for extensive pollution.

14.Bollihope Burn.

The site at Bollihope is relatively free from heavy metals, although there are many disused mine workings close by. The area is grazed by sheep.

15.R.Wear.

The Wear at Westgate is a fast-flowing site dominated by *Lemanea*.

16.Swinhope Burn.

This stream was sampled at Swinhope Head near to an old disused mine workings. The area near to the stream was used for cattle pasture.

17.Kilhope Burn.

A small acid upland stream in a moorland area.

C.Alston Moor

18.R.Nent., Alston

This site was sampled just above the confluence with the R.South Tyne. The Nent has a high concentration of Zn and extensive growth of *Stigeoclonium.*

19.R.South Tyne.

The site at Alston was just above the confluence with the R.Nent. It is a medium-sized fast-flowing upland river dominated by *lemanea* and *Rhynchostegium.*

20.R.Nent.

This site lies between Nenthead and Alston.

D.Allendale

22.R.West Allen.

The site at Coalcleugh was an upland small stream polluted by Zn in an area of moorland and rough grazing.

23.R.East Allen, above Allenheads.

This is a small site draining from upper fells and a conifer plantation.

24.R.East Allen, below Allenheads.

This site receives inflows from disused mine workings.

21.R.North Tyne

This site at Wark was a wide site that is relatively free from pollution. The flow regime is regulated by Kielder Water.

3.2.2. Site Criteria

Table 3.1 overleaf lists sites in order of sampling. It indicates date of sampling, water quality and altitude of sites. Further details of sites are given in Appendix A.

Table 3.1. Details of sites sampled

Site no.	Site Name	Date	Grid Ref.	Water Quality	Altitude(m)
1	Twyzell Burn	13.5.92	NZ257517	D	60
2	Coalford Burn	13.5.92	NZ343474	C	100
3	Sherburn Hall Beck	13.5.92	NZ320434	C	80
4	Old Durham Burn	13.5.92	NZ311424	C	60
5	R.Deerness, U.M	26.5.92	NZ226422	A	90
6	R.Deerness. E.H	26.5.92	NZ156403	A	180
7	Cong Burn	26.5.92	NZ236496	A	90
8	R.Team	26.5.92	NZ226540	D	70
9	R.Skerne, F	2.6.92	NZ377306	B	90
10	R.Skerne, C.M	2.6.92	NZ291207	C	60
11	R.Tees, B.Br.	2.6.92	NZ270125	A	40
12	R.Tees, H	2.6.92	NZ312101	B	30
13	Houselop Beck	17.6.92	NZ103383	A	190
14	Bollihope Burn	17.6.92	NZ005350	A	250
15	R.Wear, W	17.6.92	NY908380	A	250
16	Swinhope Burn	17.6.92	NY897347	A	410
17	Kilhope Burn	17.6.92	NY809432	A	530
18	R.Nent, A	25.6.92	NY716467	A	270
19	R.South Tyne, A	25.6.92	NY716467	A	270
20	R.Nent, N	25.6.92	NY767448	A	375
21	R.North Tyne, W	14.7.92	NY863769	A	83
22	R.West Allen, C	14.7.92	NY802453	A	530
23	R.East Allen, A	14.7.92	NY858448	A	400
24	R.East Allen, bl.A	14.7.92	NY856458	A	450
25	R.Browney	14.7.92	NZ222454	B	100

Details of physical and chemical measurements at study sites are listed in Tables 3.2 and 3.3.

Table 3.2 Details of physical measurements at sites

						Dissolved Oxygen	
Site No.	**pH**	**Temp °C**	**Current ms^{-1}**	**Alk meq l^{-1}**	**Cond. µS cm^{-1}**	**Oxygen %**	**Oxygen mg l^{-1}**
1	7.5	12.6	0.346	16.97	1100	100	11.0
2	7.8	12.3	0.102	33.90	1910	106	11.2
3	7.8	15.8	0.192	55.90	2250	136	13.5
4	7.6	15.7	0.275	61.90	2250	127	12.9
5	5.6	13.8	0.399	26.90	1353	120	15.5
6	7.8	11.2	0.573	13.97	939	107	12.0
7	7.8	13.3	0.041	5.50	1155	106	11.4
8	9.0	16.9	0.071	3.50	769	113	11.3
9	7.7	13.8	0.037	11.97	1160	27	2.8
10	7.0	16.5	0.026	7.48	924	91	8.0
11	7.1	18.2	0.610	3.00	195	108	11.8
12	7.5	18.6	0.297	3.99	381	97	9.1
13	8.0	10.2	0.131	1.50	200	115	12.5
14	7.6	13.1	0.240	4.00	224	107	11.4
15	7.8	12.4	0.193	6.00	322	109	11.6
16	8.0	16.7	0.169	5.00	217	103	10.3
17	8.1	18.4	0.102	0.50	340	104	9.6
18	8.3	12.4	0.566	8.00	559	114	12.4
19	7.6	14.7	0.420	8.50	327	U/A	U/A
20	8.3	15.9	0.454	6.50	616	120	11.7
21	8.2	15.0	0.742	3.00	137	107	10.7
22	7.6	12.4	0.089	7.00	377	108	11.0
23	7.4	14.6	0.279	1.50	132	93	9.1
24	7.6	13.8	0.274	5.20	180	108	10.8
25	7.7	15.1	0.324	9.70	666	106	10.8

Alk. = Alkalinity , Cond. = Conductivity , U/A = unavailable

Table 3.3 Details of site chemical measurements

Site No.	BOD_5 (mgl^{-1} O)	NO_3-N ($mg\ l^{-1}$)	NH_4-N ($mg\ l^{-1}$)	FRP ($mg\ l^{-1}$)	FUP ($mg\ l^{-}$
1	52.0	12.420	0.060	3.054	4.596
2	19.0	1.680	0.030	0.016	0.022
3	20.0	1.700	0.030	0.035	0.045
4	21.0	1.130	0.050	0.027	0.040
5	<1.0	1.100	U/A	<0.001	<0.001
6	1.0	0.680	U/A	0.067	0.073
7	<1.0	0.850	U/A	<0.001	<0.001
8	2.0	14.200	U/A	1.070	1.120
9	2.6	0.630	0.040	0.694	0.827
10	3.7	11.000	0.780	2.567	3.086
11	<1.0	0.720	0.030	0.063	0.040
12	2.5	3.600	0.190	0.719	0.846
13	<1.0	1.100	<0.005	<0.001	0.016
14	<1.0	0.450	<0.005	<0.001	<0.001
15	<1.0	0.300	<0.005	0.006	0.003
16	2.3	<0.005	<0.005	<0.001	0.002
17	<1.0	<0.005	<0.005	<0.001	<0.001
18	<1.0	0.140	<0.005	0.003	0.003
19	3.4	<0.005	<0.005	0.004	0.004
20	2.2	0.140	<0.005	0.008	0.006
21	4.6	0.380	0.030	0.003	0.040
22	2.4	0.250	0.020	<0.001	0.032
23	6.6	0.490	0.010	0.093	0.118
24	5.1	0.750	0.020	0.092	0.116
25	5.1	4.050	0.050	0.039	0.990

BOD_5 = Five Day Biochemical Oxygen Demand

NO_3-N = Nitrogen as nitrate

NO_2-N = Nitrogen as nitrite

NH_4-N = Nitrogen as ammonia

FRP = Filtrable Reactive Phosphorus

FUP = Filtrable Unreactive Phosphorus

U/A = unavailable

CHAPTER 4

RESULTS OF MACROINVERTEBRATE SAMPLING

4.1 Faunal Composition

A total of forty one families (except Class Oligochaeta) were obtained from all sites, with between 3 and 22 families found per site (Appendix B)

4.2 Biotic Indices

BMWP scores, ASPT and the numbers of taxa per site (Table 4.1) showed wide variation. BMWP scores varied from 6 (at the R.Team) and 144 (at the R.North.Tyne), ASPT scores from 2.00 (at the R.Team) and 7.31 (at the R.South Tyne) and the numbers of taxa from 3-22.

Table 4.1. Biotic scores, number of taxa and Simpson's diversity index (D) for invertebrate samples from all sites.

Site No.	No. Taxa	BMWP	ASPT	D
1	6	22	3.67	0.285
2	5	15	3.00	0.160
3	10	41	4.10	0.770
4	8	33	4.13	0.315
5	19	113	4.95	0.462
6	11	78	7.09	0.882
7	6	29	4.83	0.419
8	3	6	2.00	0.343
9	4	15	3.75	0.369
10	4	9	2.25	0.115
11	11	77	7.00	0.863
12	10	58	5.80	0.641
13	18	108	6.00	0.768
14	19	130	6.84	0.726
15	9	52	5.78	0.847
16	15	100	6.67	0.591
17	5	31	6.20	0.725
18	8	46	5.75	0.188
19	13	95	7.31	0.817
20	3	17	5.67	0.625
21	22	144	6.55	0.851
22	9	57	6.33	0.741
23	13	75	5.77	0.782
24	13	77	5.92	0.757
25	17	96	5.65	0.725

The mean BMWP scores and ASPT for each of the four water quality classes as determined by the NRA, Northumbrian Region were plotted (Fig.4.1 and Fig.4.2)

The mean values show that BMWP scores tend to fall with decreasing water quality as do ASPT values, although there is a lot of variation in both the range and about the mean.

4.3 Diversity Index

The value of Simpson's Index was calculated for invertebrate diversity at all sites (Table 4.1). Again, there was alot of variation, with values of D from 0.160 (at Coalford Burn) to 0.882 (at the R.Deerness at East Hedleyhope). There is a lot of variation. The mean diversity for each water quality category (Fig. 4.3) also tends to fall with decreasing water quality.

In order to give a better impression of how communities are structured the relative density of macroinvertebrate families in two populations are shown as percentages of the total number of individuals counted. The relative density of macroinvertebrates at a clean site i.e.water quality class A, th R.North Tyne at Wark (Fig 4.4) indicates a large amount of families with no one showing great dominance over the others. However at a grossly polluted site D, Twyzell Burn (Fig 4.5) there are a small number of families with one showing dominance over the others, Chironomidae, a low scoring group.

4.4 Correlation between Biotic Scores, Diversity Index and Physical and Chemical Parameters

Pearson's product moment correlation coefficient was calculated for most of the environmental variables studied (Table 4.2). Dissolved oxygen, temperature and pH data were not considered to encompass a wide enough range to use in the matrix.

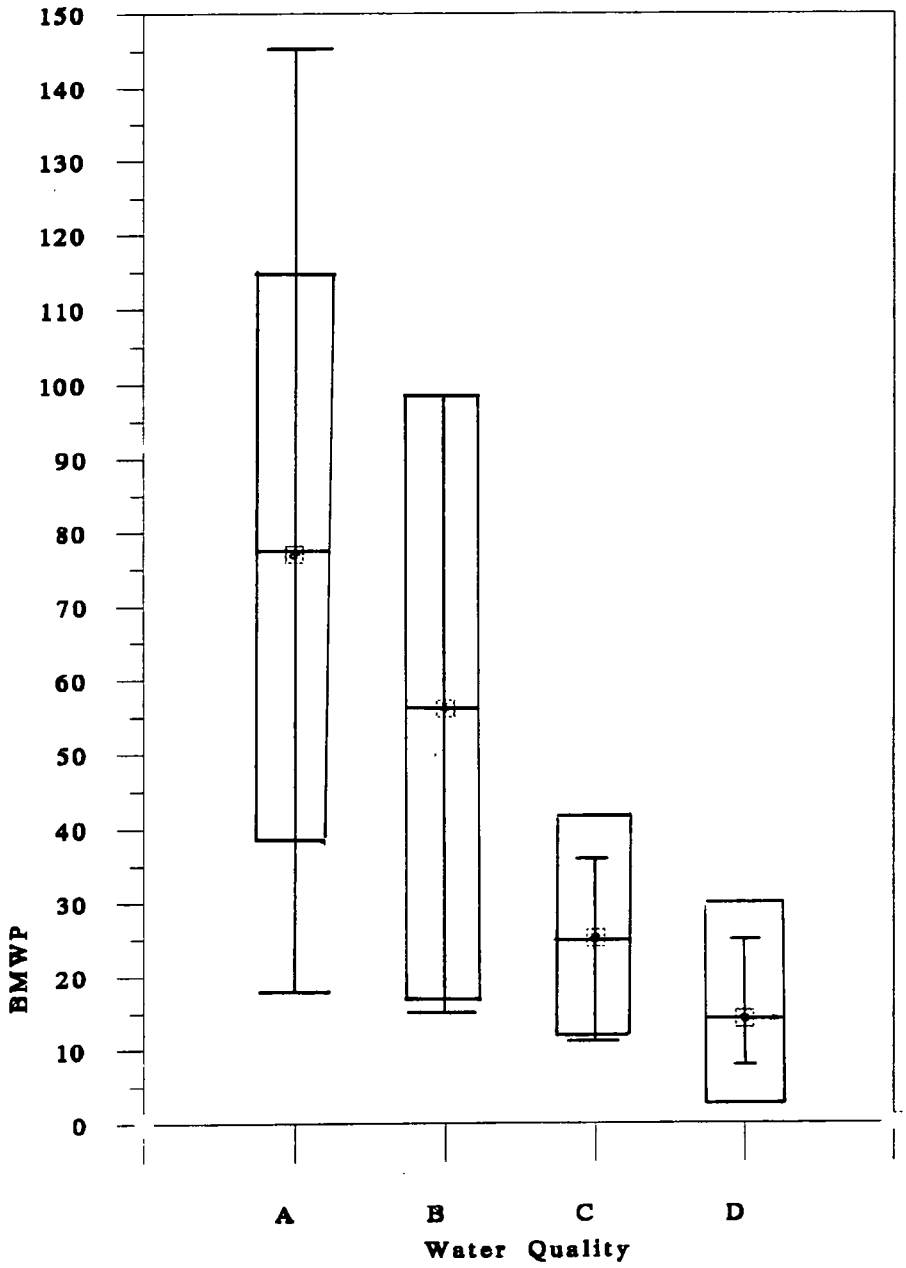


Fig 4.1 Range, mean+S.D for BMWP values plotted against water quality

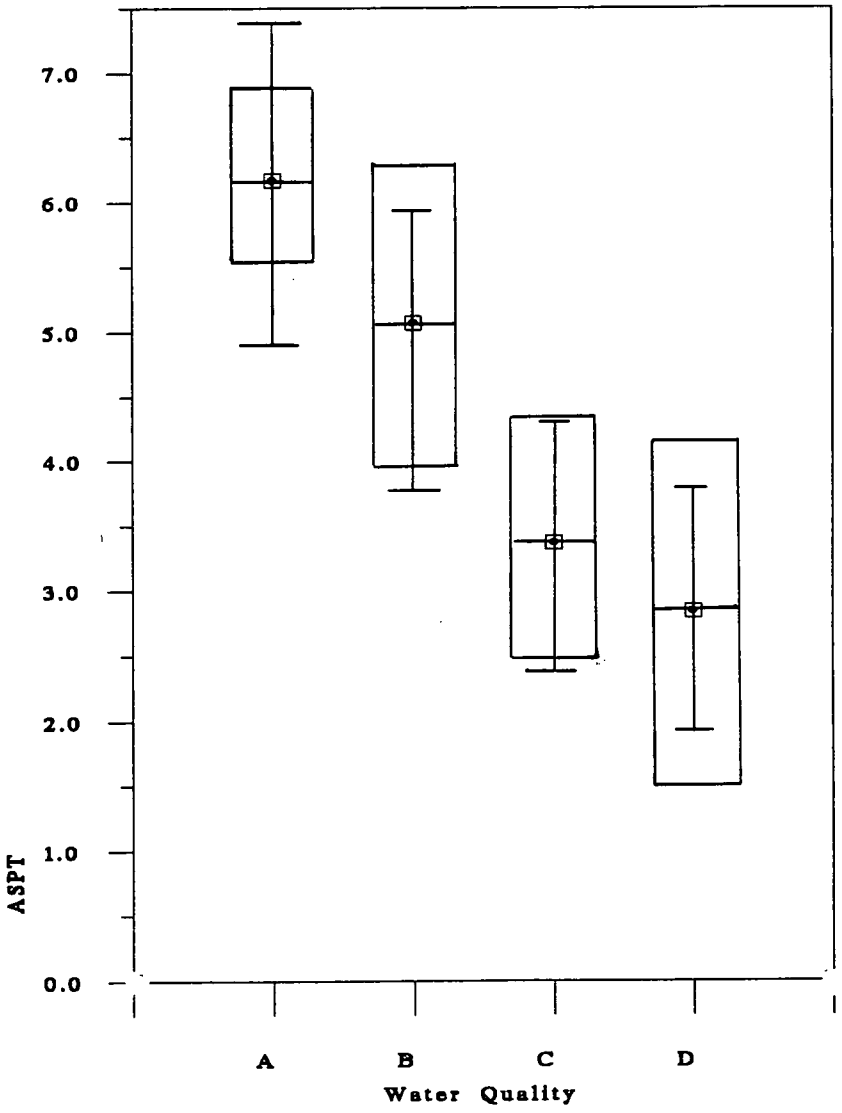


Fig 4.2. Range, mean+S.D for ASPT values against water quality

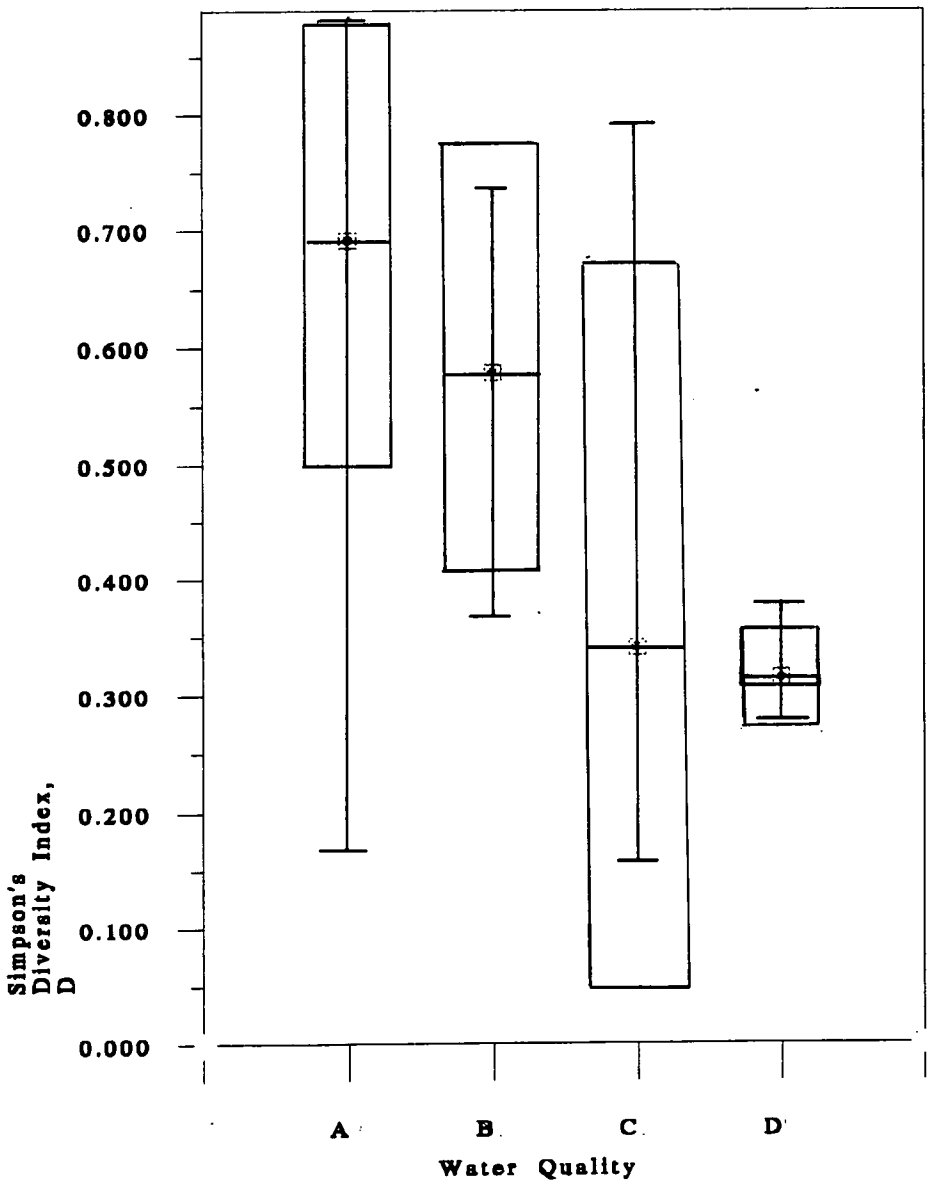


Fig.4.3 Range, mean+S.D for values of Simpson's diversity index for invertebrate communities against water quality

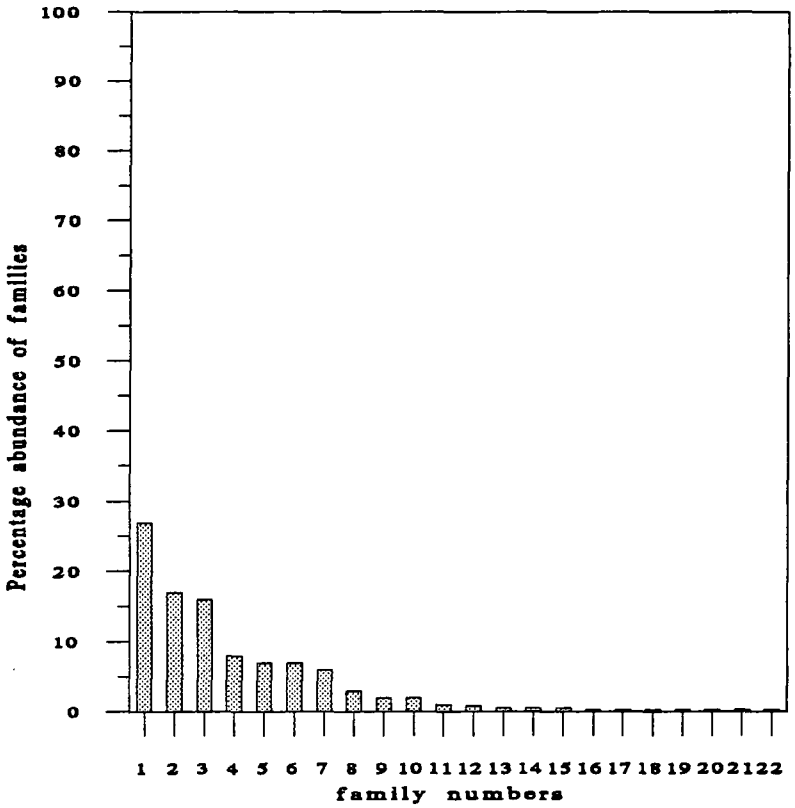


Fig 4.4 The community diversity at R.North Tyne, a clean site

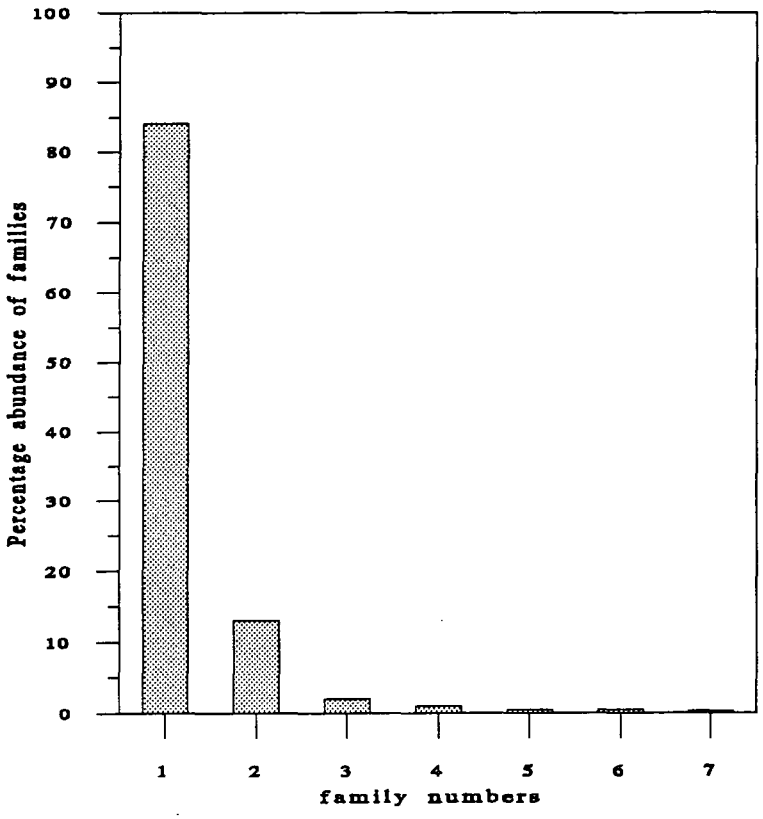


Fig 4.5 The community diversity at Twyzell Burn, a polluted site

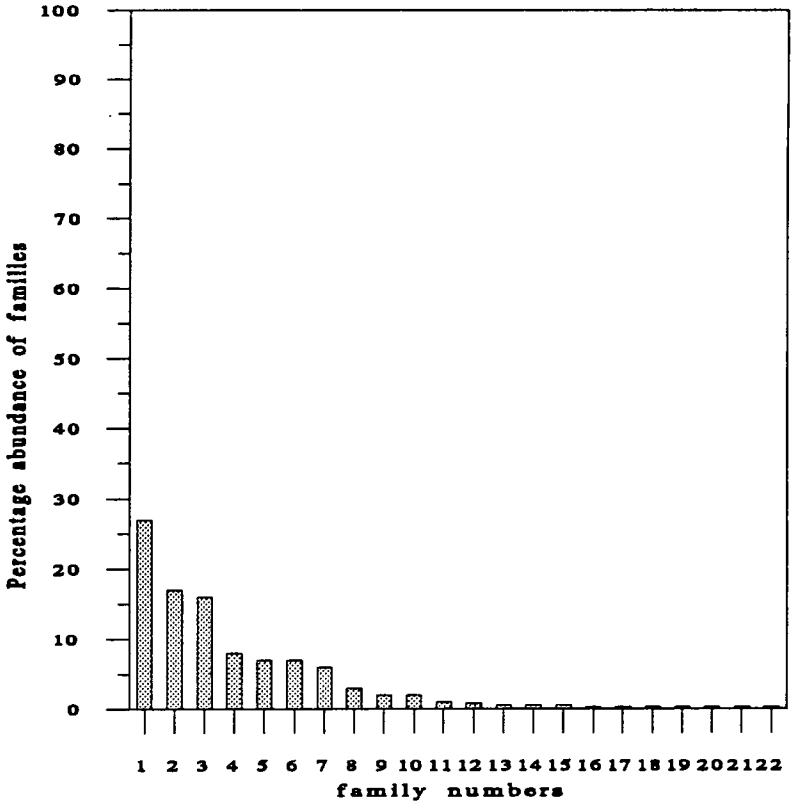


Fig 4.4 The community diversity at R.North Tyne, a clean site

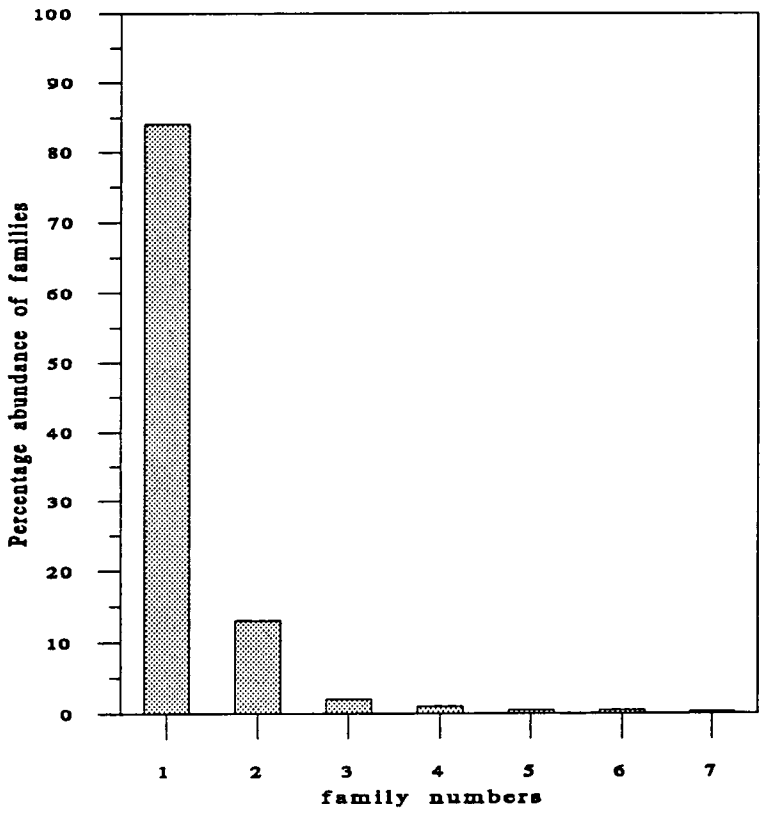


Fig 4.5 The community diversity at Twyzell Burn, a polluted site

Table 4.2 Correlation Matrix between invertebrate biotic scores, diversity and important physical and chemical parameters. Correlation scores are shown in the right half of the table, probability levels on the right. (** P < 0.01, * P < 0.05, N.S not significant, DF = 23, Alk. = Alkalinity, Cond = Conductivity).

	BMWP	ASPT	D	BOD	NH_4-N	NO_3-N	FRP	FUP	Alk.	Cond.
BMWP	1.000	**	**	N.S	*	*	*	N.S	N.S	*
ASPT	0.751	1.000	**	*	**	**	**	**	N.S	**
D	0.623	0.769	1.000	N.S	*	**	**	*	N.S	**
BOD	0.317	-0.436	0.349							
NH_4-N	-0.443	-0.679	-0.453							
NO_3-N	-0.443	-0.717	-0.505							
FRP	-0.448	-0.607	-0.526							
FUP	-0.383	-0.550	-0.478							
Alk.	-0.240	-0.367	-0.292							
Cond.	-0.483	-0.605	-0.526							

The relationship between the different indexes applied to invertebrate scores show a good agreement. BMWP was positively correlated strongly with ASPT (Fig 4.6) and D (Fig 4.7).

The relationship between nutrient levels and the biotic indexes is as expected in waters receiving organic enrichment. NH_4, NO_3, FRP all were negatively correlated, though at different levels, with BMWP, ASPT and D. BOD only correlated significantly ($P<0.05$) with one index, ASPT. FUP correlated highly with ASPT and D but not with BMWP.

The relationship between the physical measures taken showed that conductivity correlated significantly

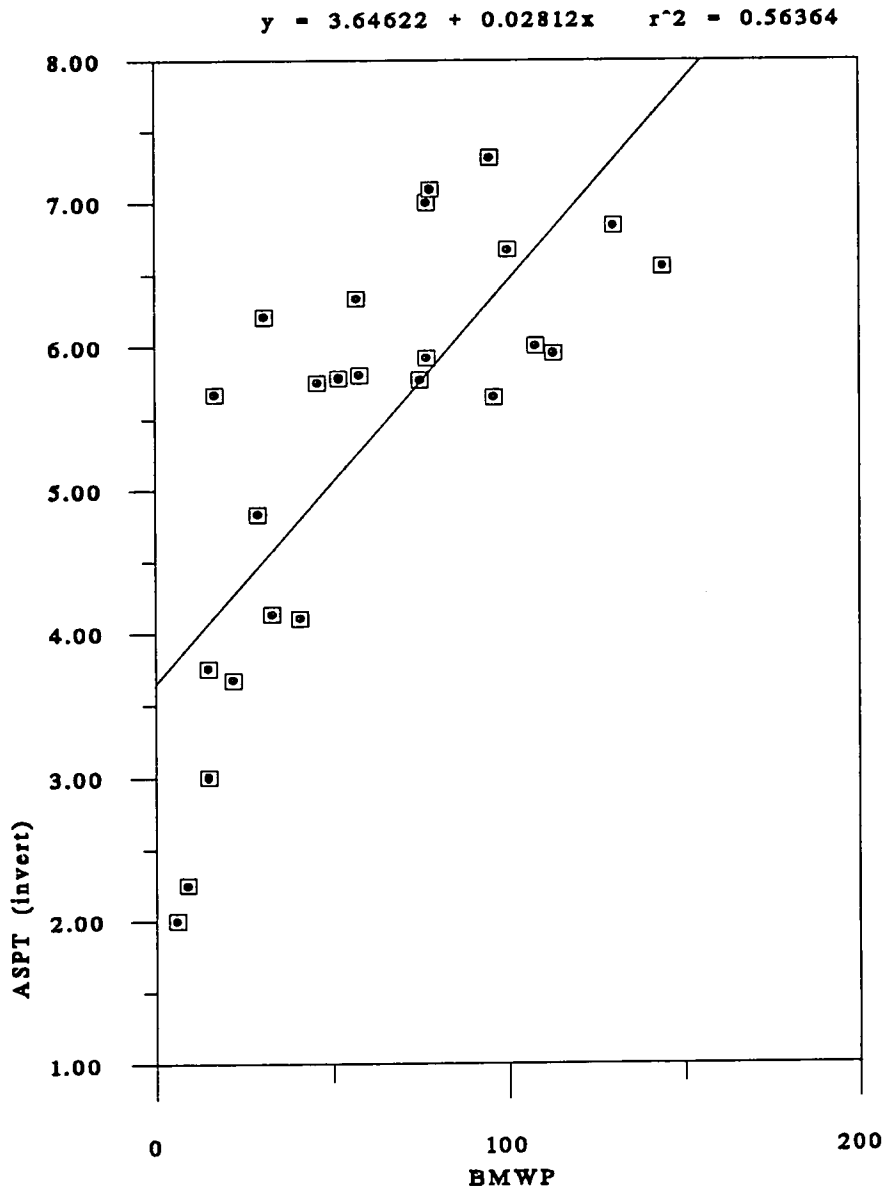


Fig 4.6 Correlation between BMWP scores and ASPT values for all invertebrate data

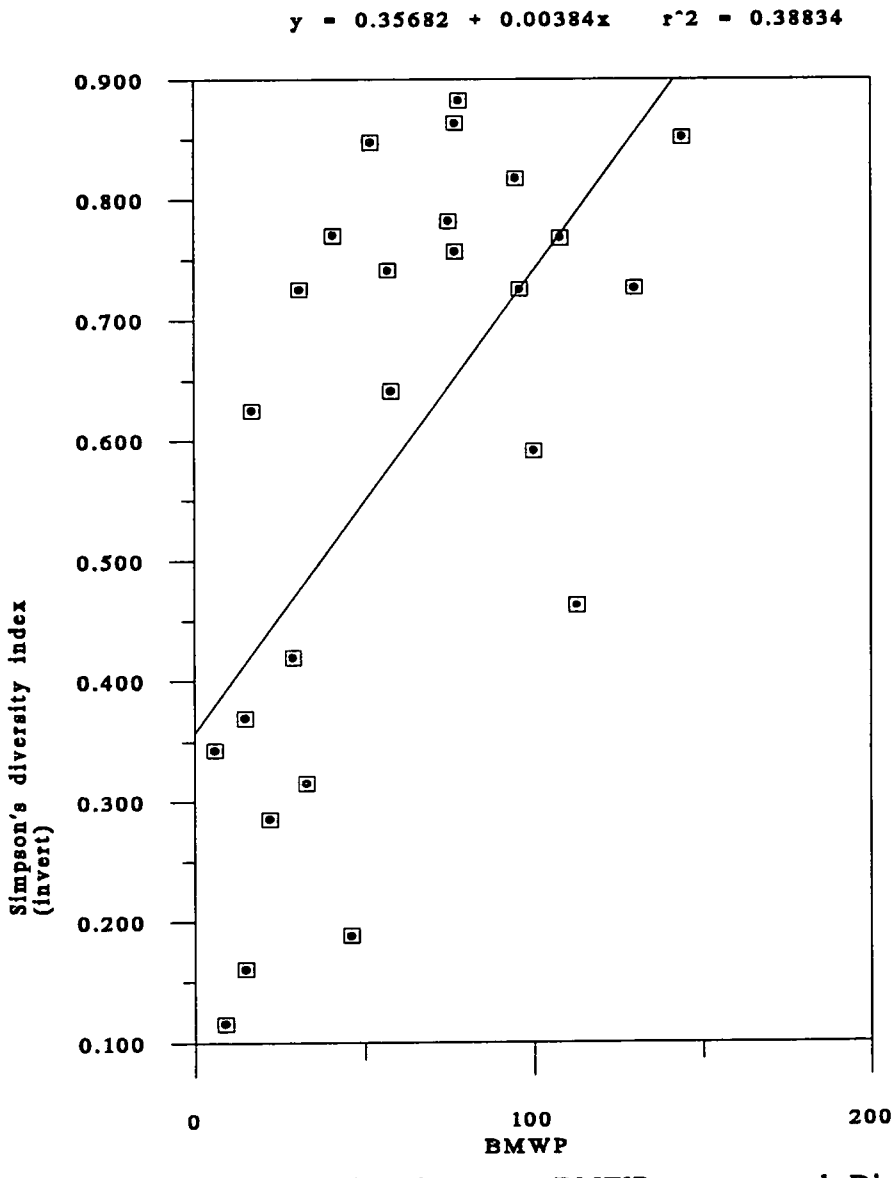


Fig.4.7 Correlation between BMWP scores and Diversity index (invert)

with all the indices, thought to be an artefact of the study where most of the polluted sites were found on Permian limestone, the hard water there lead to high conductivities. Alkalinity was not significantly correlated with any of the invertebrate indices.

4.5 Site Classification

TWINSPAN classified the 25 sites according to the abundances of the 41 families found at them. The classification was taken to four levels (Fig 4.8) to be consistent with the method used for the diatoms, which if it had been left at three levels resulted in an unsatisfactory very large group of sites. The resulting classification is presented as a dendrogram along with median values for selected environmental variables.

At level 1 the TWINSPAN classification produced two groupings; the upland sites and the lowland sites, except for two sites, both lowland sites, the R.North Tyne at Wark which can be regarded as quite upland in character and Cong Burn. The latter has a high conductivity and a low BMWP score, 29. It was separated out into this position presumably due to the presence of the f.Rhyacophilidae at the site. The indicator species for the positive sites (Asellidae, Polycentropodae and Oligichaeta) all have low individual BMWP scores and are common to the lower reaches of rivers. The fifteen negative sites were in turn separated according to indicator species common to clean upland streams (Leuctridae, Ryacophilidae). These both have high BMWP values.

The divisions at level 2 separated each of these groups into a group composed largely of polluted sites and one composed largely of unpolluted sites. The positive division into group 5 contains upland sites with lower conductivity values and lower alkalinity values but higher BMWP scores. The sites in group 4 are mainly polluted with heavy metals and, as there are veins are associated with the Carboniferous limestone these also tend to be harder.

On the other side of the division at level 2 there is a separation into the less polluted lowland sites (group 6) and the sites with very high conductivity, alkalinity, BOD and less taxa and lower BMWP

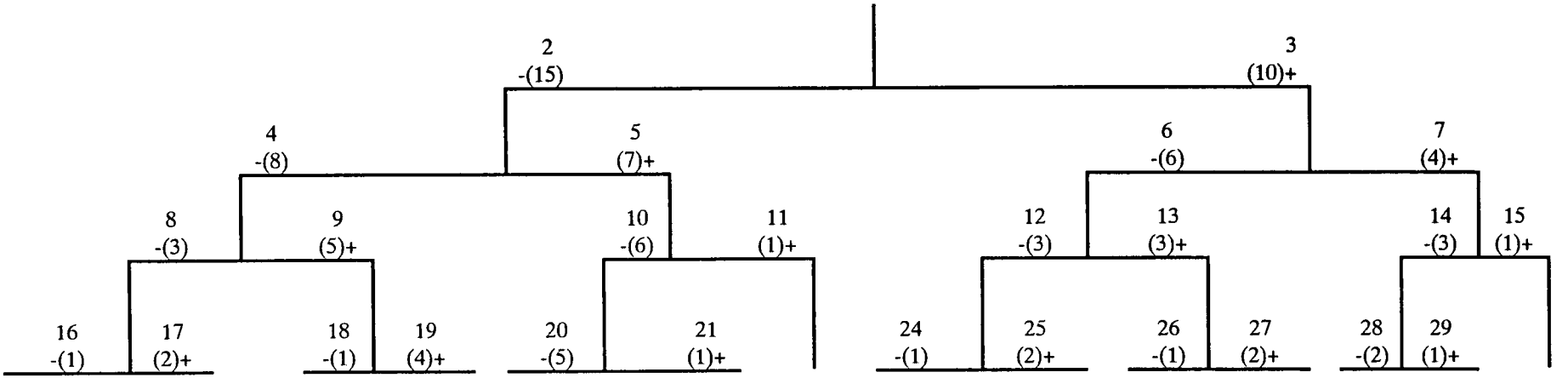
scores, the organically polluted lowland sites (group 7). The relationship between organic pollution and conductivity may, as in section 4.4 represent an artefact of the sampling programme.

The indicator species used at division 2 follow the observed classification with Heptageniidae, a high scoring invertebrate characteristic of upland clean sites used to separate the sites into group 4 and 5, by being the positive indicator for sites in the cleaner group 5. The positive indicator for the very polluted sites in group 7 was Gammaridae, a low scoring species characteristic of the more polluted sites.

The classification at levels 3 and 4 separated sites into six and seven divisions respectively. At level 3 the sites were grouped according to narrower divisions of water chemistry and each associated with a particular invertebrate assemblage.

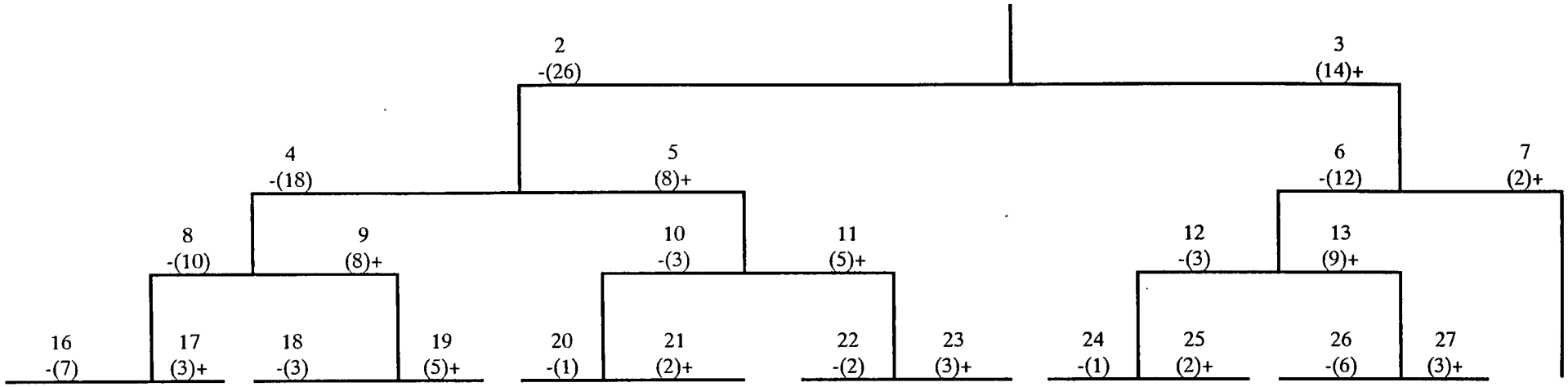
4.6 Distribution of species at sites

The classification of species obtained by the TWINSPAN analysis (Fig.4.9) illustrate the results of the site classification in section 4.5. Those families with high BMWP values (group 1 or 2 of the BMWP classification) are those families that are classified out into the left hand side of the dendrogram, i.e the families indicative of cleaner conditions usually found in the upland reaches of rivers (Trichoptera, Plecoptera, Ephemeroptera and some Dipteran families). Those invertebrates classed as groups tolerant of pollution and common of the lower reaches of rivers (Hirudinea, Oligochaeta, Gastrapoda and other Dipterans) are found to the right of the dendrogram



Median values														
Altitude (m)	90	278	530	335	270	250	83	90	95	30	65	60	100	80
Width (m)	5.0	3.5	1.0	4.9	7.0	4.0	96.4	4.0	3.3	40.0	8.0	4.0	2.0	2.0
BOD_5 mg l^{-1}	+	1.6	2.4	+	2.3	+	4.6	+	3.9	2.5	2.9	36.5	19.0	20.0
FRP mg l^{-1}	+	0.038	+	0.005	0.004	+	0.003	+	0.366	0.719	1.819	1.541	0.016	0.035
Cond.µS cm^{-1}	1155	778	377	331	200	224	137	1353	913	381	847	1675	1910	2250
Alkal.meq l^{-1}	5.50	10.24	7.00	3.75	5.00	4.00	3.00	26.90	10.84	3.99	5.49	39.44	33.90	55.90
BMWP	29	48	57	49	95	130	144	113	56	58	8	28	15	41
No.Taxa	6	7	9	8	13	19	22	19	10	10	3	7	5	10

Fig4.8 Dendrogram of TWINSPAN classification to four levels using invertebrate families. The group number at every division with numbers of families in that group shown in parenthesis. Median physical, chemical and biotic scores are shown underneath.



	Lepidost. Sericost. Libellul. Philopot. Ancycl. Hydropt. Hydrphil.	Perldidae Perlidae Chloroperl.	Leuctrid. Goerid. Sialid.	Nemourid Rhyacoph. Polycentro. Dytiscid. Simul.	Elmid.	Siphlonur. Baetid.	Heptagen Psychomy.	Limnephil. Hydropsy. Tipulid.	Lymnaeid.	Leptophleb. Ephemerel.	Leptocerid. Caenidae Haliplidae Glossopho. Erpobdel. Aselidae	Gammar. Sphaerid. Oligocha.	Hydrobi. Chironom.
Median Value													
Individ.BMWP score	9	10	10	8	6	7	9	6	4	10	5	4	3

Fig.4.9 TWINSPAN classification to four levels of invertebrate families. The number of each group are shown and the numbers of invertebrate families in each group are shown in parenthesis.

CHAPTER 5

RESULTS OF DIATOM SAMPLING

5.1 Floral Composition

A total of 18 genera were obtained from all sites with between 5 and 13 being found per site (Appendix C).

5.2 Biotic Indexes

The Generic Diatom Index (GDI) varied from 2.686, at Cong Burn to 4.857, at the R.Wear at Westgate. ASPT values calculated varied from 0.269, at Cong Burn to 0.810, at the R.West Allen with numbers of taxa varying as mentioned in 5.1. (Table 5.1).

Table 5.1 The numbers of taxa found at each site along with the Generic Diatom Index (GDI) values, ASPT and Simpson's diversity index, D for each site.

Site No.	No. Taxa	G.D.I	ASPT	D
1	6	3.384	0.564	0.696
2	8	4.306	0.538	0.732
3	9	3.039	0.338	0.626
4	7	3.096	0.442	0.491
5	9	3.122	0.347	0.652
6	10	4.588	0.459	0.403
7	10	2.686	0.269	0.749
8	9	3.240	0.360	0.600
9	9	3.351	0.372	0.766
10	5	2.907	0.581	0.552
11	12	4.000	0.333	0.809
12	13	3.622	0.279	0.762
13	7	4.245	0.606	0.441
14	9	3.867	0.483	0.653
15	9	4.299	0.478	0.527
16	8	4.340	0.482	0.560
17	8	4.729	0.591	0.210
18	10	3.985	0.399	0.563
19	9	4.196	0.466	0.654
20	8	4.814	0.602	0.330
21	9	3.891	0.432	0.780
22	6	4.857	0.810	0.178
23	9	4.068	0.452	0.807
24	9	3.876	0.431	0.765
25	10	3.801	0.380	0.801

The mean GDI scores when plooted against water quality do show a decrease in value as water quality decreases (Fig 5.1), although, as in 4.2 there is a lot of variation, ASPT does not exhibit such a clear relationship with mean values falling from class A to B but rising for classes C and D. (Fig. 5.2). This suggests an inability of ASPT values to give a clear indication of water quality; however the numbers of sites falling into water quality classes was very uneven with class A comprising 16 of the study sites; B comprising 3 sites and C and D classes containing 4 sites and 2 sites respectively. The numbers of taxa does not exhibit a very wide variation (5 to 13) which could account for the failure of ASPT values to reflect water quality classes effectively.

5.3 Diversity Index

Simpson's Index values for diatom data at all sites exhibits a wide variation 0.178, at R.West Allen to 0.809, at the R.Tees at Hurworth (Table 5.1).

The mean value for Simpson's diversity index does not decrease with decreasing water quality but remains around 0.600 to 0.700 for all categories of water quality (Fig 5.3). This appears to indicate a consistent diversity at even very polluted sites. The relative density of each genus in a poulation against the number of genera in the community at two different 'clean' sites (Fig. 5.4 and Fig. 5.5) indicates that although both sites, Kilhope Burn and the R.Tees at Blackwell Bridge, are classified A they exhibit very different community structures. Kilhope Burn site is dominated by a single genus, *Eunotia* and contains low numbers of a few other genera. The site at Blackwell Bridge has a large number of diatom genera without one being greatly dominated by another. A polluted site, Old Durham Burn was found to be dominated by one genus, *Navicula* and to contains low numbers of other genera. The use of diversity measures as an indication of water quality at the level of identification used in this study does not seen to be valid.

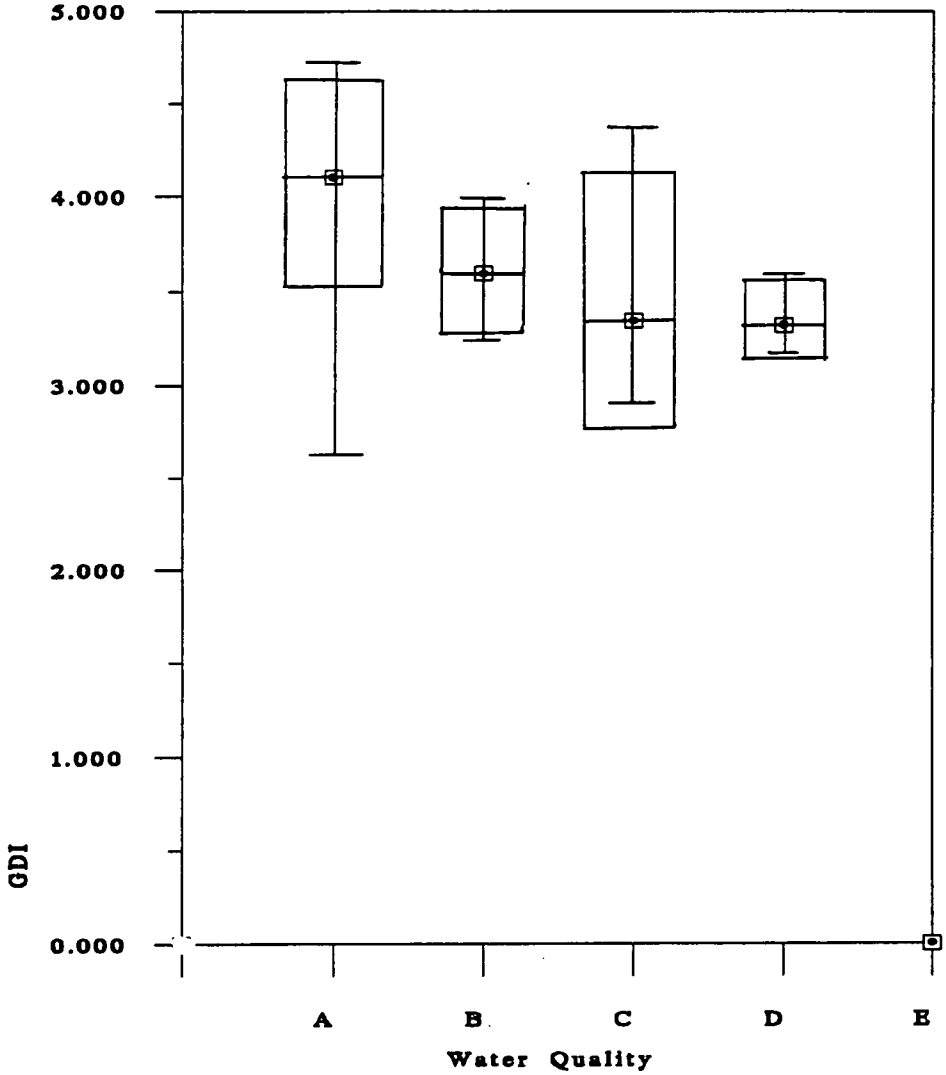


Fig 5.1 Range,mean+S.D for GDI at different water qualities

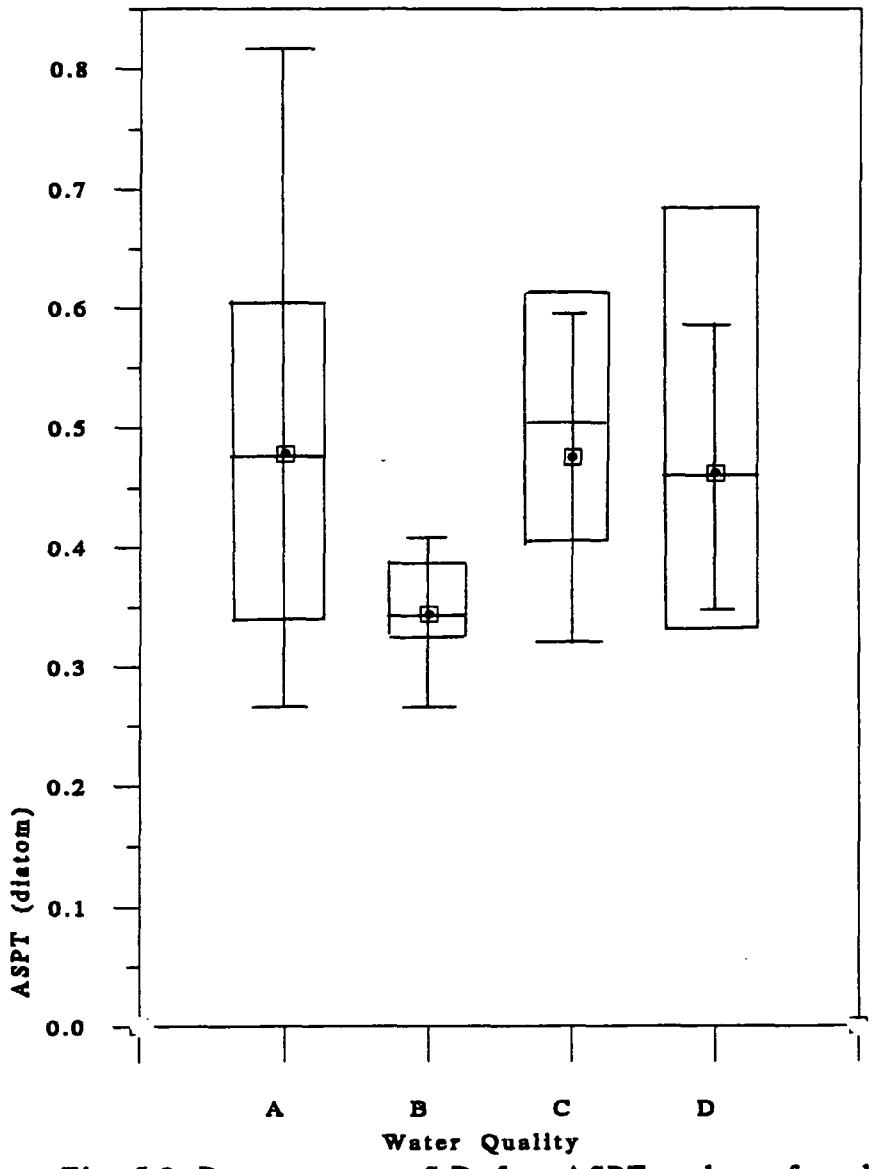


Fig 5.2 Range, mean+S.D for ASPT values for diatom genera against water quality

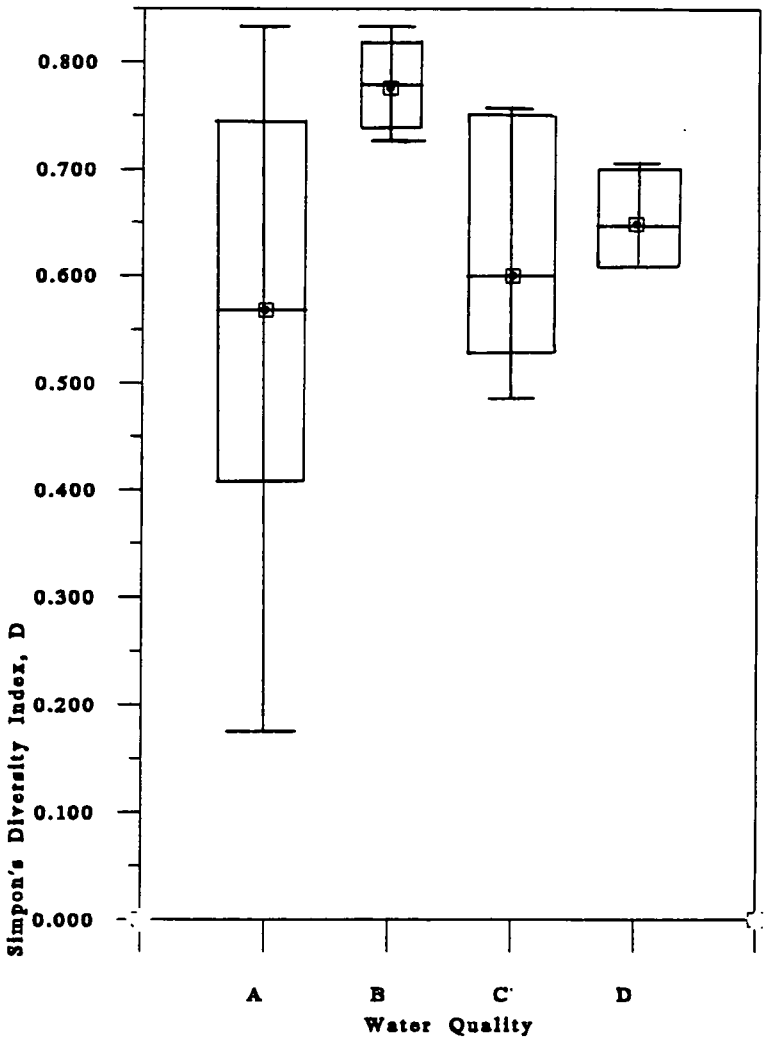


Fig.5.3 Range, mean+S.D for values of Simpson's diversity index for diatom communities against water qualities

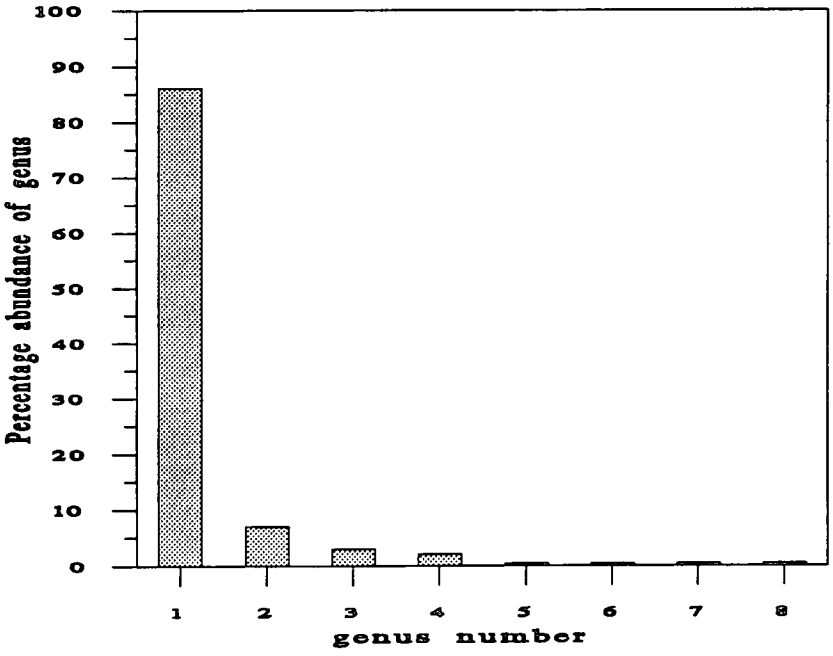


Fig.5.4 Community structure of diatoms at Kilhope

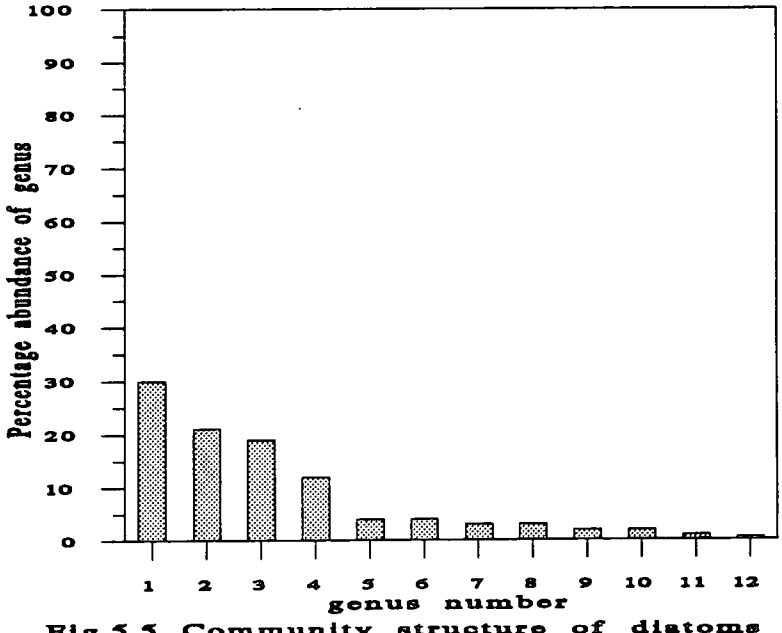


Fig.5.5 Community structure of diatoms at R.Tees at Blackwell Bridge

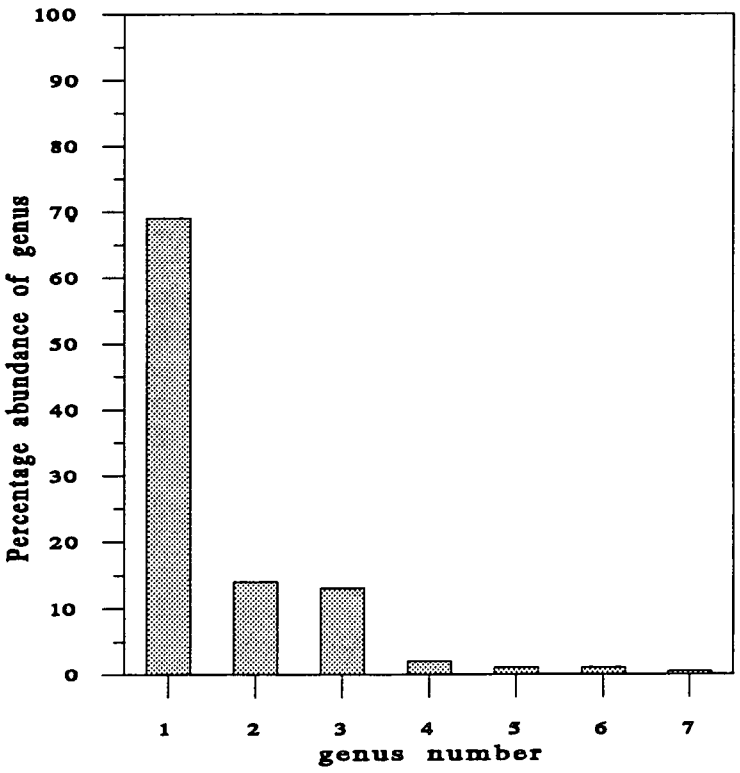


Fig.5.6 Community structure of diatoms at Old Durham Burn

5.4 Correlations between GDI, ASPT and Diversity with physical and chemical parameters

A correlation matrix was consructed for data, as in 4.4, (Table 5.2)

Table 5.2 Correlation Matrix between GDI, ASPT, D and physical and chemical parameters. Pearson's product-moment correlation coefficients are shown in the left half of the table, significant probablity values in the right half, (** P < 0.01, * P < 0.05, N.S - not significant, DF = 23, Alk. = Alkalinity, Cond = Conductivity).

	G.D.I	ASPT	D	BOD	NH_4-N	NO_3-N	FRP	FUP	Alk.	Cond.
GDI	1.000	**	**	N.S	*	*	*	N.S	N.S	**
ASPT	0.650	1.000	**	N.S	N.S	N.S	N.S	N.S	N.S	N.S
D	-0.523	-0.750	1.000	N.S	N.S	N.S	N.S	N.S	N.S	N.S
BOD	-0.269	-0.045	-0.163							
NH_4-N	-0.454	-0.140	-0.165							
NO_3-N	-0.468	-0.117	-0.128							
FRP	-0.422	0.012	0.117							
FUP	-0.392	0.005	0.156							
Alk.	-0.391	-0.155	0.000							
Cond.	-0.528	-0.222	0.036							

The agreement between biotic indices calculated was good. ASPT and G.D.I values were strongly correlated (P < 0.01). Diversity correlated with both GDI and ASPT (P < 0.01). NH_4-N, NO_3-N and FRP all correlated strongly (P < 0.05) with GDI, though not with BOD_5.

The relationship with conductivity and GDI also indicates a high level of significance (P < 0.01).As in 4.4 this is thought to be an artefact of the sampling program . There were no other significant correlations.

5.5 Site Classification

Sites were classified as for the invertebrates, using TWINSPAN to four levels, the results were illustrated in the form of a dendrogram together with physical, chemical and biological parameters deemed important. (Fig 5.7)

The division at level 1 split off three upland sites based on the presence of *Eunotia* as a positive indicator. At level 2 the division basically separates the 22 sites in the first negative division at level 1 into lowland and upland sites. The indicator species used for the division are those having a clear preference for cleaner upland reaches of rivers (*Achnanthes* and *Tabellaria*) and a genus common to nutrient rich waters and tolerant of high nitrogen and pollution levels, *Nitzschia*.

The divisions at group 3 split the three upland groups separated by level 1 on the basis of narrower water chemistry characteristics. West Allen at Coacleugh and the East Allen at Allenheads both having a higher alkalinity level than Kilhope Burn. At the same division the separation of group 4, the upland sites divided them into sites, with or without heavy metals.

At levels 3 and 4 there were six and nine divisions respectively. At level 3 TWINSPAN divided sites according to their water chemistry and each group was characterised by a particular diatom assemblage. At level 4 the divisions consisted of smaller site groupings each characterised by a narrower water chemistry range and a dominant diatom genera.

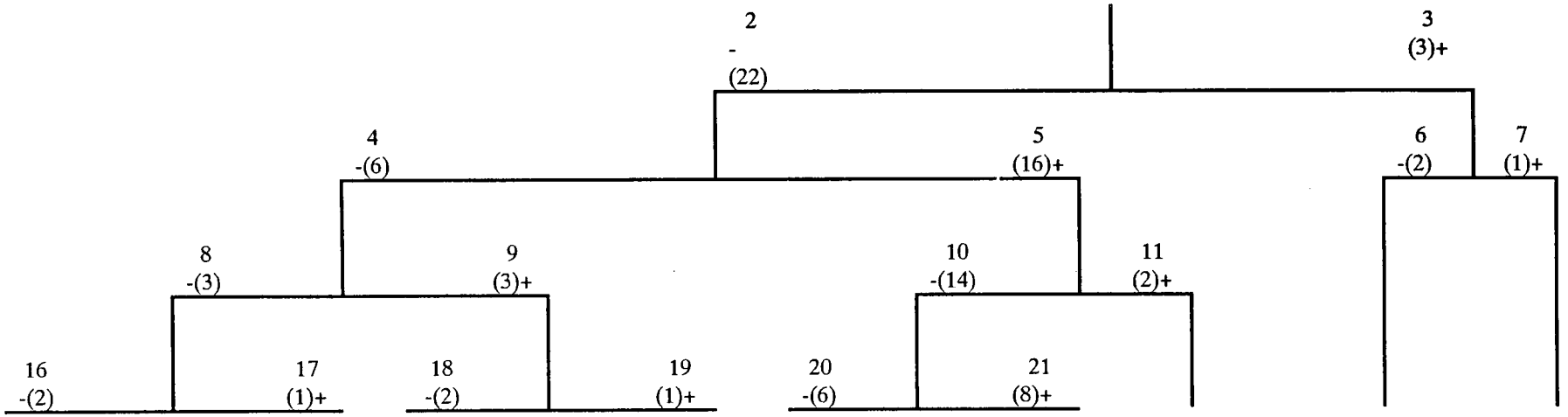
The classification system of Round was applied to the site divisions to assess whether the TWINSPAN classification followed his zonation. The sites can be seen to fall into initially zone1/2 and zones 2-5, with the first division at level 1. At level 4 it seems the sites were divided into upland sites falling into zone 3 (group 16 & 17) the less clean upland sites, dominated by the genus *Achnanthes* and zone 2 (group 18 & 19) the cleaner upland sites, dominated also by *Achnanthes*. The lowland sites were divided into three main groups: zone 4/5 (group 20) polluted sites dominated by tolerant genera *Navicula* and *Nitzschia*; zone 3 (group 21) moderately nutrient enriched waters, dominated by *Navicula, Achnanthes,*

Cocconeis and *Gomphonema* and zone 4/5 (group 22/23) the very polluted lowland sites, dominated by *Navicula*.

5.6 Distribution of Genera at sites

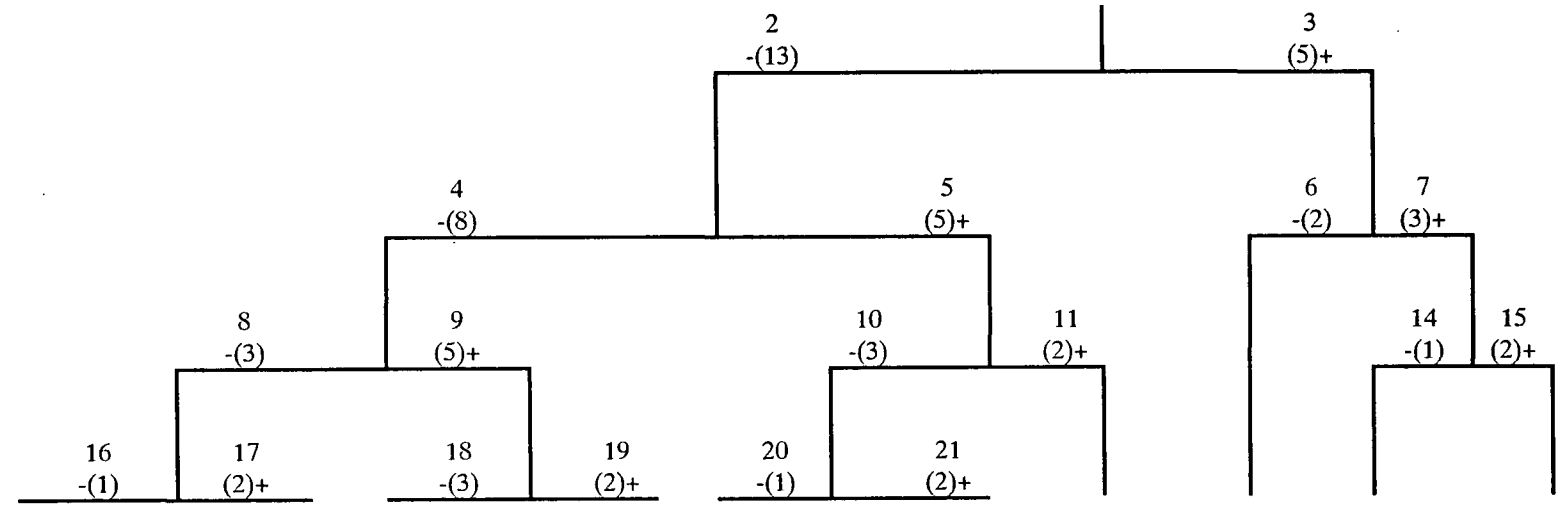
The classification of genera by TWINSPAN is illusrated in the form of a dendrogram , Fig.5.8.

The genera were basically divided as for the sites with those genera preferring upland cleaner reaches seperated from the genera tolerant of high pollution levels. The taxa also followed Round's system.



	R.Deerness, EH R.Nent, N	R.Nent, A	R.Wear, W Swinhope Burn	Bollihope Burn	Coalford Burn R.Deerness, UM Cong Burn R.Team R.Skerne, FB R.Tees, H	Twyzell Burn R.Skerne, CM R.Tees, Bl Br. Houselop Beck S.Tyne, Alston N.Tyne, Wark E.Allen,bl. A R.Browney	Sherburn Beck Old Durham Burn	W.Allen, C E.Allen, A	Kilhope Burn
Median values									
Altitude (m)	278	270	330	250	90	92	70	465	530
Width (m)	3.5	6.8	5.3	4.0	4.0	9.0	2.5	2.0	3.0
FRP mg l^{-1}	0.038	0.003	0.003	+	0.365	0.066	0.031	0.047	+
BOD_5 mg l^{-1} O	1.6	+	1.2	+	2.3	4.2	19.5	4.5	+
Conductivity µS cm^{-1}	778	559	270	224	1157	263	2250	255	340
Alkalinity meq l^{-1}	10.24	8.00	5.50	4.00	8.74	6.34	58.90	4.25	0.50
GDI	4.701	3.985	4.320	3.867	3.296	3.884	3.068	4.463	4.729
No.Taxa	9	10	9	9	9	9	8	8	8

Fig.5.7 Dendrogram of TWINSPAN classification to four levels using diatom genera.The group number is shown at every division with numbers of genera in that group shown in parenthesis. Median physical, chemical and biotic scores are shown underneath.



Median values									
S score	3	4	3.5	5	3	2.5	3.5	5	4.5

Fig.5.8 TWINSPAN classification to four levels of diatom genera. The number of groups are shown and numbers of genera in the groups are shown in parenthesis The median value of the S score, the genera tolerance levels used in calculating the GDI are illustrated beneath the dendrogram.

CHAPTER 6

A COMPARISON OF MACROINVERTEBRATE AND DIATOM RESULTS

6.1 Introduction

Although some similarities between invertebrate and diatom results may already be apparent from reading chapters 4 and 5, a formal analysis of this relationship is also necessary in order to clarify the results.

6.2 Biotic scores and Diversity Indices

The Biotic and diversity scores obtained from both data sets were correlated using Pearson's product-moment correlation coefficient. A correlation matrix was constructed (Table 6.1)

Table 6.1 Correlation matrix of Biotic scores and Diversity indices for invertebrate and diatom data. Pearson's product-moment correlation coefficient is shown on the left half of the table, probability on the right. (** P < 0.01, * P < 0.05, N.S - not significant, DF(n-2) = 23).

	BMWP	ASPT(invert)	D (invert)	GDI	ASPT (diat)	D (diat)
BMWP	1.000	**	**	N.S	N.S	N.S
ASPT(invert)	0.751	1.000	**	**	N.S	N.S
D (invert)	0.623	0.769	1.000	*	N.S	N.S
GDI	0.223	0.588	0.499	1.000	**	**
ASPT (diat)	-0.049	0.099	-0.075	0.650	1.000	**
D (diat)	0.204	0.131	0.081	0.523	0.750	1.000

BMWP was found not to exhibit a significant correlation with any of the diatom indices. The correlation between BMWP and GDI although it was not a significant relationship is of interest to the results and is shown in Fig.6.1. The graph does identify a positive relationship and a general trend of increasing GDI

with increasing BMWP score. The outlying points to the graph are the R.Deerness at East Hedleyhope, a site with a high BMWP score but a low GDI; Kilhope Burn an acidic site with a high GDI (due to the dominance of *Eunotia,* a very tolerant genus) but a low BMWP score because the sampling site did not support a wide range of invertebrate species therefore only five taxa were found; the R.Nent at Nentsberry and the R.West Allen at Coalcleugh both had very low numbers of taxa, 3 and 9 respectively but scored high in the GDI because of the abundance of *Achnanthes* at both sites.

ASPT (invert) did correlate positively ($P<0.01$) with the GDI (Fig.6.2) as did Simpson's diversity index (Fig.6.3) for the invertebrate data ($P<0.05$).

6.3 Site Classification

The comparison between TWINSPAN classifications (Fig.6.4) is not a strict statistical comparison between data sets but is intended to illustrate the general agreements. The positions of some of the diatom groups has been changed slightly in order to facilitate this. There is a good match between the site classifications with the exception of the three upland sites separated out in level 1 of the diatom classification. The invertebrate analysis failed to separate these sites on the basis of invertebrate families present. Both methods gave good classifications of sites into upland and lowland polluted and unpolluted groups.

6.4 Ordination of sites

Detrended correspondence analysis was used to assess the difference in importance of environmental gradients between the two data sets. The eigenvalues produced (Table 6.2)are thought to be an indication of the relative importance of the different axes although because further analyses were not performed these environmental variables were not identified. The correlations produced between data sets, even if significant, only indicate a general agreement because the axes may represent different parameters.

Table 6.2 Summary of results for DCA for both data sets

	Invertebrates			
	Axis 1	**Axis 2**	**Axis 3**	**Axis 4**
Eigenvalues	0.591	0.303	0.131	0.081
% variance	53.400	27.400	11.800	7.300
	Diatoms			
	Axis 1	**Axis 2**	**Axis 3**	**Axis 4**
Eigenvalues	0.787	0.278	0.124	0.056
% variance	63.200	22.300	9.900	4.500

Correlation between axes of diatoms and invertebrates for all 25 sites were calculated (Table 6.3) as a correlation matrix. The only significant correlations existed between axes 2 and axes 4, and between axis 2 (D) and axis 3(I). However, as axes 3 and 4 explain relatively low levels of variance (Table 6.3), only the relationship between the axes 2 is likely to be of real significance. The lack of correlation between the primary axes of variation is of interest because it suggests that the main factor controlling community composition is different for invertebrates and for diatoms.

Table 6.3 Pearson's correlation coefficient between axes calculated by DECORANA (*= P < 0.05, I = invertebrate D= diatom)

	Axis 1 (I)	**Axis 2 (I)**	**Axis 3 (I)**	**Axis 4 (I)**
Axis 1 (D)	0.065	0.241	0.218	-0.340
Axis 2 (D)	-0.122	0.461*	-0.449*	-0.278
Axis 3 (D)	-0.218	-0.019	0.028	-0.157
Axis 4 (D)	0.016	-0.329	-0.157	-0.474*

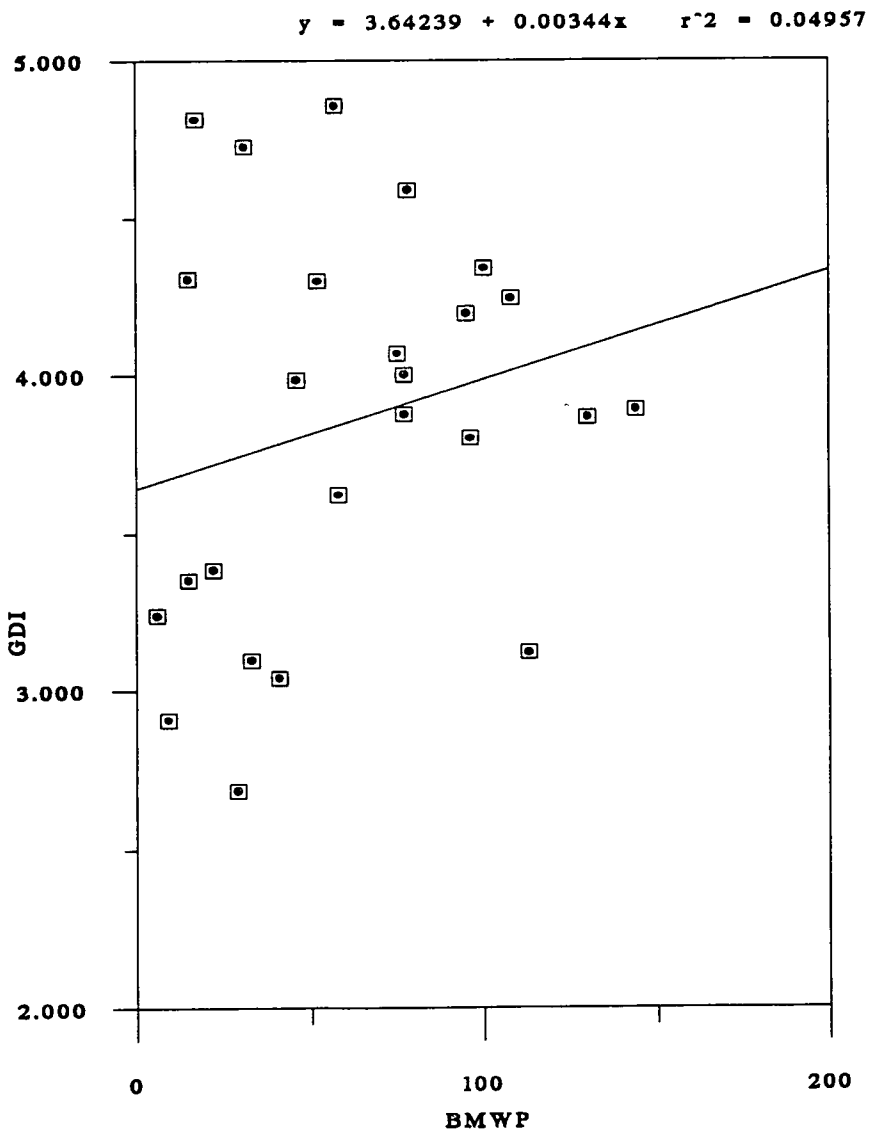


Fig.6.1 Correlation between BMWP scores and GDI scores

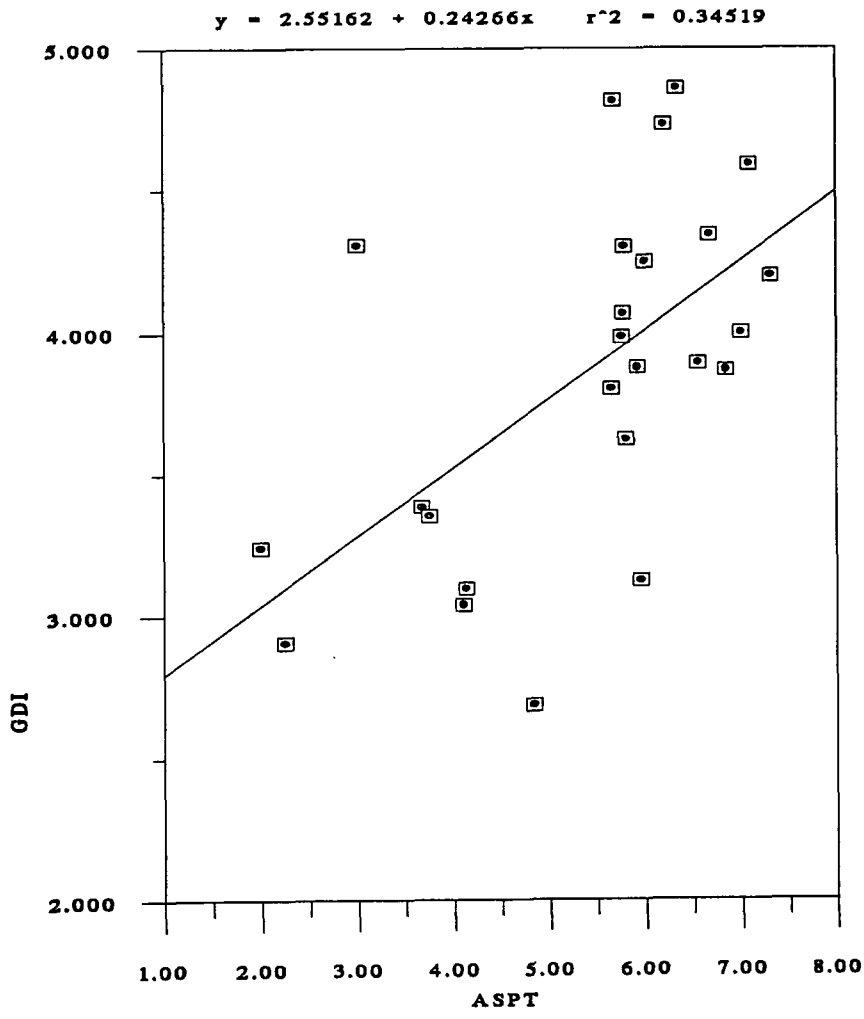


Fig 6.2 Correlation between GDI and ASPT (invert)

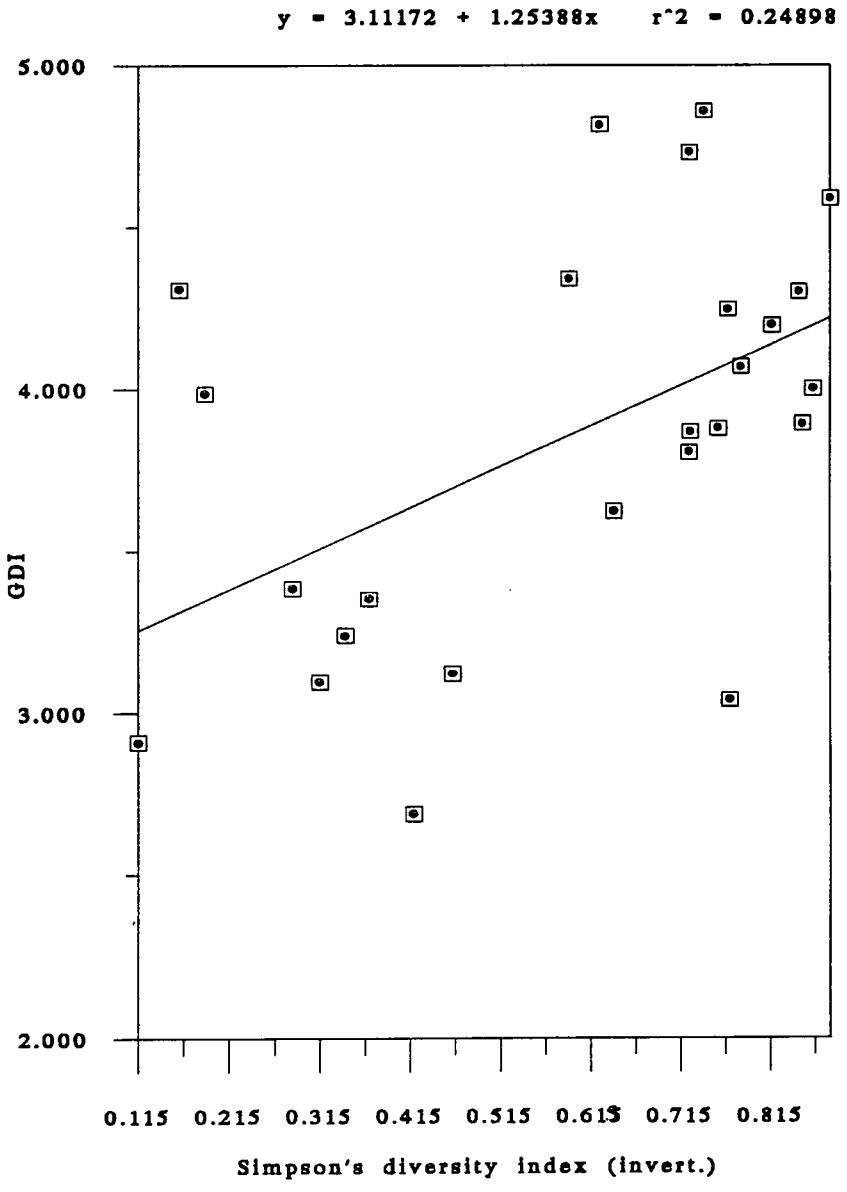


Fig.6.3 Correlation between Simpson's diversity index (invert) and GDI scores

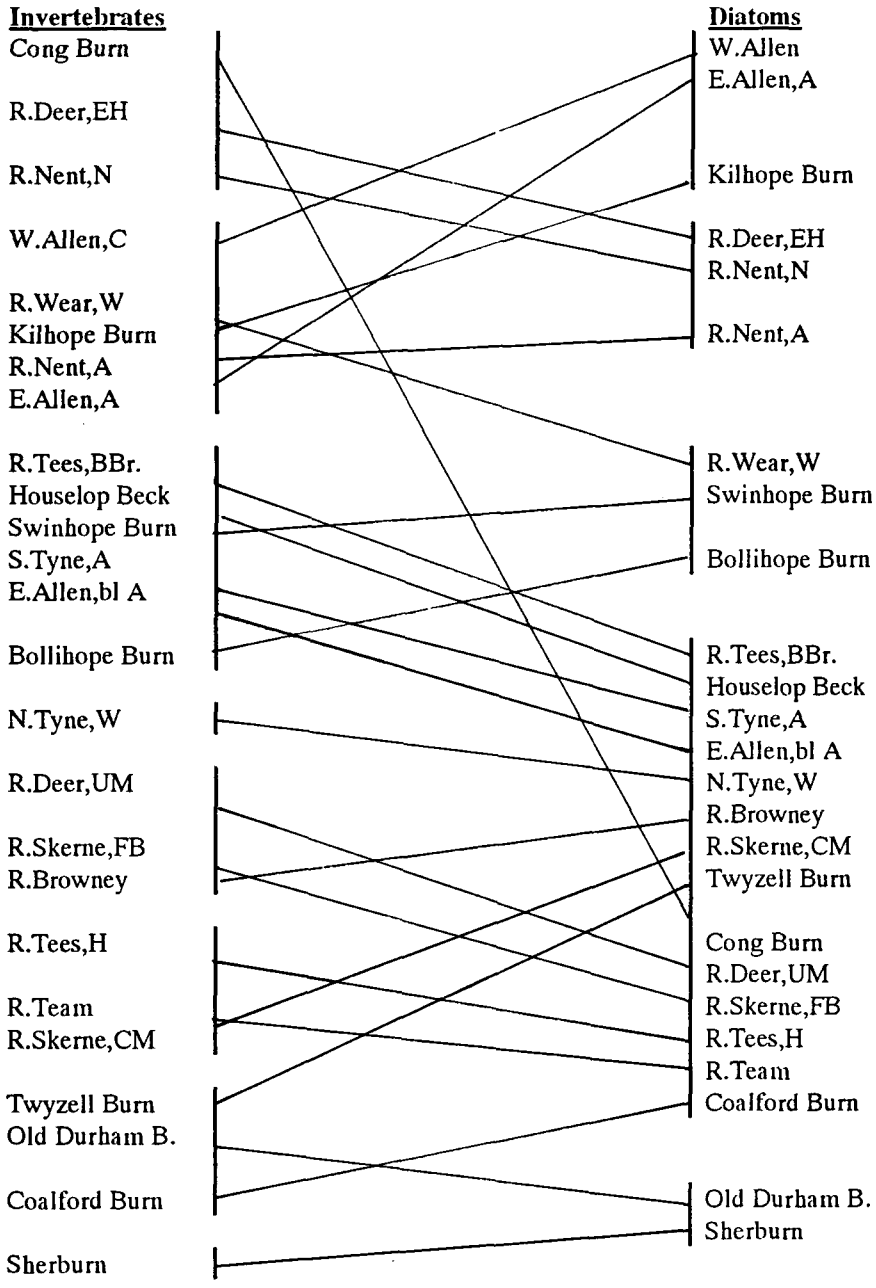


Fig. 6.4 Comparison between TWINSPAN classifications for invertebrates and diatoms

CHAPTER 7

DISCUSSION

7.1 Introduction

The discussion is organised into three sections in order to discuss the results of each set of data separately before comparing the two methods.

7.2 Macroinvertebrates

The results of the sampling program using macroinvertebrates, as discussed in chapter 4 show the success of these animals in biological monitoring of water quality. However there was considerable variation in the BMWP scores (Table 4.1), even at clean sites (6 to 144) Although such low scores may have been due to a sporadic pollution event, it is more likely that it demonstrates the extent to which the method requires an intuitive 'feel' of where within a reach to sample. This takes some weeks to develop (A.Lewis, pers.comm.).

A study conducted by Furse et al., (1981) to quantify the method of pond net sampling for biological monitoring (using invertebrates) compared the results of three experienced scientists taking two samples per site. The method used the three minute kick sample and a 900 μm mesh net. The mean taxa caught did vary between operators but was between 31.9 to 58.3 families per sample, these are considerably higher than the numbers in this study (Table 4.1) but that study was performed on very 'taxa rich' chalk streams. The effort put into sampling was found to affect BMWP but not ASPT, because the numbers of taxa do not show such an additive result. An increase in sampling effort does lead to substantial increases in BMWP score: 62.3% of the full catch after 3 minutes and 87.3% after 9 minutes (Furse et el., 1981)

The present study was performed over two seasons, spring and summer. Sample data appeared to have no appreciable effect on scores but as no two sites were sampled in both seasons then there can be no direct comparison. Seasonal effects are thought to be slight with respect to both BMWP and ASPT (Armitage et al., 1983), compared to the considerable variation in each expected with different environmental features. (Wright et al.,1989). ASPT values are usually highest in the upland reaches of

rivers (Armitage et al.,1983), a result also observed in this study. However in this study BMWP scores were also higher at the upland sites, in contrast to the findings of Armitage et al.,1983. This may be an artefact of the relative small data set used in this study.

The wide variation between BMWP scores, ASPT and Simpson's diversity index values within water quality classes may reflect the fact that the BMWP system was designed to monitoring organic pollution whilst many other types of pollution can affect overall water quality. Heavy metals at high concentrations in streams do have an adverse affect on fauna and flora. The sites at the R.Nent are polluted with heavy metals which is one reason why levels of invertebrate fauna are low at these sites (Armitage & Blackburn, 1985). Massive growths of *Stigeoclonium* in response to elevated levels of zinc also reduces the diversity and abundance of invertebrate fauna in the R.Nent (Armitage, 1979). The effects of coal waste on invertebrates has also a deleterious effect on community structure(Scullion & Edwards, 1980) as can be seen at East Rainton and Cong Burn.

This problem is over come to some extent by examining the correlations between the indexes calculated for the invertebrate communities and chemical data, a significant relationship was found between BMWP, ASPT and nutrient levels indicative of waters receiving organic enrichment (NH_4-N, NO_3-N, FRP FUP and BOD_5) (Table 4.2). The classification into class A does contain a wide range of conditions not measured in this study. The relationship between physical measures (conductivity and alkalinity) and the indices applied to the invertebrate families shows a significant correlation between the indices and conductivity only. This result is thought to be an artefact of the sampling program, where most of the grossly polluted sites were found on the Permian limestone (3.2) and so the hard water found at these sites tends to lead to high conductivities.

Some of the limitations of pollution indexes such as BMWP are illustrated by the TWINSPAN classification. The primary split was into upland and the lowland sites, (section 4.5), suggesting that environmental features were overriding water quality However, splits at subsequent levels did seem to be strongly affected by water quality. At level 3 the separation is into eight ecological groups according to their water chemistry. The separation at the forth level is into site groups characterised by narrower

chemical characteristics. Each site group is associated with a specific invertebrate assemblage. The invertebrate family abundances seem able, when classified using statistical techniques, and associated with corresponding physio-chemical parameters, to be grouped into assemblages which are indicative of specific ranges of water quality.

7.3 Diatoms

The values of the Generic diatom index showed much less variation between scores than shown by BMWP, with values varying from 2.686 to 4.857 (Table 5.1). It is assumed that this is due to the use of genus as the taxonomic level required for the index. The mean values of the GDI when plotted against water quality did exhibit a clear relationship with lower mean GDI values found at polluted sites (Fig.5.1). There was considerable variation about the mean and in the range, which is thought to be partly a reflection of the low numbers of samples taken in each water quality category, as indicated in 7.2. The results are not as clear cut as observed during studies in France (Coste et al.,1991)

The numbers of taxa varied little (Table 5.1) between sites presumably because of the taxonomic level used. Although *Navicula* is represented by a single score in the method (Table 2.1), there were often 6-7 separate species present in any one particular sample (M.G Kelly & J.R Carter, unpublished observation)

Division of GDI by number of taxa allowed an ASPT to be calculated as for invertebrates. Although this is not a routine part of the method (Rumeau & Coste, 1991), it allows a direct comparison with ASPT calculated for invertebrates. It was calculated from the total GDI score not from the individual S scores. The results in this study show it to be unreliable as an estimate of water quality. There was little agreement with ASPT values when plotted against water quality (Fig. 5.2) but it would certainly be worth repeating the exercise when all taxa had been identified to species.

Similarly the values of Simpson's diversity index did not have a clear relationship with water quality (Fig.5.3). Again this could be a sign that diatom monitoring should be conducted at a species level; however the fact that invertebrate families do respond does suggest that this is a natural taxonomic level

at which to work. However in a study of diversity and its relation to water quality by Archibald (1972) diatoms were identified to a species level and a variety of diversity indices applied. The results indicated that a decrease in diversity value with the deterioration of water quality did not follow and clean water samples were found with diversity values lying between those of moderately enriched and polluted sites. The results of this study seem to agree with those of Archibald and indicate that using the diversity of diatom communities at a genus level is not a good tool in monitoring water quality. Kilhope Burn (Fig 5.4), for example, lacks any obvious sources of pollution yet it has a community structure more commonly associated with a polluted environment (Magurran ,1988)

The indication so far is that only GDI of the measures of diatom communities is a reliable estimate of organic pollution. The correlations between all the indexes used and chemical parameters reflected this. The only significant correlations between indices and chemical parameters were obtained using GDI values (Table5.2). which correlated significantly with nutrient levels indicative of organic enrichment (NH_4-N, NO_3-N and FRP). However it did not correlated significantly with BOD_5, a key indicator of organic pollution. The only significant correlation between indexes and physical parameters was between GDI and conductivity. This is thought to be for the same reasons as mentioned in section 7.1.

The classification of the 25 sites using TWINSPAN produced different results from those for invertebrates. The separation at level 1 was into three geographical groups, the acidic upland sites, the upland sites and the lowland sites. The acidic upland sites had significantly different floras characterised by the presence of *Eunotia*, a typical indicator species of acid waters (Batterbee, 1984). At level three and four the separation was approximately the same as invertebrates. At this level there is a specific assemblage of diatom genera associated with each group. The classification of diatom genera using statistical techniques and using corresponding physio-chemical parameters does produce assemblages which are indicative of specific ranges of water quality. and correspond approximately to the groups proposed by Round (1992).

7.4 Comparison of diatom and invertebrate methods of monitoring water quality

From the points already discussed it is clear that although there are some similarities between invertebrate and diatom results, there are also some serious anomolies. These were examined more closely by a series of statistical analyses. BMWP was found not to correlate with any of the diatom indices although ASPT and Simpson's diversity index do correlate significantly with GDI (Table 6.1) indicating some agreement between the two methods. Neither ASPT or Simpson's diversity index for the diatoms correlates with any of the invertebrate scores. All invertebrate scores and GDI were found to be good indicators of organic pollution. However GDI failed to show a significant correlation with BOD_5, a common indicator of organic pollution.

Both data sets enabled sites to be grouped according to the assemblages of families or genera present and according to specific ranges of chemical parameters. The comparison of classifications although not intended to be a strict statistical comparison does allow similarities to be seen. The two classifications do present broadly similar site groupings. The division at level 1 using both data sets grouped sites largely according to their geography but in the classification using invertebrates the three upland acidic sites were not separated into a distinct group. This indicates a failure of invertebrates to act as indicators for certain chemical conditions.

Ordination of the two data sets enabled further comparisons to be made. The correlation between axes 2 was significant but between axes 1 it was not (Table 6.3). This indicates some degree of similarity between the two methods, however because further statistical methods were not used it is not known what environmental parameters the axes described by ordination are representing and indeed they could be two different sets of environmental parameters. It is particularly significant that the primary axis of variation for the two sets of data was different as it underlines the extent to which the two communities are responding in different ways to environmental change. Thus it is likely that both are providing complementary information in water monitoring programs.

7.5 Concluding comment

The two methods of monitoring water quality used in this study both provide the necessary criteria to be good indicators of environmental quality. Whilst the use of invertebrates as expected provided a good system , for monitoring organic pollution the diatoms also performed well in classifying water qualities but only using the GDI. The prime benefits of the diatoms are that identification at a generic level necessary in order to calculate the index was easier to learn and less time consuming compared to the time taken to learn the invertebrate families, because counts of only ±30 genera and 200 frustules on a slide needed to be made, reducing the time spent sorting through samples on collection. This, however has to be balanced against the longer time involved in sample preparation. It is also clear that the indices for diatoms, largely derived on the continent; clearly need some 'fine tuning ' before they can be recommended for use in Britain. There are costs and advantages of using both systems whilst invertebrates are likely to remain as the primary tool for the foreseeable future there are clearly costs and advantages of using both systems. Closer examination of diatom based methods are to be strongly encouraged.

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APPENDIX A. Details of Sites

Ai. Reach descriptions

1. TWYZELL BURN - 10m downstream of sewage pipe.

2. COALFORD BURN - 10m upstream of road bridge.

3. SHERBURN HALL BECK - 10m upstream of bridge close to the road.

4. OLD DURHAM BURN - 10m upstream of road bridge.

5. R.DEERNESS, USHAW MOOR (0005-41) - 50-60m above road bridge.

6. R.DEERNESS, EAST HEDLEYHOPE (0005-21) - reach next to road.

7. CONG BURN (0023-21) - immediately below bridge in a ponded area.

8. R.TEAM (0024-31) - immediately below footbridge.

9. R.SKERNE, FISHBURN (0025-10) - 10-20m above bridge.

10. R.SKERNE, COATHAM MUNDEVILLE (0025-50) - 4m downstream of bridge to 3m upstream incorporating raised brick portion of bridge.

11. R.TEES, BLACKWELL BRIDGE (0009-65) - 10m below Blackwell Bridge.

12. R.TEES, HURWORTH (0009-80) - 20m down from bridge.

13. HOUSELOP BECK (0007-21) - 10m below tunnel.

14. BOLLIHOPE BURN (0015-23) - 10m upstream of bridge.

15. R.WEAR, WESTGATE (0008-15) - 10m upstream of bridge.

16. SWINHOPE BURN (0018-31) - 10m immediately below bridge.

17. KILHOPE BURN (0013-12) - 5m above and below a small falls.

18. R.NENT, ALSTON (0048-99) - immediately above confluence with the S.Tyne.

19. R.SOUTH TYNE (0055-30) - 10m immediately above confluence with R.Nent.

20. R.NENT, NENTSBERRY (0048-20) - 10-20m below bridge.

21. R.NORTH TYNE, WARK (0043-20) - immediately downstream of bridge.

22. R.WEST ALLEN, COALCLEUGH (0085-10) - 15m down from old wooden plank bridge.

23. R.WEST ALLEN, ALLENHEADS (0081-06) - 10m upstream from plantation parking.

24. R.EAST ALLEN, BELOW ALLENHEADS (0081-16) - immediately below wooden footbridge near to old disused mine, just out of the village.

25. R.BROWNEY (0014-40) - immediately downstream of road bridge.

Aii. Width, mean depth, degree of shading and substratum composition of sites, where B= boulders, C=cobbles, P=pebbles, G=gravel, S=sand, Si=silt and Cl=clay.

	Substratum Composition (%)						
Site no.	**B and C**	**P and G**	**S**	**Si and Cl**	**Width (m)**	**mean depth (cm)**	**Shading (1-5)**
1	60	40	0	0	5.0	25	4.0
2	10	70	10	10	2.0	7	5.0
3	50	40	0	10	2.0	18	0.0
4	30	40	0	30	3.0	21	1.0
5	50	30	0	20	4.0	24	1.0
6	20	60	10	10	2.5	12	1.0
7	0	20	40	40	5.0	40	5.0
8	30	20	25	25	4.0	60	3.0
9	20	0	30	50	2.5	25	3.0
10	60	30	0	10	12.0	27	0.5
11	80	20	0	0	48.0	37	0.5
12	80	10	0	10	40.0	37	0.5
13	40	10	0	50	7.0	11	4.0
14	50	50	0	0	4.0	90	0.0
15	80	20	0	0	9.0	33	1.0
16	70	30	0	0	1.5	10	0.0
17	70	20	0	0	3.0	10	0.0
18	60	30	10	0	6.8	11	1.0
19	50	40	10	0	11.0	12	0.0
20	70	25	5	0	4.5	9	0.0
21	70	30	0	0	96.4	33	1.0
22	70	20	0	10	1.0	11	0.0
23	70	20	0	10	2.2	11	1.0
24	70	30	0	0	2.3	11	4.0
25	80	20	0	0	4.0	22	4.5

APPENDIX B. The numbers of invertebrate families at sites.

	Site No.														
Family name	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Siphlonuridae					1						3		2	1	
Heptageniidae					2						3	1	3	56	
Leptophlebiidae					1								2		
Ephemerellidae														7	
Ephemeridae					1										
Leuctridae						5					3			5	7
Perlodidae					1						1				
Perlidae						2								1	
Chloroperlidae														1	
Leptoceridae					3							2			
Goeridae						2									
Lepidostamatidae															
Sericostomatidae															
Libellulidae														1	
Psychomyiidae					2						2				
Philopotammidae													4	1	
Caenidae					1									1	
Nemouridae						4	1					2	1		1
Rhyacophilidae						2	1				1	1	3	2	4
Polycentropodidae				1								2	3	2	3
Limnephilidae			2		3	2							1		
Ancylidae											3		2		
Hydroptilidae															
Gammaridae	63	65	50	36	4								15	1	
Haliplidae			1			1			2						
Dytiscidae	2				1	3	1		1				12	4	5
Hydrophilidae													1		
Elmidae	1				1		1				3		1	2	
Hydropsychidae	1		9	3	8						8	55	5	5	2
Tipulidae	4		5	1		1								6	
Simuliidae					1										1
Baetidae			7	1							1		63	9	5
Sialidae															
Hydrobiidae			38		1		21								
Lymnaeidae								1					2		
Sphaeriidae		2													
Glossiphoniidae		1	1		1										
Erpobdellidae					1					1		7			
Asellidae				1	5				65	1		3			
Chironomidae	400	1	42	350	176	4	3	26	220	64	1	60	62	3	1
Oligochaeta	8	2	5	32	31			6		2		5	7	3	

Table of invertebrate familiy site data continued.

	Site no.									
Family name	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**
Siphlonuridae	1			1		104	2	1	6	176
Heptageniidae	4			7		45				210
Leptophlebiidae										
Ephemerellidae										
Ephemeridae										
Leuctridae	4	1	2	15	1	38	11	5	4	3
Perlodidae										
Perlidae	2			1		1				
Chloroperlidae				1		2	3	2		
Leptoceridae						3				5
Goeridae										
Lepidostamatidae				1		3				
Sericostomatidae						1				
Libellulidae										
Psychomyiidae						2				
Philopotammidae	3									
Caenidae										1
Nemouridae	1									
Rhyacophilidae	1	1	2			14		1	10	5
Polycentropodidae	1	2	3	1		1	11	1	2	
Limnephilidae				1						
Ancylidae									1	
Hydroptilidae						3				
Gammaridae						12				2
Haliplidae							1			1
Dytiscidae	52						3	26	19	1
Hydrophilidae										
Elmidae	3			4		20		2	1	4
Hydropsychidae	2		1	1		169	1			
Tipulidae	1		1		1	4		3	10	3
Simuliidae	1		1	1		2				
Baetidae	4			6		46		7	39	1
Sialidae								1		
Hydrobiidae						97				
Lymnaeidae										
Sphaeriidae										
Glossiphoniidae							1			1
Erpobdellidae						1				5
Asellidae										111
Chironomidae	2	2	103	15	2	47	12	29	68	35
Oligochaeta						8		4	2	41

APPENDIX C. The numbers of Diatom genera at sites.

	Site No.													
Genus	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Achnanthes	85	18	4	2	21	172	11	23	17	30	22	27	172	120
Amphora									11					
Cocconeis											7	68	1	1
Cyclotella					11				7		2	5		
Cymbella					7	1	3	2	34		42	47	9	
Diatoma		3			11	19	27	10	23		17	4		6
Eunotia														
Fragellaria		3				2	3				7	6		
Gomphonema	17	49	26	29	13		69	18	19	125	20	8	9	48
Gyrosigma			2											
Meridion	3	60	10	5	4	5	3	2			4	8	1	3
Navicula	85	82	129	146	127	14	107	148	95	25	100	44	12	31
Nitzschia	42	1	24	27	26	3	99	1	17	15	6	6	31	9
Pinnularia														
Rhoicosphemia			5								3			
Suriella	1	5	11	2	6	5	4	4	2		4	2		
Synedra			10	1		5	1	39		2	1	1		9
Tabellaria						1								

Appendix C cont.

	Site no.										
Genus	15	16	17	18	19	20	21	22	23	24	25
Achnanthes	150	152		140	156	172	76	200	63	98	61
Amphora				40							
Cocconeis			1				23				75
Cyclotella											3
Cymbella	13	15		7	1	11	24			25	3
Diatoma	4	3		16	3	14			5		
Eunotia			190					1	18	2	
Fragellaria	4	3		3	13		1	10	10	2	5
Gomphonema	27	19	5	15	24	4	25		19	14	17
Gyrosigma											
Meridion		1		4	3	17	3	2			7
Navicula	23	34	16	15	13		45	5	57	42	43
Nitzschia	2		1		13		12	3	1	17	11
Pinnularia			1								
Rhoicosphemia		2									
Suriella	1			12	6	2				29	6
Synedra	1	9	1			1	1		12	6	
Tabellaria			7	1		1			46		