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THE EFFECTS OF SALINE PUMPING WATER ON FRESHWATER INVERTEBRATE COMMUNITIES

by

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Thesis submitted to the Council for National Academic Awards in partial fulfilment of the requirements for the Degree of Doctor of Philosophy

Sponsoring Establishment

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November 1989

DECLARATIONS

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

2. The author has not received, and has not been registered for any other higher degree awarded by a University or the Council for National Academic Awards during the course of this work, nor has any part of it been previously submitted in partial fulfilment of a higher degree.

3. The author has attended conferences and programmes of study relevent 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..... L.M. Bird .....

(Candidate)

Signed.....

(Director of Studies)

ABSTRACT

THE EFFECTS OF SALINE PUMPING WATER ON FRESHWATER INVERTEBRATE COMMUNITIES

Linda M Bird

Saline effluents associated with coal mining affect many rivers in the English East Midlands. The effects of one such effluent, with a salinity close to that of sea water, were investigated by invertebrate sampling and chemical monitoring.

Mathematical analyses showed that elevated salinity reduced faunal diversity and altered species composition. Some species were indicative of increased salinity, including *Lumbricillus rivalis*, *Paranais litoralis* and *Gammarus tigrinus*.

A computer program was devised to predict species lists for saline affected sites from environmental data. The program was tested and used to predict changes in fauna which would result from an increase in salinity.

Life history studies in the field and laboratory were designed to explain the distribution of several species in relation to salinity. *Gammarus tigrinus* was more *r* selected than *G. pulex*. It was also able to reproduce in fresh water and would probably remain in the rivers of the Midlands if saline pollution was eliminated. *Tubifex tubifex* was unable to reproduce above a salinity of 56mM NaCl. *Lumbricillus rivalis* had optimal reproductive success at 56mM NaCl, and was found to replace Tubificidae at saline sites.

In physiological experiments the salinity tolerance of freshwater species such as *Gammarus pulex* was similar in NaCl solution and in sea water. The estuarine *Gammarus tigrinus* and *Gammarus zaddachi* were better able to tolerate salinity in seawater, when 10mM potassium was present.

Recommendations were made for less harmful disposal of saline effluents. The most economical method may be to release dilute effluents, provided the concentration in the receiving river remains below 14mM chloride.

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ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr C E Terrell-Nield for his guidance and encouragement during the course of this research. I would also like to thank Dr N A A Macfarlane and Mr J Adcock for their advice and support.

I am indebted to the Department of Life Sciences, Nottingham Polytechnic, for providing the finance and laboratory facilities for this research.

Particular thanks are due to Dr. R. Jenkinson and all the staff at Creswell Crags Visitor Centre for providing formal collaboration, laboratory facilities and much practical assistance and advice during the field work stages of the research.

I would like to thank Mrs M. Richards for assistance with radioactive tracer experiments, and Mr M. Hutchins for the photographic reproduction of all the figures. I would also like to thank my colleagues at Nottingham Polytechnic, whose companionship and support during my research was greatly appreciated.

Last but not least I would like to thank my husband, Dr Graham Bird, for his great support throughout the whole of the project and during the writing of this thesis.

CHAPTER ONE

General Introduction

1:1 Introduction

The history of river pollution in Britain probably dates back to the establishment of the first large settled communities, which poured raw sewage into the rivers from a localised source. One of the earliest written records of river pollution, however, from Spencer's "Faerie Queene" and dating from the 16th Century, concerns mine pollution rather than domestic wastes, and describes the River Dart in Cornwall as "nigh choked with sands of tinny mines" (Hynes, 1970). This is significant in a country whose industrial wealth was based on large reserves of coal, iron ore and other minerals which provided power and raw materials for the newly developing industries during the Industrial Revolution.

There is thus a long history of pollution from mines of all types in Britain, and concern about the condition of rivers polluted by both domestic and industrial wastes resulted in the first Act designed to limit such pollution, which was passed by Parliament in 1874. This Act and several others which followed, were only partially successful, and when the regional Water Authorities were formed in 1974, with a statutory duty to control polluting inputs into rivers, many water bodies in the industrial areas of Britain were still in very poor condition, with discharges from old mines and factories dating back for more than a century.

These older mines and factories were able to release wastes into rivers with no thought as to their effect on

water quality, and the subsequent reduction of the pollution load from their effluents has proved difficult and expensive. In the late 1980's, many discharges remain which are unacceptable in quality, causing gross pollution, but which have no easy method of improvement. Many of these discharges originate from coal mines, which must not only dispose of large quantities of solid spoil, but must continuously pump out water from workings to allow coal to be mined.

There have been many studies on the effects of the acid coal-mine drainage commonly found in Wales, North-West England and North America (eg Roback and Richardson, 1969; Learner *et al.*, 1971; Greenfield and Ireland, 1978; Larrick *et al.*, 1979; Scullion and Edwards, 1980; Weatherley and Ormerod, 1987) but very little published work is available on the effluents discharged by the numerous collieries in the English East Midlands, which are typically neutral in pH and saline. The East Midlands includes the counties of Nottinghamshire and Derbyshire, together with parts of South Yorkshire, North Leicestershire and Lincolnshire. There has been some work on the effects of saline effluents in North America (Claasen, 1926), but these are due to the potassium extraction industry, and oil production, rather than to coal-mining.

A literature search of biological data bases revealed only one reference to saline mine effluents in Britain (Beadle and Cragg, 1940) which briefly described a saline stream in County Durham, although there were papers on saline ponds and streams in Cheshire (Savage, 1981; 1982), and the saline River Weaver in Lancashire (Holland, 1976), and references to saline pollution in other countries (eg North America; Claasen, 1929; Sitter,

1947: West Germany; Wachs, 1963). It thus appeared that saline coal-mine pumping water was a problem of the English East Midlands which had received little previous attention.

Researchers on coal efffluents mention the orange-coloured deposits of ferric hydroxide (ochre) which stain river beds and in some instances directly affect the distribution of invertebrates due to smothering (Greenfield and Ireland, 1978). Fresh deposits of solid colliery waste are neutral in pH in most areas, but after some years of weathering, most coal-tip wastes become more acid due to the oxidation of iron pyrites (FeS) which produces sulphuric acid and iron oxides. This process is enhanced by the action of sulphur and iron bacteria. The iron becomes soluble in acidic solutions and contributes to the toxicity of the effluents. In some areas, the run-off from coal tips is treated with hydrated lime to neutralise it before it is released into rivers. In the East Midlands, this process occurs naturally, due to the high concentration of calcium ions in the ground-water, dissolved from surrounding limestone.

1:2 Coal-field Geology in the East Midlands

The major rock formations in the area are shown in Table 1:1. The East Midlands Coalfield covers an area of 2,400 square miles (104km^2) and is the western part of a coal measures basin of unknown extent. The coal measures reach a thickness of 5,000 feet (1667m), and in the West are close to the surface, while to the east they are buried under increasingly thick layers of Permo-Triassic or Jurassic rocks (Ministry of Fuel and Power, 1945). The

TABLE 1:1

Major Rock Formations in the North Midland Coalfield
(Ministry of Fuel and Power Report, 1945)

Geological Period	Rock Formation	Range of Depth (m) in the East Midlands
Recent	clays, sands and gravels	0-57
Jurassic	clays, limestones and sands	above 467
Permo/ Trias	New Red Sandstone	up to 667
	Triassic marls and sandstones, Permian limestones and marls with basal sands or braccia	
Carboniferous	"Upper Coal Measures" mudstones, sandstones and thin coal	up to 200
	"Middle and Lower Coal Measures" mudstones and sandstones with many coal seams	up to 1667
	Millstone Grit grey mudstones and sandstones with thin coal	33-1667
	Carboniferous Limestone	283-1667

All depths are converted from feet as in the original text to metres (m)

individual beds of coal were originally laid down as approximately level sheets which extended over a much wider area than that mined at present. After the coal measures were formed earth movements elevated the strata and buckled them into a series of ridges. This accompanied a period of arid conditions over the uplifted area of land, and at this time there was rapid erosion, removing coal and Millstone Grit from the surface, in some areas down to the Carboniferous Limestone. This left the land as an almost flat plain with limestone outcrops and coal preserved in depressions. In the succeeding downward movements of the land these eroded sediments were covered with Permian and Triassic rocks, which were continental deposits, in contrast to the Carboniferous formations, which were primarily estuarine and marine in origin. Overlying the coal measures is a roof of marine shells consisting mainly of *Lingula mytiloides*, *Posidoniella minor*, *Pterinopecten papyraceus*, *Gastrioceras carbonarium*, *G. listeri* and various other gastropods.

Over this 'roof' are two beds of Permian Limestone separated by red marl and sandstone. This limestone is 44% magnesium carbonate, with beds of gypsum, both of which are commercially quarried in Nottinghamshire and Derbyshire. These limestones may also contain beds of rock salt up to 20 feet (7m) thick. This assemblage of rocks indicates that they were laid down in a land-locked basin similar to the present Caspian Sea, surrounded by hot desert.

Overlying the Permian limestones are Triassic sandstones, which cover two-thirds of the coalfield. The more recent deposits above this are black shales covered by glacial drifts.

1:3 Formation of Saline Ground-Water in Coal Measures

Most of the ground-waters in the East Midlands are saline, especially where circulation of underground water is restricted due to the low permeabilty of surrounding rocks and/or the lack of a natural outlet for drainage (Downing and Howitt, 1969). This salinity increases from the SW to NE of the area. Many authors consider that such saline waters developed from sea water trapped in marine sediments at the time of their formation (eg White, 1957), although others (eg Chebotarev, 1955; Schoeller, 1962 and Anderson, 1967) point out that high salinities can be achieved through solution of soluble materials in the rocks, such as the rock salt found in the Permo-Triassic formations in the Midlands. Anderson suggested that the chemical composition of saline groundwaters in the Midlands was not identical to that of ancient sea water, and in particular that the waters contained much more calcium chloride than would be expected. Downing and Howitt (1969), however, considered that the groundwaters of the Midlands were essentially marine in origin, and changes in chemical composition were due to flushing of the pore water from the limestone during periods when the strata were uplifted, and their dilution by rainfall. The changes in ionic ratios found in the ground-water when compared with sea water (Table 1:2) are believed to be due to reactions between the original sea-water and the sediments, commencing soon after deposition and continuing for a considerable period. These changes involved the reduction of sulphates (Emery and Rittenburg, 1952) and a decrease in the magnesium and potassium concentration (Chave, 1960). The

TABLE 1:2

Proportions of Major Ions found in Standard Sea Water Compared to Mine Drainage Water from Creswell Colliery

Ion	Sea Water (mM)	Mine Drainage Water (mM)
Na^+	459	326
Cl^-	535	602
Mg^{2+}	53	19
Ca^{2+}	10	122
K^+	10	0.6
SO_4^{2+}	28	1.5
Br^+	1.0	14
Na^+/Cl^- ratio	0.56	0.35
K^+/Cl^- ratio	0.02	0.001
Mg^{2+}/Cl^-	0.07	0.02
SO_4^{2+}/Cl^- ratio	0.14	0.006
Ca^{2+}/Cl^- ratio	0.02	0.06

Source of figures for standard sea water was Nicol (1967)

groundwater can also be concentrated in geological strata such as clays which act as semi-permeable membranes (Sitter, 1947).

Saline ground-waters are an extremely common companion to coal and oil-bearing rocks in the east of Britain, and in many countries throughout the world such as the Americas, Belgium, India and New Zealand (Hodges, 1983). In Britain, there were several brine springs in North-Eastern England which disappeared from the surface when coal began to be mined in the area, to become a problem in mine workings throughout the area (Hutton, 1831). These brines are, however, able to be discharged directly into the sea in most cases.

1:4 History of Coal Mining in the East Midlands

The North Nottinghamshire and South Derbyshire area is underlain by large reserves of coal. Some of this has been mined since Roman times in the Western part of the area, around Chesterfield, where the coal outcrops to the surface (Figure 1:1). This is termed the 'exposed coalfield' and regularly shallow mining in this area began in the Middle Ages onwards (Area 1 on Figure 1:1). Indeed, in 1552, the first horse-drawn railway in Britain was built at Wollaton near Nottingham to carry coal between Wollaton and Strelley by Sir Percival Willoughby, who owned the Wollaton Estate (Griffin, 1971).

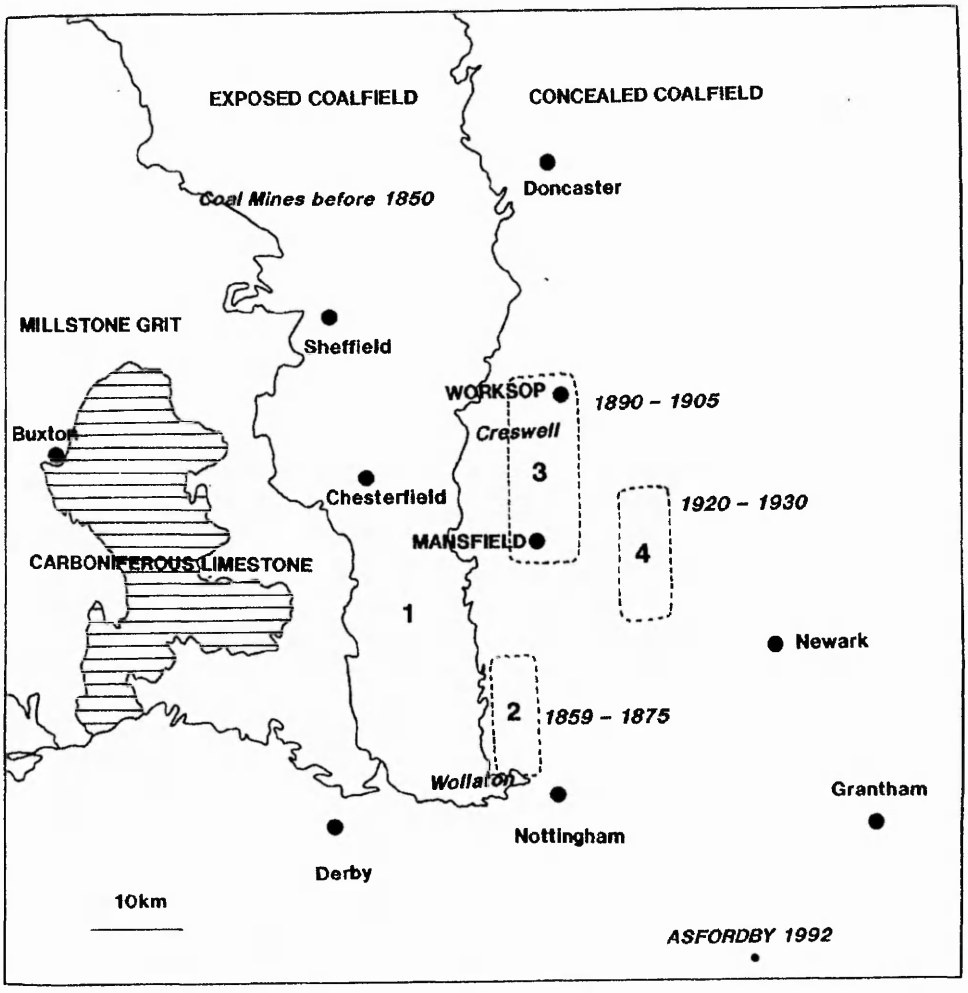
By the middle of the eighteenth century 100,000-150,000 tons of coal were being burnt annually in Britain (Griffin, 1971). This coal came from shallow mines (less than 700ft, 233m deep) on the exposed coalfield. By the mid nineteenth century, however, most of the coal in these areas was exhausted, and new mines were sunk

FIGURE 1:1

Map showing the East Midlands Coalfield with major towns marked.

Areas enclosed by dotted lines indicate the position of coal mines opened between the dates given.

Area 1 - Coal Mines worked bfore 1850
Area 2 - Coal Mines opened 1859 - 1875
Area 3 - Coal Mines opened 1890 - 1905
Area 4 - Coal Mines opened 1920 - 1930


EXPOSED COALFIELD
CONCEALED COALFIELD
Doncaster
Coal Mines before 1850
MILLSTONE GRIT
Sheffield
WORKSOP
1890 – 1905
Creswell
Buxton
3
Chesterfield
1920 – 1930
MANSFIELD
4
CARBONIFEROUS LIMESTONE
1
Newark
2
1859 – 1875
Wollaton
Grantham
Nottingham
Derby
10km
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progressively further east on the concealed coalfield in the Leen Valley (Green, 1935) (Area 2 on Figure 1:1). The shafts for these mines were sunk to an average of 850 feet (283m) (Williams, 1962) between 1859, when Shireoaks Colliery was begun (Anon, 1956) and 1875, when the pit at Newstead Abbey was opened.

Between 1890 and 1896, five new collieries were opened in the Mansfield area to the East of the Leen Valley (Area 3 on Figure 1:1). These were followed by Gedling, Mansfield, and Sherwood between 1900 and 1905. After this eleven new pits were sunk in the Sherwood Forest area (Area 4 on Figure 1:1), for example those at Clipstone, Ollerton and Thoresby (Waller, 1983). Some of these collieries needed to sink very deep shafts (2,640 ft, 880m) to reach the coal of the "Top Hard" seam, which earlier collieries to the west had mined into at a much shallower depth. By 1945, there were 92 collieries in Nottinghamshire and Derbyshire, 26 in the older exposed area and 66 in the newer concealed field, where each successive wave of pits was opened further to the east.

The removal of the water which floods into coal seams as soon as the water table is reached was a gradually worsening problem for these mines as they were sunk progressively deeper. The earliest mines in Nottinghamshire, such as Wollaton, used a system of small conduits which connected with larger drainage channels or adits. This system was never very satisfactory, however, and beam and other pumping engines were installed in all mines as they became available. The need for adequate drainage became increasingly urgent as shafts were sunk deeper, encountering water at higher pressure which flooded seams very quickly if not removed. The newer mines also needed to cope with the problem of flooding

from older, shallow workings which had been abandoned and were encountered as new shafts were sunk.

In very shallow mines, the drainage water was fresh or slightly saline, but as mines were sunk deeper, brine springs were encountered, for example in a boring at Ollerton (Wilson, 1926) a feeder of strong briny water was encountered at a depth of 987 feet (328m) and overflowed into the hole at a rate of 50 gallons per hour until operations ceased. The water, saline or otherwise, was pumped to the surface and then either piped to the nearest river or directed into lagoons and drainage channels which drain into the nearest river. Many of the mines were situated at the head of valleys, and the streams which were available to take away the mine drainage water were very small.

One such colliery was at Creswell, and the workings at this colliery produced streams of vey concentrated brine which was discharges directly into a small stream, Millwood Brook.

1:5 Millwood Brook and the History of Creswell Colliery

The river system chosen for the field work for this project was the River Idle catchment, which includes many rivers which flow through mining areas, such as the Maun, Meden and Poulter, Rainworth Water, Vicar Water and Millwood Brook.

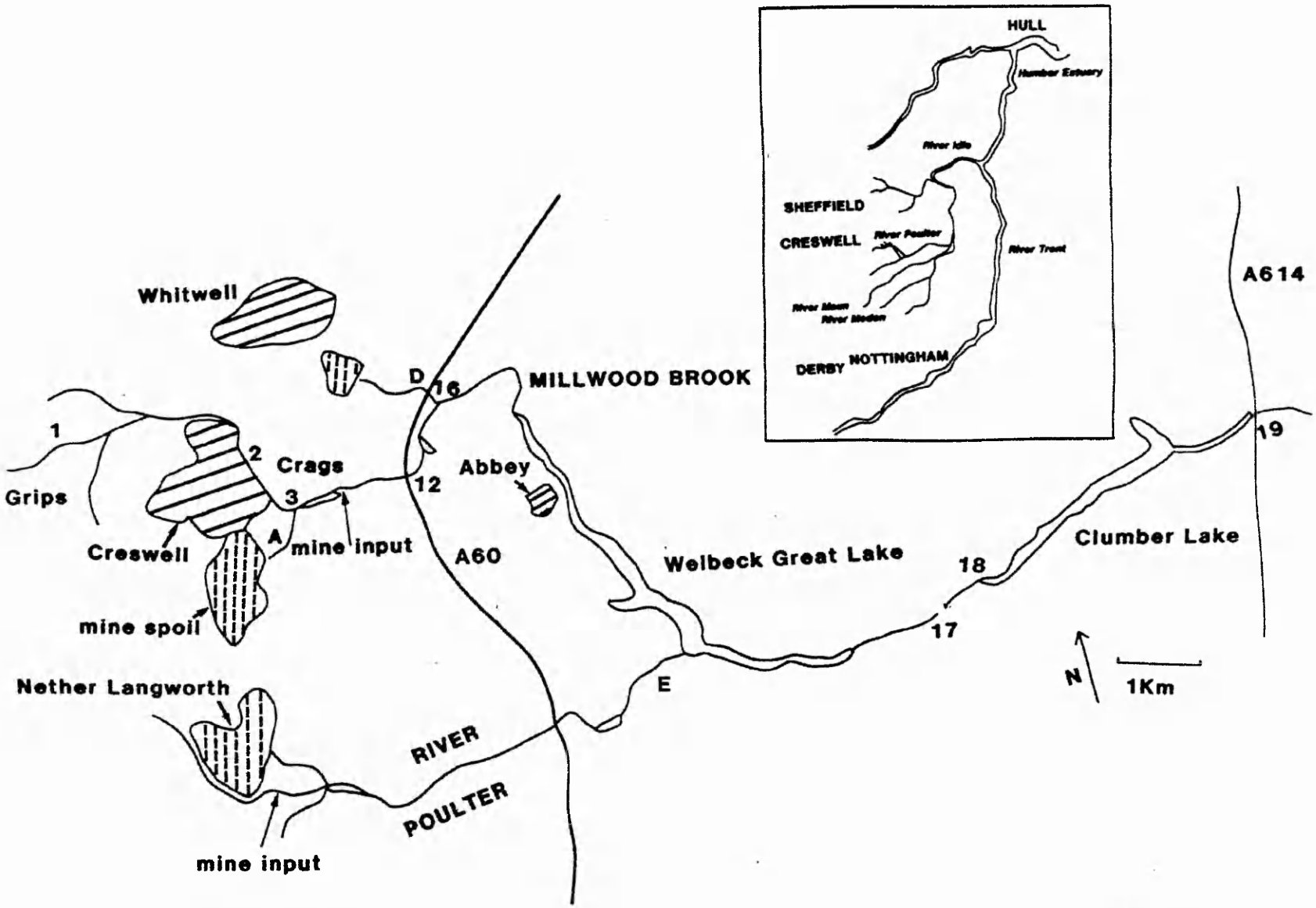
Millwood Brook was chosen for detailed survey work as this small river receives a quantity of pumped mine drainage water which is very large in relation to its total flow, and the salinity levels close to the discharge are thus very high, allowing the effects of a sudden increase in salinity followed by a decline to be

FIGURE 1:2

Sketch Map showing Millwood Brook and the River Poulter together with the position of Creswell, Nether Langwith and Whitwell.

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 M1, M2, M3, M12, M16, M17, M18, M19, D and E are shown, and the position of the mine drainage water inputs indicated by arrows. (The positions of other sites are shown in Figure 1:3)


HULL
Humber Estuary
River Idle
SHEFFIELD
CRESWELL
River Poulter
River Trent
River Idle
River Meden
DERBY
NOTTINGHAM
A614
Whitwell
D
16
MILLWOOD BROOK
1
2
Crags
Grips
3
12
Abbey
19
A
mine input
Creswell
A60
Welbeck Great Lake
18
Clumber Lake
mine spoil
17
N
1Km
Nether Langworth
E
RIVER
POULTER
mine input

investigated in the same water course.

Millwood Brook is a tributary of the River Poulter (Figure 1:2), and it receives pumped mine drainage water as it flows through Creswell Crags, a Site of Special Scientific Interest, due to archaeological remains of early human occupation in a series of Permian limestone caves which cut into the walls of the gorge. There is an archaeological research team based at a modern visitor centre, and the whole area is a popular tourist attraction, receiving 250,000 visitors per year, as it is close to two large centres of population, Sheffield and Nottingham.

All the visitors to Creswell Crags must walk alongside Millwood Brook to reach the caves (see Figure 1:3). The obvious pollution of the river provides a contrast to the pleasantly wooded gorge through which it flows, and provokes many comments from visitors to the area. In addition to mine effluent flowing from a large concrete pipe, which stains the river bed orange and produces steam in winter (Plate 1), there is also, on the opposite bank to the footpath, a water reclamation (sewage) works. This works receives waste from Creswell Village (population approximately 5,200), and has three discharge points on the river, one directly opposite the orange effluent input, one 75m below this input and one 130m downstream (see Figure 1:3).

Previous work on the river has been carried out by Creswell, 1984 and Dodd and Terrell-Nield, 1985, investigating the fauna and chemistry of the section of river at Creswell Crags. They found that the effluent was non-acid, with a pH above 6.5, and extremely saline, with a salinity approximately equal to that of sea water. The pumped drainage water was also released at a temperature

FIGURE 1:3

Sketch Map showing Millwood Brook in the area of Creswell Crags.

The positions of sites M3 to M12, A and B are shown. Arrows indicate the direction of flow.

The position of inputs of Mine Pumping water (M), water from Crags Pond (C) and sewage effluent (S) are indicated by letter.

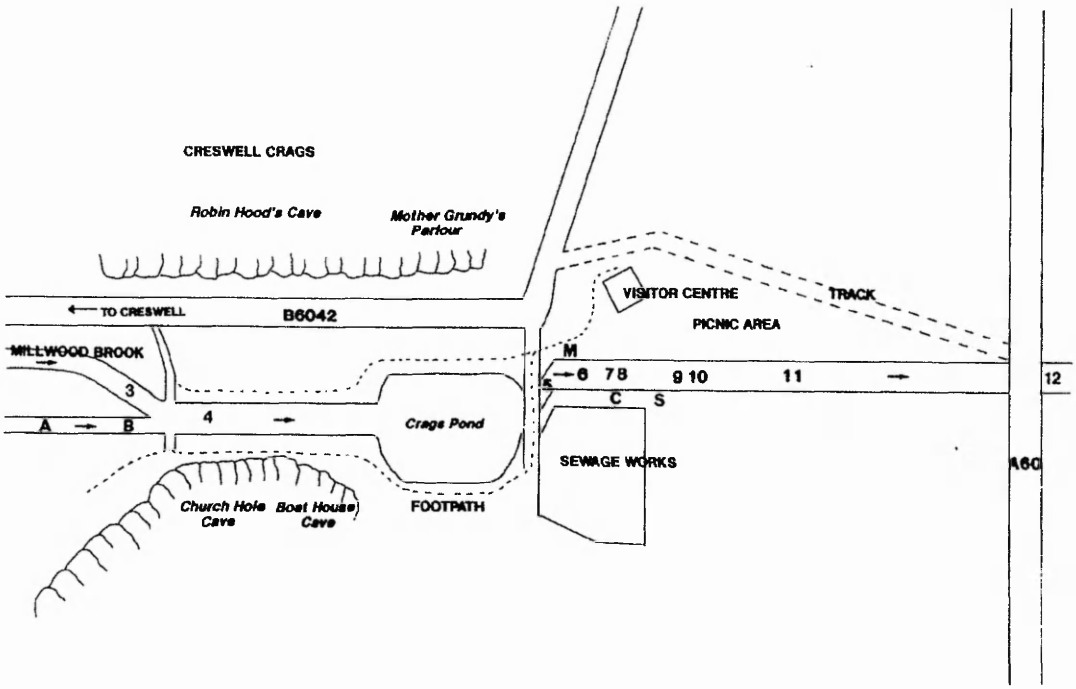

CRESWELL CRAGS
Robin Hood's Cave
Mother Grundy's Parlour
TO CRESWELL
B6042
VISITOR CENTRE
TRACK
PICNIC AREA
MILLWOOD BROOK
3
4
A
B
M
6 7 8
9 10
11
12
C
S
Crags Pond
SEWAGE WORKS
460
Church Hole Cave
Boat House Cave
FOOTPATH

PLATE 1

Views of Millwood Brook looking upstream towards the Pumped Mine Drainage Water outfall, which can be seen on the right of the pictures. The storm sewage outfall pipe can be seen on the left of photograph 1.

Photograph 1 was taken in July and photograph 2 in February

1

2

PLATE 2

Sewage litter from the storm sewage outfall on the bed of Millwood Brook.

Photograph 1 was taken 5m below the Pumped Mine Drainage Water outfall, and photograph 2 40m below, two weeks after the sewage was released.

1

2

of approximately 20°C, winter and summer. This was due to the depth at which the water was encountered, as groundwater heats with depth.

Parts of the stream are also affected by effluents from the Creswell Water Reclamation Works, which introduces three discharges into the river. The first, released directly opposite the mine pumping water input (see figure 1:3) is intermittent, and used after sudden heavy rain or overloading at the works, and is the least aesthetically acceptable input as it discharged completely untreated sewage into the river (Plate 2). The third effluent consisted of 1-5Ml/day of treated sewage with a consent standard BOD (Biochemical Oxygen Demand) of 10-15mg/l O_2 (Severn-Trent Water Authority Report 1987), and the second is a flow of river water diverted from Crags Pond through the Water Reclamation Works area and released 75m below the mine input.

In addition to the discharges received at Creswell Crags, Millwood Brook, along its 14km length from its source at Markland Grips (SK 503 747) to its exit from Welbeck Great Lake (SK 547 751), where it joins the River Poulter, has consents given for 27 discharges. Of these, 16 are from water treatment works, 10 from industry and one from farm drainage. Of the 10 industrial discharges, three are from Steetley Minerals, a company which quarries limestone from near Creswell Crags, and the others saline effluents from the collieries at Creswell and Whitwell. There are chemical standards required by the Severn-Trent Water Authority for all these effluents, but surprisingly no limit is set for the level of either sodium or chloride released by collieries, despite these two chemicals forming the major components of the effluents.

The coal-mining rights at Creswell were purchased from the Duke of Portland in 1888 by Emerson Muschamp Bainbridge (Williams 1962), who founded the Bolsover Colliery Company (Griffin 1971). The shafts for the mine were sunk between 1896-1898, and in 1898 the colliery was producing 3,000 to 3,200 tons of coal a day. Up to 1908, the colliery held the world record for production (3,800 tons/day), until it was overtaken in that year by Warsop. The main seam of high quality "Top Hard" coal was reached at 1095 ft (365m), and salty water was encountered early in the digging of the shafts. The water was originally discharged to the Millwood Brook via an open channel, but later a pipeline was constructed under the valley to discharge the water below Crags Pond, where it is situated today.

A "model village" was built to house the workers at the colliery, which originally had over 1,000 workers, and still employed 939 in 1983. Most of the coal from Creswell (92%) now goes to the C.E.G.B. power stations in the Trent Valley, and the colliery has an annual output of 671,000 tons (NUM Nottingham Area Report 1983).

1:6 Aims of the Research

This research project was undertaken to describe and explain the effects of saline mine effluents on the rivers of the English East Midlands. In the first phase of the work, rivers were to be surveyed to discover the distribution of saline affected water-courses and the invertebrate animals present in saline and non-saline rivers. Detailed field sampling was to be carried out on Millwood Brook.

These field results were then to be modelled using

mathematical techniques, to devise formulae to predict the effects of increased salinity on freshwater communities, which could then be used in setting limits for future saline discharges.

The next stage in the work was to be an attempt to explain the distribution of species in saline affected rivers using life-history studies, which were to be carried out in both the field and the laboratory. Four species were to be chosen for these studies, two which were "saline-tolerant" and two which were "saline-intolerant". The life histories and effects of increasing salinity on the reproduction of the four species were then to be used to explain their distribution in relation to salinity.

The toxicity of saline solutions to invertebrates was then to be investigated, and finally the physiological effects of increasing salinity were to be studied in the laboratory, using measurements of physiological stress such as respiration, mobility and sodium balance.

CHAPTER 2

Invertebrate and Chemical Surveys of Millwood Brook and the River Idle Catchment

2:1 General Introduction

Benthic invertebrate surveys are important in the biological monitoring of the effects of pollution on rivers (Hellawell, 1986). This approach is usually chosen as, unlike fish, invertebrates cannot voluntarily move away from a source of pollution and rapidly return once conditions ameliorate. Some invertebrates are more tolerant of pollution of various types than others, and these tend to remain below a source of pollution while others are killed. Thus a study of the composition of the invertebrate fauna above and below a source of pollution can provide much information regarding the severity of pollution, and the length of time over which it has occurred. In addition, invertebrates are present in all but the most polluted river sites, can be easily collected and handled in the laboratory, and much information has been published concerning their identification and ecology.

Chemical samples are usually collected to supplement invertebrate samples in order to provide information on the specific nature of any pollutants present. These, however, have the limitation of only providing information about the condition of the river water at a single instant, and only those pollutants known or suspected to be present can be measured.

A detailed invertebrate survey of Millwood Brook in the

vicinity of Creswell Crags was therefore planned, and a more general survey of the rivers in the River Idle catchment was undertaken to discover the extent of the effects of saline mine effluents in this area.

2:2 Millwood Brook

2:2:1 Methods for qualitative and quantitative invertebrate sampling and chemical analysis

Twenty-four sites were selected for invertebrate sampling, nineteen on Millwood Brook and five on inputs and tributaries. Sites were selected on the basis of their distance from a source of pollution and their ease of access. Most sites were in riffle sections (areas of the river bed where stones break the surface of the water) and all were shallow enough to be sampled on foot. Sites were further apart away from the input of pumped mine drainage water and closer together below this point to detect any rapid changes in fauna. Details of the sites are given under the "Site Descriptions" heading.
Once a month between April 1986 and April 1987, 500ml of river 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 a PTIO portable conductivity meter, a Corning model 7 pH meter and a Corning 925 "chloride" analyser. The last measures total halide ion concentration and although the results are calculated in terms of chloride alone, about 5% of the halide present is known to be bromide (Dodd and Terrell-Nield, 1985). Calcium was measured on a Corning 940 calcium analyser, which uses a fluoresence reaction,

and sodium concentrations were measured in July and December using a Corning-Eel flame photometer. Water temperature was measured in the field using a standard mercury thermometer. Dissolved oxygen concentration of the water was measured in the field in December and July using a portable oxygen meter.

To analyse the river water, sediment, pumped mine drainage water and sewage effluent for metals and other ions, several samples were analysed by electron-probe microanalysis. This method records the presence of all elements with an atomic number greater than 16, and gives an indication of the relative proportions of these ions. 100ml samples of river water from sites M1 and M5 were evaporated to dryness, in addition to 100ml samples of mine drainage water and sewage effluent, and a sample of the orange sediment collected from the surface of rocks below the mine drainage water input. Small samples of these were placed on carbon stubs and analysed by the electron-probe analyser, which was connected to a scanning electron microscope and computer, calculating peaks of activity corresponding to each element present in the sample.

The invertebrate fauna at each site was sampled in April, July and October 1986, using a standard kick and search technique. Three samples were taken from each site as it was shown by Furse *et al.* (1984) that this method of sampling produced a more representative species list than samples taken in only one season.

Kick sampling is used by most freshwater biologists to obtain benthic samples and essentially consists of placing a fine-mesh (900*um* mesh size) net downstream of the operator on the river bed, and then disturbing the sediment with the foot to a depth of several centimetres,

allowing displaced animals to be washed into the net. This technique is most effective in shallow water where animals are not washed over the top of the net, and where the substratum can be clearly seen, so that kick samples can be taken at different habitats within a sample site. In addition, a manual search of the underside of rocks and debris such as tree branches is carried out to ensure capture of clinging animals such as flatworms and leeches etc, which might not be dislodged by kicking.

The method was tested by Furse *et al.* (1980), who found that a three-minute sample from a site could collect 60% of the species which were collected from an eighteen minute sample.

Since many of the sites sampled in this survey were already poor in both species and numbers, it was decided to sample for three minutes only at each site to minimise the disturbance. Furse *et al.* (1980) also found that species-level identification reduced the numbers of "mis-classified" sites, so all invertebrates collected in the survey were identified as far as possible, to species where keys were available, and to the lowest level possible when species-level keys were not. The exception this rule were the Sphaeriidae and Hyracarina, whose identification was considered to be specialised.

All samples were sorted live and the invertebrates preserved in 70% alcohol for identification (using the keys listed in Armitage *et al.*, 1980), with the exception of the enchytraeids and planarians, which were identified alive.

To assess the numbers of oligochaetes and gammarids present in different stretches of river, core samples were taken during the summer at sites M1, M3, M4, M5, M6, M7, M8, M9, M11, and M12. The corer used consisted of a

FIGURE 2:1

Sketch Map showing Millwood Brook and the River Poulter together with the position of Creswell, Nether Langwith and Whitwell.

The position of sampling sites M1, M2, M3, M12, M16, M17, M18, M19, D and E are shown, and the position of the mine drainage water inputs indicated by arrows. (The positions of other sites are shown in Figure 2:3)

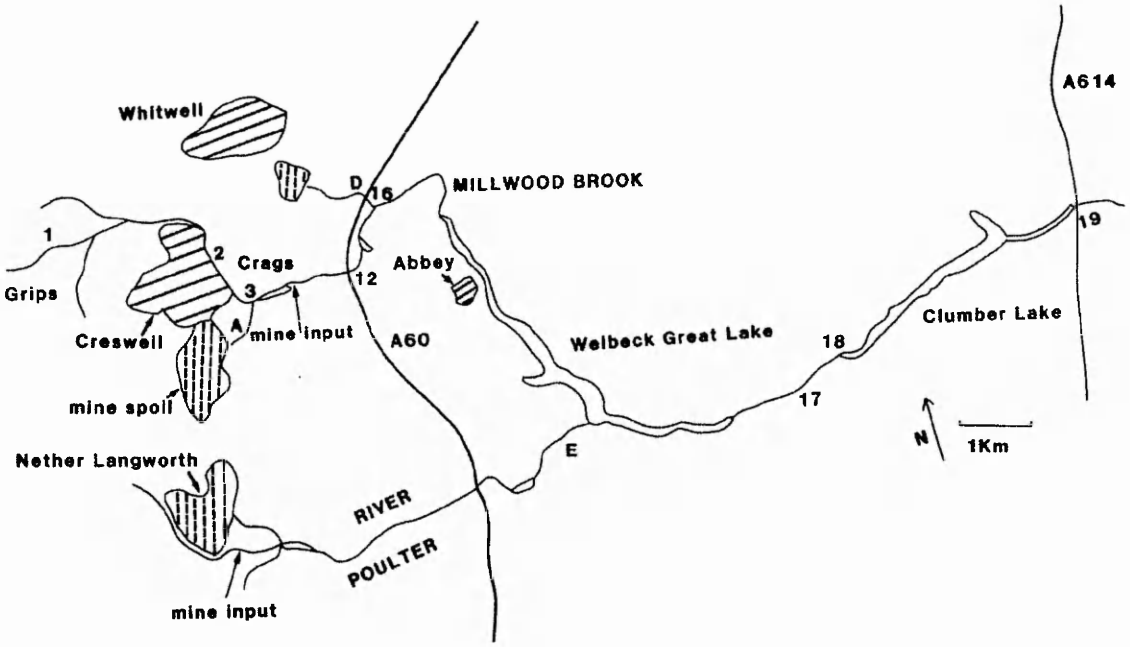

Whitwell
D
16
MILLWOOD BROOK
A614
1
2
Crags
3
12
Abbey
19
Grips
Creswell
A
mine input
A60
Welbeck Great Lake
18
Clumber Lake
mine spoil
17
N
1Km
E
Nether Langworth
RIVER
POULTER
mine input

FIGURE 2:2

Sketch Map showing Millwood Brook in the area of Creswell Crags.

The positions of sites M3 to M12, A and B are shown. Arrows indicate the direction of flow.

The position of inputs of Mine Pumping water (M), water from Crags Pond (C) and sewage effluent (S) are indicated by letter.

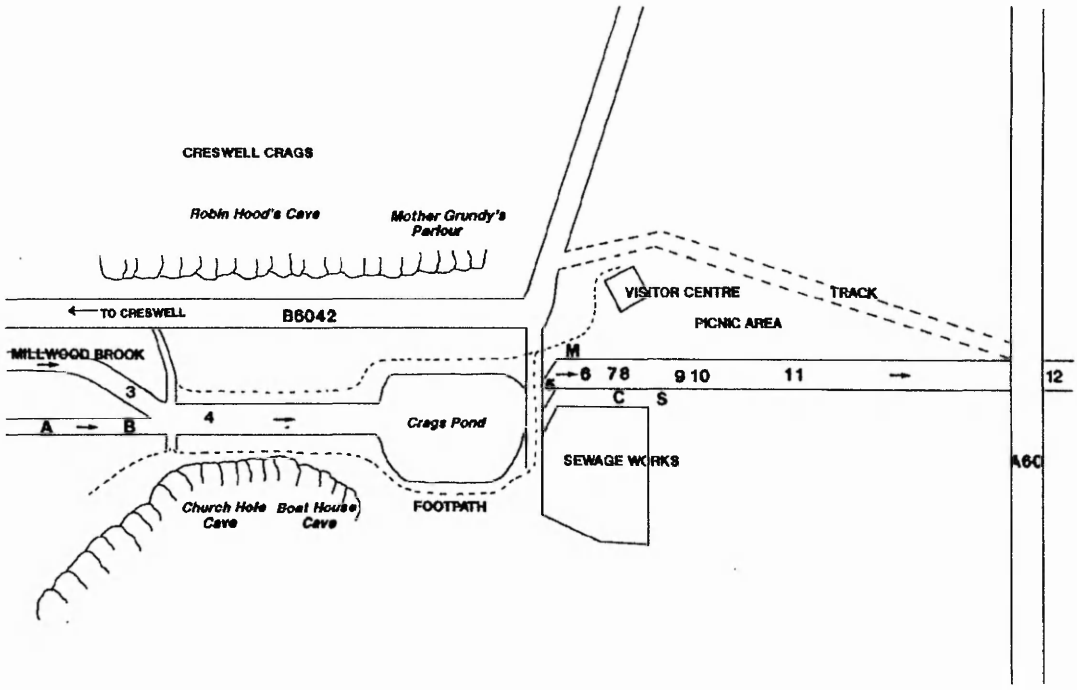
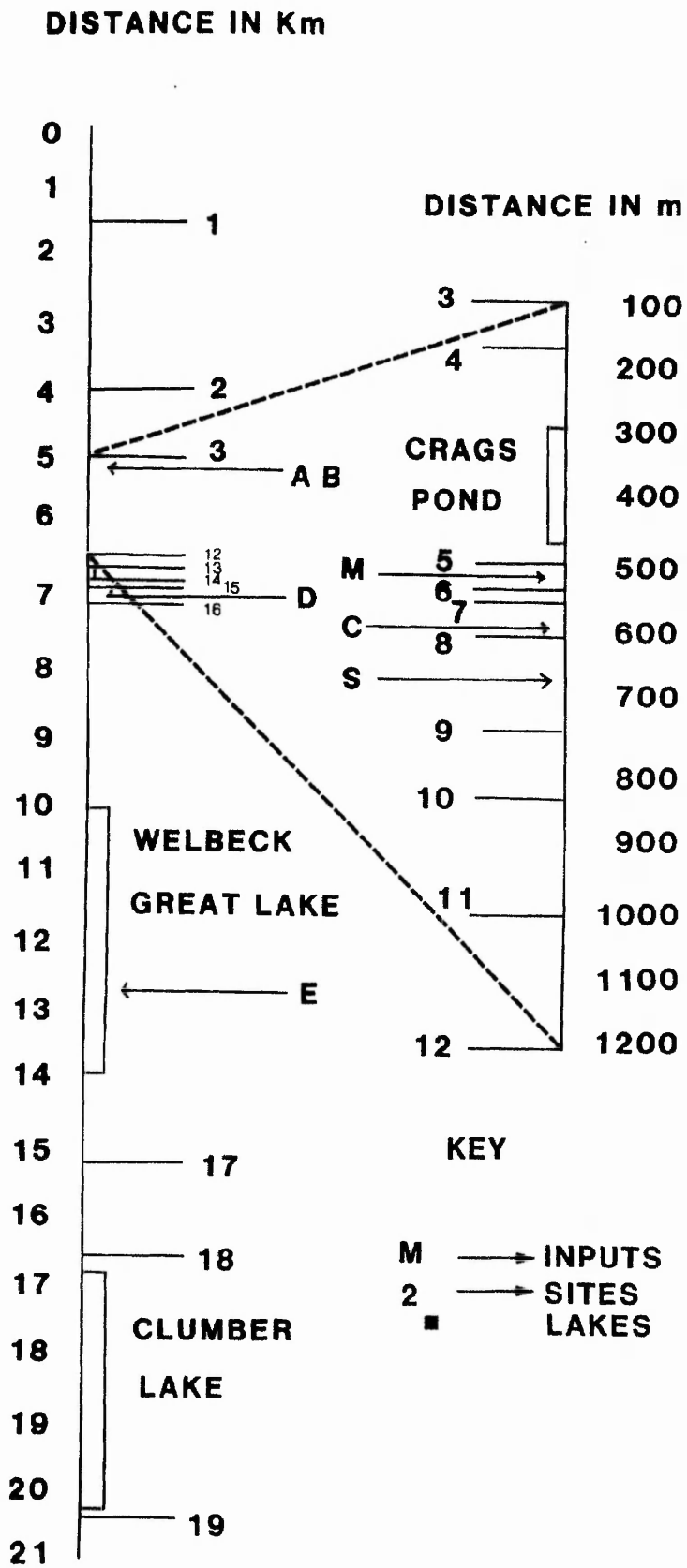

CRESWELL CRAGS
Robin Hood's Cave
Mother Grundy's Parlour
TO CRESWELL
B6042
VISITOR CENTRE
TRACK
PICNIC AREA
MILLWOOD BROOK
3
4
A
B
M
6 7 8
9 10
11
12
C
S
Crags Pond
SEWAGE WORKS
A60
Church Hole Cave
Boat House Cave
FOOTPATH

FIGURE 2:3

Diagram to show the distance from source of all sampling sites and inputs on Millwood Brook.

Sites are indicated by number and inputs by letter. The section between 5 and 6 Km from source is shown expanded, as sites are closer together in this area.


DISTANCE IN Km
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
DISTANCE IN m
100
200
300
400
500
600
700
800
900
1000
1100
1200
1
2
3
A B
D
E
17
18
19
3
4
CRAGS
POND
M
5
6
7
C
8
S
9
10
11
12
WELBECK
GREAT LAKE
CLUMBER
LAKE
KEY
M → INPUTS
2 → SITES
LAKES

10cm diameter plastic pipe pushed into the sediment and removed with a 150μm sieve held beneath the pipe. Core samples were taken in preference to kick samples for numerical analysis as the volume sampled by a corer can be precisely measured, and the corer can also be pushed into the substrate to a fixed depth, in this case 5cm. The cross-sectional area of the corer was 78.5cm^2, and the volume sampled was thus 393cm^3. Three core samples were taken at each site in August 1986, and were sorted live, as before. The total number of gammarids and oligochaetes from each core was counted.

2:2:3 Site descriptions

The 24 sample sites were numbered 1 to 19 (M1-M19) on Millwood Brook and A to E for sites on other streams and rivers. The positions of the sites are illustrated in Figures 2:1, 2:2 and 2:3. In addition, Table 2:1 shows the main physical characteristics of each site, together with grid references. The dominant substrate type at each site was graded on a 5-point scale, where 1 = silt, 2 = sand and clay, 3 = gravel, 4 = pebbles and cobbles and 5 = large stones and boulders.

Millwood Brook is formed from springs in a limestone gorge, Markland Grips. ("Grip" is a local term for gorge). The stream is initially small (1.65m wide and 13cm deep), with a clay bed supporting growths of *Apium spp.* (Fool's Cress). Site 1 (M1) was situated in Markland Grips. This small stream is joined by a similar stream from Hollinghill Grips and flows towards Creswell, at Clowne receiving the input from a sewage works below

TABLE 2:1

Grid Reference, Width, Depth, Flow Rate and Substrate Type of Millwood Brook and Tributary Sites Measured in June 1986

Site	Grid Ref.	Width m	Depth cm	Flow $m^3 s^{-1}$	Substrate Type
1	SK503747	1.65	13	0.20	CLAY
2	SK526746	2.40	15	0.80	ROCKS
3	SK534742	2.2	18	0.89	ROCKS
B	SK533740	1.8	37	0.40	SILT
4	SK534742	3.20	44	0.50	SILT/ROCKS
5	SK537743	3.00	20	1.56	ROCKS
M	SK537743	1.40	7	0.13	CONCRETE/ORANGE
6	SK537743	3.00	30	0.71	ROCKS/ORANGE
7	SK537743	4.00	25	0.57	PEBBLES/ORANGE
C	SK537743	3.80	24	0.57	PEBBLES
8	SK537743	3.95	18	0.73	PEBBLES/ORANGE
9	SK538743	3.50	17	0.52	SILT
10	SK538743	3.20	25	0.52	SILT
11	SK543745	2.80	29	1.13	ROCKS/SILT
12	SK546745	3.00	27	1.43	ROCKS/PEBBLES
13	SK548749	3.50	22	0.56	PEBBLES
14	SK547751	4.00	115	0.52	SILT
15	SK549755	5.00	45	0.68	STONES/SILT
D	SK548755	1.10	11	0.33	CLAY
16	SK549756	3.50	24	0.56	PEBBLES/STONES
E	SK574719	6.90	66	0.82	SILT
17	SK606727	6.00	50	1.08	SAND/PEBBLES
18	SK616734	10.40	57	1.63	SAND/PEBBLES
19	SK649757	10.80	28	0.85	PEBBLES

which the river flows in a much more rocky bed supporting growths of the moss *Fontinalis* spp. Site M2 was situated in this region.

Site M3 was at the head of the limestone valley, Creswell Crags, and just downstream of this site the river is joined by a stream of mine spoil tip runoff and coal washing water from the mine at Creswell, and also water from a spring at the head of the valley. Sites A and B were situated on this stream, which was excavated by the Coal Board to receive run-off water. This stream flows continuously in all but the driest weather, although it is classified as only intermittent in flow. The mine spoil water at site A is very variable in both quality and quantity, occasionally receiving mine drainage water directly. It also introduces large amounts of silt and coal dust to the main stream.

Millwood Brook then flows in a silty and rocky channel through Creswell Crags and empties into Crags Pond, an artificial enclosure at the end of the valley, which was built in 1860 to provide a duck shoot. Site M4 was situated half-way along the valley. Crags pond receives water at a rate of 20$l sec^{-1}$, and has an average output of 10$l sec^{-1}$, although this can be as low as 4$l sec^{-1}$ in dry weather, and higher in periods of very heavy rain. There is clearly some leakage from the pond into the substrata, and in wet weather there is a substantial amount of water in 'Boat House Cave' (Figure 2:2).

The water from Crags Pond flows over a concrete spillway into a very rocky channel. Site M5 was situated 15m below the pond, where the river bed consisted of large rocks with very little silt. 17m below Crags Pond, pumped mine drainage water is introduced into the river from a large pipe along a concrete slipway (Plate 1). The drainage

water flows at a rate of between 30-40lsec^{-1} and deposits an orange sediment which encrusts the pebbly river substratum for 100m downstream from its entry. Sites M6, M7 and M8 were situated 5, 50 and 80m respectively below this discharge. Large growths of *Enteromorpha linza* and *Enteromorpha intestinalis* were present in summer below this point, with no growths of the *Fontinalis* spp. and *Apium* spp. seen above the minewater entry. 75m below the minewater input, however, there is an outflow of water from Crags Pond which has been ducted through the water treatment works, (Site C), and there is a small area of *Fontinalis* spp. (1m in length) immediately below this outfall

130m downstream from the mine effluent there is a discharge of effluent from Creswell Water Reclamation Works into the river. Below this input the sediment becomes a black, glutinous and anaerobic layer over 1m deep, sometimes with solid sewage visible. The river flows between steep, made-up banks in woodland and is sluggish and offensive in odour. Sites M9 and M10 were situated in this area. These conditions continue for another 200m until the river widens and becomes more stoney bottomed, although all stones are coated in organic slime (Site M11).

The river is then channelled under the A60 road and enters Welbeck Estate, where it flows into a small lake. Site M12 was situated at the point where the river emerged from under the A60, and site M13 above the lake. Downstream of this lake the river channel is artificial and has a deep, silty sediment (Sites M14 and M15). It is joined between sites M14 and M15 by a small, swift stream flowing from coal tips at Whitwell (Input site D), which also receives the effluent from Steetly Minerals and a

small sewage works. The river then widens into a natural channel with shallow water (10-20cm) and riffles (Site M16).

The river flows across Welbeck Estate, until, just above the head of Welbeck Great lake, Millwood Brook joins the River Poulter (Input site E) and from this point takes that name, flowing into a complex series of managed lakes and channels in Clumber Park. Site M17 was situated just outside Welbeck Estate, and site M18 at the entrance to Clumber Estate, in a woodland area. On leaving Clumber Park the river flows under the A614 road and emerges into a broad, shallow and pebbly bed, site M19, which was the final sampling point in this survey.

2:2:4 Results

2:2:4:1 Chemical Analysis

The mean values and range for the five chemical parameters measured monthly are shown in Figures 2:4. These graphs illustrate the sudden rise in temperature, chloride, sodium and calcium ion concentration caused by the input of pumped mine drainage water between sites M5 and M6, and their gradual decrease through the survey area as the river received diluting water from several sources (Sites S,C and D). The July and December figures for temperature are shown in Figure 2:4(f) as these represented the maximum and minimum recorded values for temperature during the year.

At its source, Millwood Brook had a conductivity of 0.96 mS/cm and a Cl^- ion concentration of 2.07mM, which had a very low variability. These values did not greatly increase downstream of this area until the entry of the

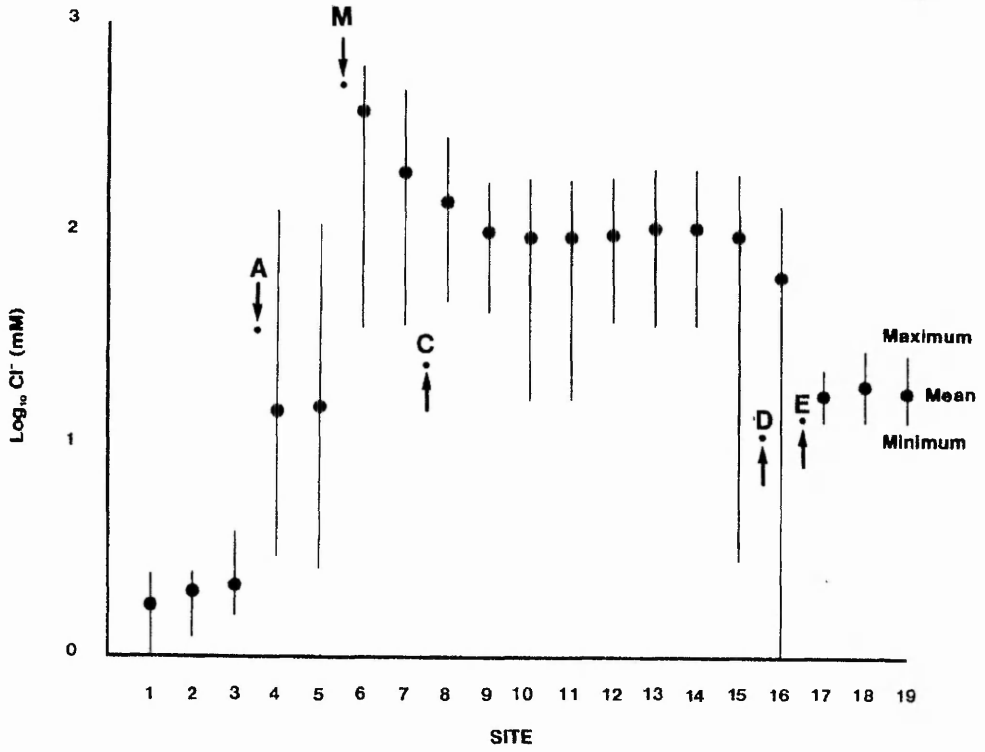
FIGURE 2:4

Mean, Maximum and Minimum values of environmental variables at Millwood Brook Sites M1 to M19, measured monthly between April 1986 and April 1987.

a) $Log_{10} Cl^-$ (mM)

Small spots and arrows indicate the mean values of inputs between sites.

b) Conductivity (mS/cm)


3
2
1
0
M
A
C
D
E
Maximum
Mean
Minimum
Log10 Cl- (mM)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
SITE

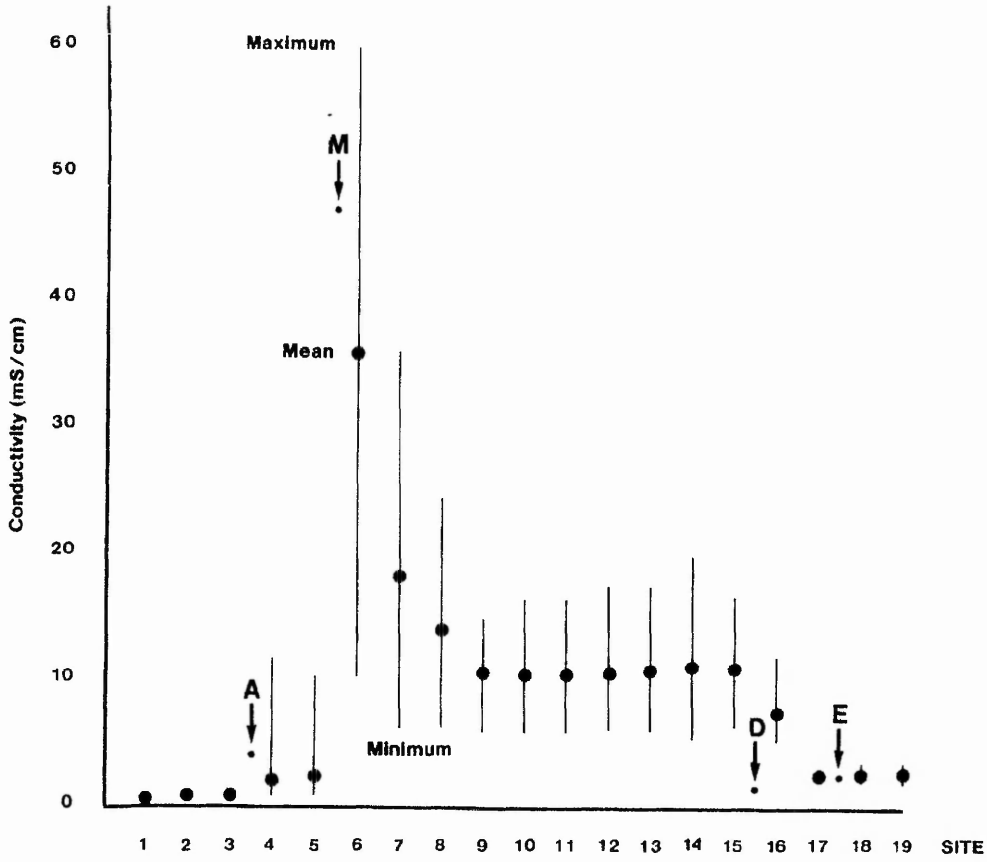
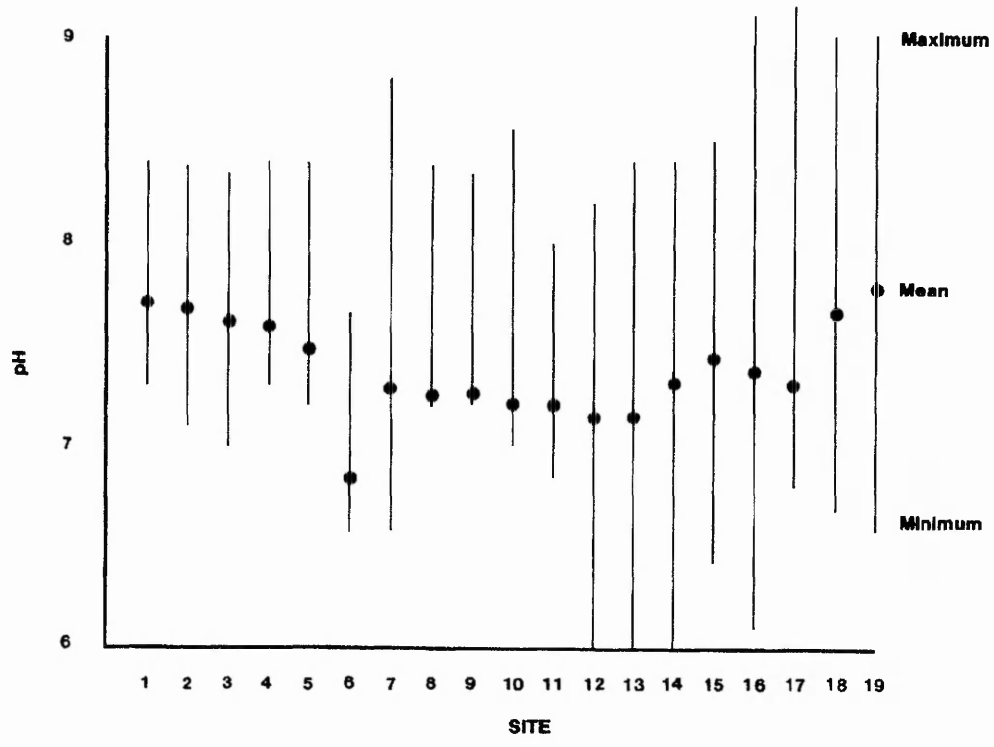

60
50
40
30
20
10
0
Maximum
M
Mean
A
Minimum
D
E
Conductivity (mS/cm)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
SITE

FIGURE 2:4

Mean, Maximum and Minimum values of environmental variables at Millwood Brook sites M1 to M19 measured monthly between April 1986 and April 1987.

c) Mean, Maximum and Minimum pH

d) Maximum and Minimum concentration of dissolved oxygen (*u*M). The maximum values were recorded in July 1986 and the minimum in December 1986.


9
8
7
6
pH
Maximum
Mean
Minimum
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
SITE

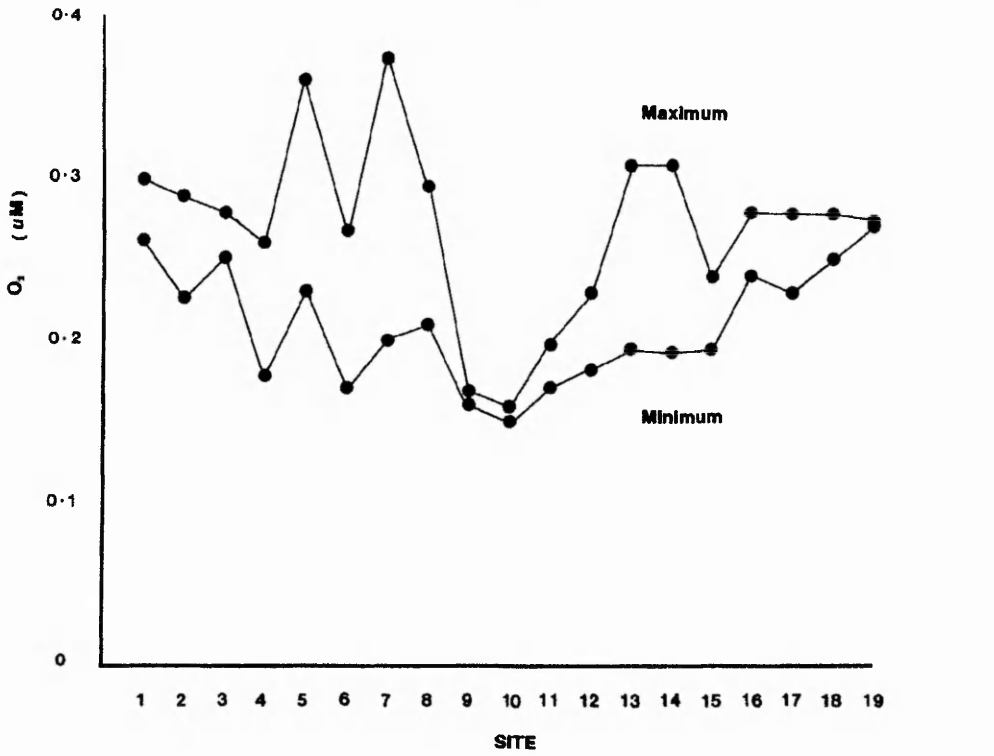

0·4
0·3
0·2
0·1
0
O_2 (uM)
Maximum
Minimum
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
SITE

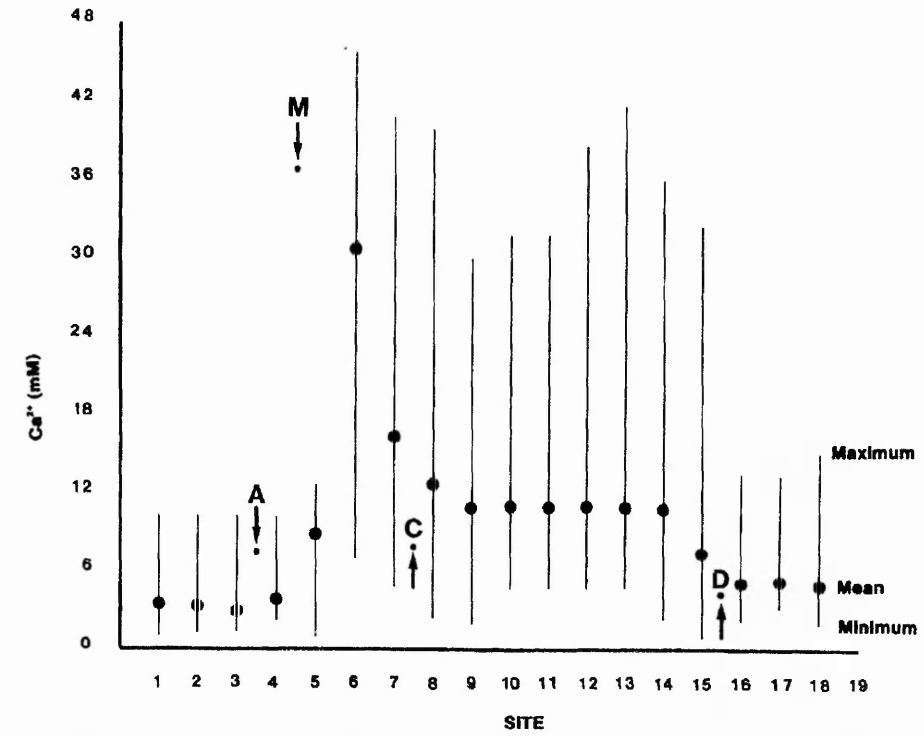
FIGURE 2:4

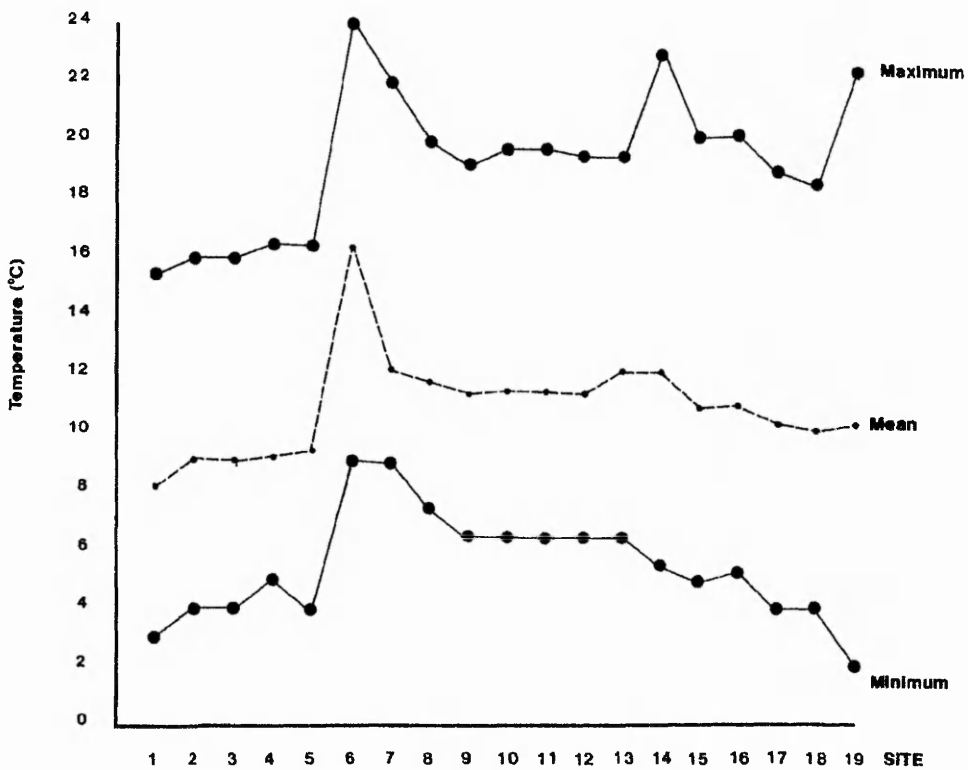
Mean, Maximum and Minimum values of environmental variables at Millwood Brook Sites M1 to M19 measured monthly between April 1986 and April 1987.

e) Mean, Maximum and Minimum concentration of Ca^{2+} (mM).

Small spots and arrows indicate the mean values of inputs between sites.

f) Mean, Maximum and Minimum Water temperature. (^0C).


48
42
36
30
24
18
12
6
0
Ca²⁺ (mM)
M
A
C
D
Maximum
Mean
Minimum
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
SITE


24
22
20
18
16
14
12
10
8
6
4
2
0
Temperature (°C)
Maximum
Mean
Minimum
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 SITE

input channel from the spoil tips at Creswell Colliery, below site B. The conductivity and chloride levels at sites A and B were very variable, conductivity at site B ranging from 1.3mS/cm to 23mS/cm during the sampling period. For much of the year, the flow in this channel consisted of run-off from the tips and had a conductivity of up to 10mS/cm, but on one occasion during year, pumped mine drainage water was released directly down this stream, increasing the conductivity to 23mS/cm, and leaving orange deposits on top of the silt in the channel. The period of release of pumped mine drainage water down this channel lasted for two weeks. The variable condition of the channel water resulted in fluctuating water chemistry downstream at site M4, where although the average conductivity was 2.67mS/cm, extreme levels of between 1.1mS/cm and 11.5mS/cm were measured.

The water released from Crags Pond had a mean conductivity of 2.4mS/cm, although a maximum value of 10.4mS/cm was recorded on one occasion after diversion of pumped mine drainage water down the channel at sites A and B. The large input of mine pumping water at site M was almost constant in volume and composition throughout the year, flowing at a rate of 30-40lsec^{-1}, only once during the experimental period being shut down to allow for the installation of new filters at Creswell Colliery. During this time the mine pumping water was diverted both to site A and to the River Poulter at Nether Langwith. (See Figure 2:1). The mine pumping water had a mean annual conductivity of 43.2mS/cm and a mean annual chloride concentration of 602.3mM. It also contained a high concentration of calcium (30.54mM) which assisted in maintaining a mean pH of 7.11 throughout the year. It can be seen from Figure 2:4(c) that pH remained between 7.1

and 8.0 at all sites. The mean annual temperature of the mine effluent was 20^{0}C, which had a considerable effect on the water temperature below the effluent, particularly in winter, when the main river flow above the pumped mine drainage water was at 8.5^{0}C, compared to a mean value of 17.5^{0}C below.

Below the mine outfall, chemical conditions fluctuated greatly depending on the amount of water flowing over from Crags Pond. In drought conditions the mine pumping water could constitute more than half the river volume, but in spate conditions it contributed less than a quarter of the flow (See Plate 1). At site 7, for example, 40m below the mine, chloride concentrations of 12mM in winter and 427mM in summer were recorded. In spate conditions the mine and river waters mixed thoroughly by this point, but in drought conditions mixing was very slow. At site 7 measurements of 5.3mS/cm conductivity and 15.6^{0}C were found in the river flow at the further side of the channel, with 29.6mS/cm conductivity and 20.4^{0}C recorded directly opposite in the mine flow.

At site C the river consistently received an input of water from Crags Pond. This water flowed rapidly out of a small pipe and before mixing with the mainstream water produced a "pocket" of water similar to that at site 4, although it did not have sufficient volume to greatly dilute the salt in the main river flow.

Just above site 9, the river received effluent of variable quality from the water treatment works, and while this reduced the average river water conductivity to 12.05 mS/cm, large quantities of organic material were added to the river from this discharge, and the appearance of the river was little improved. Figure

2:4(d) shows the oxygen content of the river water at all sites, measured in situ using a portable oxygen meter. A value of 3.5mM (10ppm) O_2 approximates to full saturation of the water. At site M1, the water was 80-100% saturated throughout the year, and at no site was a concentration below 50% measured. The sites which consistently had the lowest oxygen concentration were M10 and M11, with concentrations of 50-60% in both summer and winter. These sites were below the input from the Water Reclamation Works, where the sediment was deep and silty and there were no riffle reaches to provide extra oxygenation.

The inputs into Millwood Brook below the Water Reclamation Works (Sites D and E) were of river water lower in chloride than 16mM which served to dilute the salts in the river, which slowly improved in chemical quality while passing through the extensive lakes on Welbeck and Clumber Estates. At site 19, however, 16km below the mine input, chloride concentration was still a mean of 19mM, nine times the level found at Markland Grips.

Table 2:2 shows the results of electron-probe microanalysis of river water from site 1, site 5, the pumped mine drainage water and orange deposit found below the mine, and also the solid deposits from the sewage effluent. It should be noted that this technique does not record the presence of several major elements, including nitrogen, oxygen and carbon, and that the calculated percentages assume that the elements recorded constitute the whole of the sample.

The water from Markland Grips (Site 1) had high levels of calcium, sulphur, chlorine and magnesium, with low levels of potassium, silicon and iron. This indicated that the compounds present were calcium and magnesium sulphates

TABLE 2:2

Percentage Composition of Evaporated River Water from Sites 1 and 5 on Millwood Brook, Mine Pumping Water, Orange River-Bed Sediment and Sewage Effluent Determined by Electron Probe Microanalysis

Element	Site 1	Site 5	Mine Drainage	Orange Sediment	Sewage Effluent
Na	0	41.04	54.98	5.70	47.13
Cl	26.83	39.42	39.42	6.58	33.28
Ca	26.27	2.31	2.31	72.04	5.76
Si	0.61	0.08	0.08	2.08	0.85
Mg	12.06	1.87	1.87	0	4.41
S	31.61	0.17	0.17	0.93	6.99
Fe	0.13	0.06	0.06	7.87	0.13
K	2.44	0	0.17	0.63	1.42
Br	0	0.45	0.45	0.82	0
Zn	0	0.08	0.08	0.08	0
As	0	0.24	0.24	0.15	0
Mn	0	0	0	0	0.02
Cr	0.05	0	0	0	0
Cu	0	0.03	0.03	0.04	0

and chlorides, with probably also carbonates although these cannot be detected by this method. The low levels of sodium present were not detected by this method.
Above the pumped mine drainage water input, sodium was present at high concentrations, with a reduction in the percentage of calcium and magnesium, indicating that sodium chloride was the most important chloride present in the river water at this point. There were also very low levels of arsenic, zinc and bromine.
The pumped mine drainage water itself contained very large amounts of sodium and chloride. These were recorded in such quantity that the presence of other elements, except calcium and magnesium, appear negligible. There were very low levels of some heavy metals such as copper, arsenic, iron and zinc, and also bromine. The orange sediment removed from the mine outfall, in contrast to this, contained calcium in high concentration, with few other elements present in any great amount. Iron, however, was present in higher concentration than in the pumped mine drainage water, while sodium and chlorine were present in lower concentration. This indicated that the sediment consisted mainly of calcium carbonate, with low levels of calcium chloride and sulphate and some sodium chloride, together with oxides of iron, which accounted for the orange colour of the compound.
The sewage effluent contained quite high levels of sodium and chlorine but did not appear to introduce any heavy metals into the river.

2:2:4:2 Qualitative Invertebrate Sampling

A total of 126 invertebrate taxa were identified from the combined sites, although no individual site had more than

39 taxa. The sites at which each species was recorded are listed in Appendix A.

Figure 2:5 shows the relationship between number of taxa and conductivity for each site, which can be seen to be highly negatively correlated ($r = -0.734$), which is significant at the 1% level using Spearmann's rank order correlation.

Figure 2:6 shows the Biological Monitoring Working Party (BMWP) scores (Chesters, 1980) for each site on the mainstream above the abscissa and also on each tributary below the abscissa. The effect of each input is reflected in the scores at each site. A BMWP score of above 50 is generally taken to indicate a site of acceptable quality by Water Authorities, (Severn Trent Water Authority, 1985/86) and it can be seen from Figure 2:6 that only sites 1,2,3,5,17,18 and 19, together with input E reached this minimum standard.

At site 1, 35 species were recorded, including the stoneflies *Isoperla grammatica* (Poda) and *Amphinemura standfussi* Ris, species commonly found in the Midlands (Bird, 1982). Two species of mayfly, *Baetis rhodani* (Pictet) and *Baetis vernus* Curtis were recorded, and large numbers of beetle larvae (*Helodes* sp.). There were also large populations of *Simulium costatum* Friederichs, typical of springs, and *Gammarus pulex* (L.) present. The site had low numbers of Oligochaeta, most of those found being enchytraeids and lumbricids. The high calcium content of the water at this site resulted in most of the cased caddis, gastropods and slow moving beetles such as *Elmisaenea* (Muller) being coated in white calcareous deposits.

At site 3, 32 species were recorded. Stoneflies and *Baetis vernus* were absent, and the dominant *Simulium*

FIGURE 2:5

Total number of invertebrate taxa recorded at nineteen sampling sites on Millwood Brook (M1-M19) (Solid Line) and mean annual conductivity (mS/cm) (Dotted Line).

Mean conductivities are calculated from monthly samples collected between April 1986 and April 1987 and total taxa from kick samples collected at each site in spring, summer and autumn 1986.

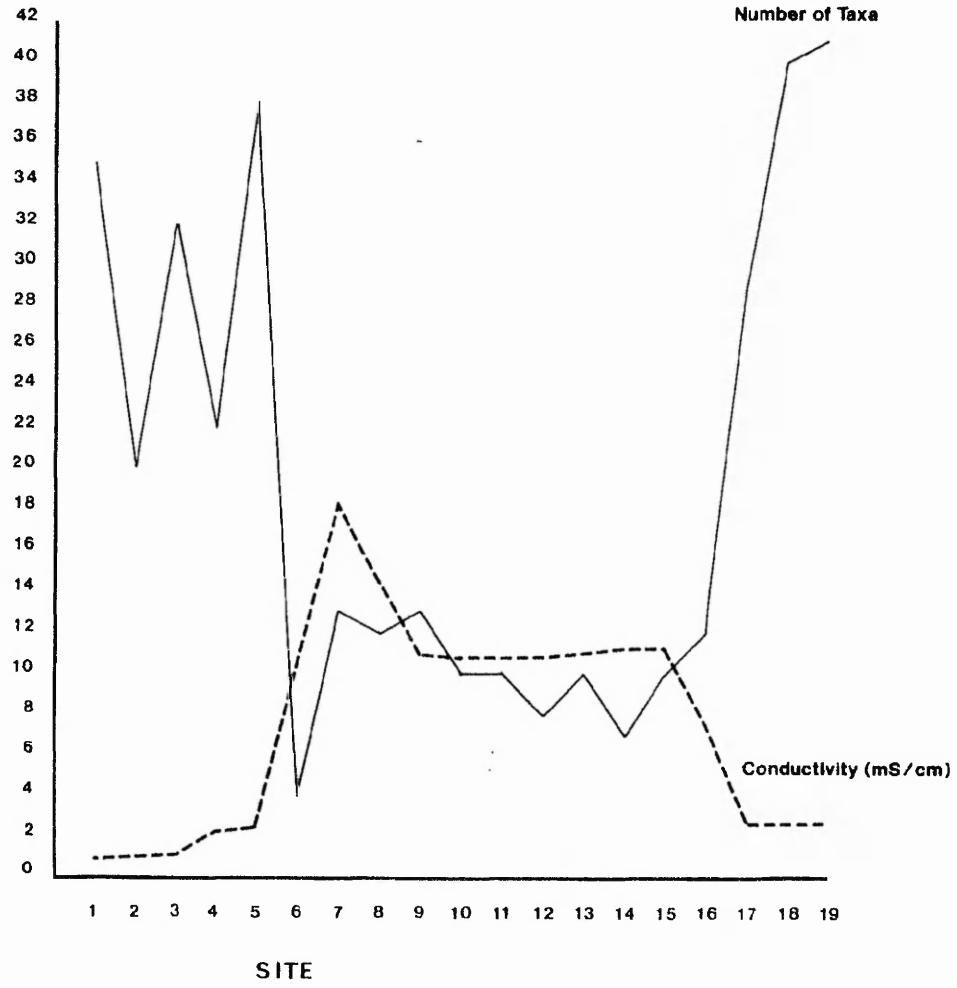
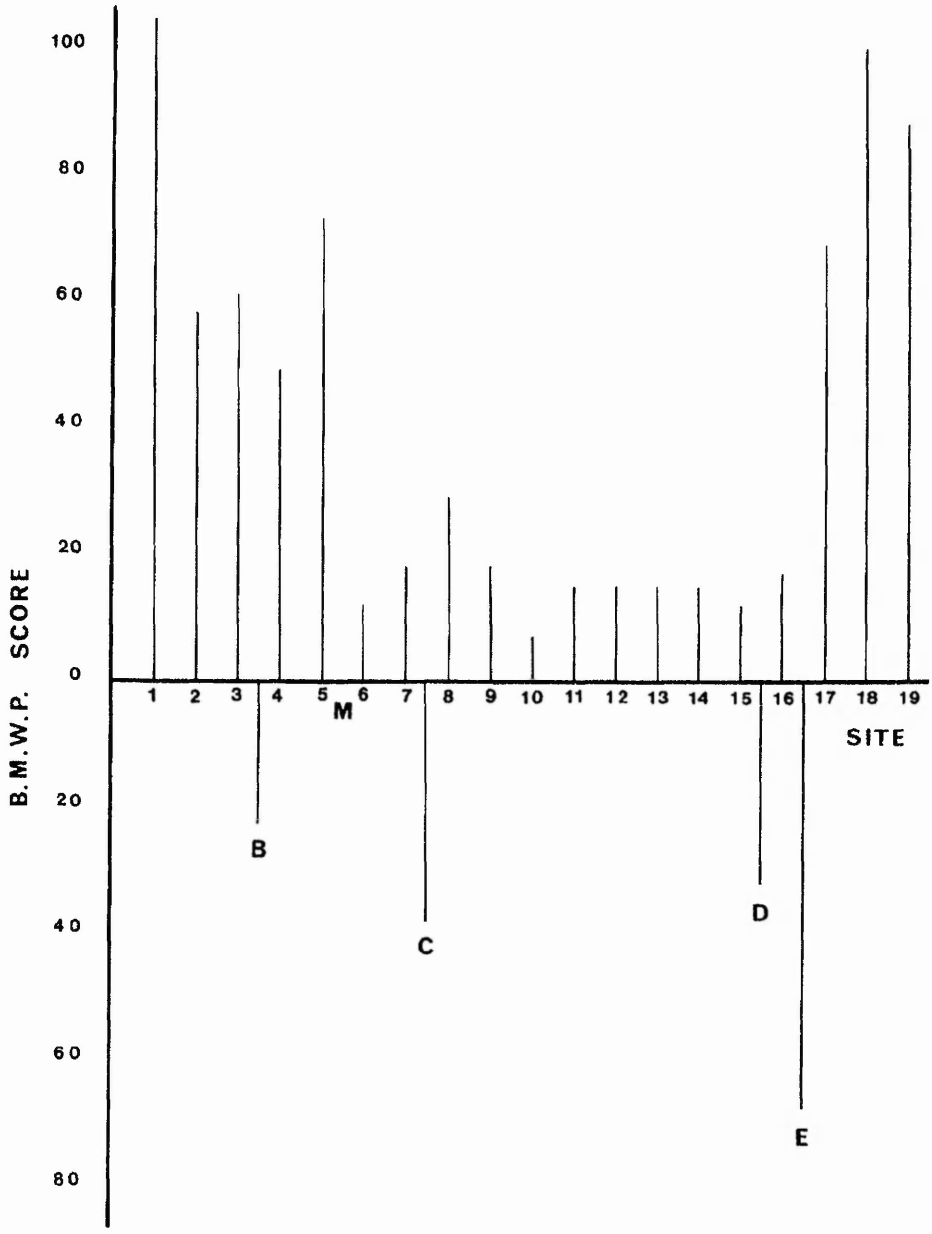

Number of Taxa
Conductivity (mS/cm)
42
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
SITE

FIGURE 2:6

BMWP Score calculated from the total numbers of families collected at nineteen sites on Millwood Brook and four tributary sites.

Lines above the abscissa represent the BMWP score for Millwood Brook sites, and lines below the abscissa the score for sites on B, C, D, and E. The position of the input of mine drainage water is indicated by M.


100
80
60
40
20
0
20
40
60
80
B.M.W.P. SCORE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
M
SITE
B
C
D
E

species was *Simulium ornatum* Meigen. There was an increased oligochaete fauna consisting mainly of Tubificidae.

Site A had a very limited fauna, with no Tubificidae despite the presence of an apparently suitable sediment and with *Chironomus* spp. the only group present in quantity. This site receives mine tip runoff water which is very variable in quality. The spring input at site B produced a much more diverse fauna in the stream, with *G. pulex* present.

At site 4, 22 species were recorded. The numerically dominant group at this site were the Tubificidae, with a mixed population consisting mainly of *Tubifex tubifex* (Muller), *Limnodrilus hoffmeisteri* Claparede and *L. udekemianus* Claparede. The *Gammarus pulex* present at this site were noted to have a high level of parasitism by the acanthocephalan genus *Polymorphus*. At site 5, directly above the minewater input, 38 species were recorded, the mayfly *Cloeon dipterum* (L.), typical of lakes, replacing *Baetis rhodani*. A large number of gastropods and flatworms were also present.

Below the mine effluent, this diverse fauna was effectively wiped out. Although *Gammarus pulex* continued to be recorded downstream of this point in kick samples, the animals were single individuals which may have been washed downstream and were not living in the area, the exception being those found in the clean water "pocket" around input C.

The depauperate fauna below the mine was dominated by the enchytraeid worm, *Lumbricillus rivalis* Levinsen. The worms were present in great numbers in the summer, clinging to the growths of *Enteromorpha* spp. Also present in small numbers were the naidids *Paranais litoralis*

(Muller) and *Nais elinguis* Muller. These three species, together with *Potamopyrgus jenkinsi* (Smith), *Asellus aquaticus* (L.), and the chironomids *Chironomus* spp. and *Cricotopus* spp. formed the major part of the fauna for the next 1km, onto Welbeck Estate. Above the lake at Welbeck (Site 13), the introduced gammarid *Gammarus tigrinus* Sexton was first found, and this species was present at almost all the remaining sites, often becoming the numerically dominant species at a site (for example, at sites 14 and 15).

The most downstream record of *Gammarus pulex* was at the A60 road bridge (Site 12) so that a distance of 300m separated the ranges of the two species in 1985-86. *Gammarus tigrinus* has, however, been recorded extending its range upstream during this survey period. In autumn 1985 and spring 1986 it was found only at the edge of the small lake on Welbeck Estate (between sites 12 and 13, but by summer 1986 it was found consistently at site 12.

Tubificidae were not found in the river between sites 5 and 17) despite an abundance of organic matter in this stretch of river, and the main oligochaete species continued to be *L. rivalis*. Tubificids did not reappear until site 18, where a mixed tubificid fauna consisting mainly of *Limnodrilus* species was found in low numbers. The numbers of tubificids increased downstream from this point, with a corresponding decline in numbers of enchytraeids.

After joining the River Poulter below Welbeck Estate, the river began to support a more diverse fauna, with 29 species recorded at site 17. The caseless caddis, *Hydropsyche angustipennis* (Curtis) was recorded for the first time at this site and was the only hydropsychid found in the sample area. At site 19, there were 39

species including leptocerid caddis such as *Athripsodes cinereus* (Curtis) and *Mystacides nigra*(L.).

2:2:4:3 Quantitative Invertebrate Sampling

The abundances of *Gammarus pulex*, *Tubifex tubifex* and *Lumbricillus rivalis* above and below the pumped mine drainage water input are shown in Table 2:3. Above the input, *G. pulex* was the dominant species, with between approximately 6000 and 9000m^{-2}, but below the mine input were not found in any of the core samples.

Tubifex tubifex increased from zero density at site M1 to a peak of 37,000m^{-2} at site M4, in silty conditions, then declined at site M5, above the mine outfall, in stoney substratum. Tubificidae were completely absent below the mine outfall.

Lumbricillus rivalis was not present at sites M1, M3, M4 and M5, but at site M6, below the input of mine pumping water, appeared in numbers of approximately 13,000 m^{-2}, and increased to a maximum of 18,000 m^{-2} at site M7. There was a decline in the abundance of this species at site M9, below the sewage outfall, where the river was very sluggish, and numbers increased again at site 12.

2:2:5 Discussion

The chemical and invertebrate data presented in this section show very clearly the effects of a grossly polluting discharge on a small stream. At the head of the stream the fauna was typical of a clean, stoney, small stream in this area of the Midlands, indicated by the

TABLE 2:3

Abundance (m^{-2}) of *Gammarus pulex*, *Tubifex tubifex* and *Lumbricillus rivalis* at three Millwood Brook Sites Estimated from three core samples per site in August 1986

Site	*Gammarus pulex*	*Tubifex tubifex*	*Lumbricillus rivalis*
1	8318	0	0
3	7080	6401	0
4	6310	37153	0
5	9120	135	0
6	0	0	13183
7	1	0	18621
8	1	0	11220
9	0	0	794
11	0	0	2630
12	0	0	4732

presence of *Gammarus pulex*, *Glossosoma spp.*, *Rhyacophila dorsalis* (Curtis), *Baetis* spp., *Amphinemura standfussi* Ris, *Nemoura cambrica* (Stephens) and *Isoperla grammatica* (Poda). When the fauna of sites 1 and 2 were classified using the 'preliminary classification of running-water sites in Great Britain' (Wright *et al.*, 1984) these sites appear in groups 19 and 27 respectively, ie. groups representative of the upper and middle reaches of clean lowland rivers with a high alkalinity. In the zone below the input of mine drainage water this fauna was replaced by a restricted and much altered one consisting of *Lumbricillus rivalis*, *Paranais litoralis*, *Asellus aquaticus* and *Chironomus* spp, which the input of low quality sewage effluent did not significantly alter. Below this zone of extreme pollution, however, recovery was seen, especially after Millwood Brook joined the River Poulter between sites 16 and 17. The fauna found at the last survey site was typical of the large, shallow, mineral rich rivers of this area of the Midlands. If this site was assumed to be unpolluted and was classified using the same system as sites M1 and M2, it would appear in group 29, a group containing sites in lower reaches of rivers in Eastern England with a high alkalinity and macrophyte cover. The species composition at this site did, however, show some alterations which could be attributed to raised salinity, with the presence of *Gammarus tigrinus* and also the water-boatman *Sigara concinna* (Fieber), which is described by Macan (1976) as possibly being associated with slightly brackish water.
The drainage water entering Millwood Brook from the coal mine can be compared quite closely with standard sea water (Nicol, 1967) (Table 2:4), although there are decreased Na^{+}/Cl^{-}, K^{+}/Cl^{-}, Mg^{2+}/Cl^{-}, $SO4^{2+}/Cl^{-}$ ratios, an

TABLE 2:4

Proportions of Major Ions found in Standard Sea Water Compared to Mine Drainage Water from Creswell Colliery

Ion	Sea Water (mM)	Mine Drainage Water (mM)
Na^+	459	326
Cl^-	535	602
Mg^{2+}	53	19
Ca^{2+}	10	122
K^+	10	0.6
SO_4^{2+}	28	1.5
Br^+	1.0	14
Na^+/Cl^- ratio	0.56	0.35
K^+/Cl^- ratio	0.02	0.001
Mg^{2+}/Cl^-	0.07	0.02
SO_4^{2+}/Cl^- ratio	0.14	0.006
Ca^{2+}/Cl^- ratio	0.02	0.06

Source of figures for standard sea water was Nicol (1967)

increase in the Ca^{2+}/Cl^{-} ratio, and also an increase in the total ionic concentration. Millwood Brook itself is a limestone stream, with high concentrations of calcium plus low concentrations of other ions such as nitrate, phosphate and sulphate. Below the mine drainage water input, conditions similar to those in a river estuary were created, with large, but less rapid fluctuations in chloride levels ranging from 12mM (approaching fresh water) to 475mM (approaching sea water).

In such conditions it was perhaps not surprising to find upper sea-shore and estuarine species, in particular the green alga *Enteromorpha*, the enchytraeid worm *Lumbricillus rivalis* and naidid worm *Paranais litoralis*. *Enteromorpha* species are found throughout the country in rivers with some salt intrusion, even when this is slight (Haslam, 1982). In Millwood Brook, *Enteromorpha* grows vigorously to produce long mats in summer, coating the river bed. Living on this algal growth were large populations of *Lumbricillus rivalis* which is common on the upper sea-shore in decaying sea-weed (Kirk, 1971), in sewage filter beds (Williams *et al.*, 1969) and in low numbers as part of the normal river fauna. Learner *et al.* (1971) noted increases in the abundance of *L. rivalis* below coal washing and sewage outfalls on the River Cynon in South Wales. It may be that the enhanced salinity combined with organic matter in well oxygenated conditions (as in Millwood Brook) favour this species over others such as *Enchytraeus coronatus* (N&C) which also occurs in sewage filter beds (Learner, 1972).

The naidid *Paranais litoralis* is most commonly regarded as an estuarine species (Learner *et al.*, 1978), although it has been recorded inland by several workers such as Wachs (1963) from the River Werra, Brinkhurst (1967) from

the Saginaw River, and by Pascar-Gluzman and Dimentman (1984) from the lower Jordan Basin in Israel, all areas with salt intrusion. The second recorded naidid species, *Nais elinguis*, is a common freshwater and estuarine inhabitant. (Learner *et al.*, 1978).

It is surprising that tubificids, generally regarded as "pollution tolerant" organisms, were so completely eliminated by the raised salinity of the water in Millwood Brook below the input of mine drainage water, and did not reappear for 20km downstream. This was despite the reduction in salinity found below the Water Reclamation Works and the organic sediment produced by sewage discharges, normally considered good conditions for Tubificidae. The river at this point also had a raised mean annual temperature compared with site M4, where Tubificidae are abundant. Aston (1973), however, has demonstrated that Tubificidae are able to survive and breed at temperatures of up to 25^{0}C. As these temperatures were not exceeded below the input of mine effluent, it does not appear that raised temperature is in itself limiting the distribution of this group, although it may contribute to their absence by increasing the toxicity of salts present in the water. Bahner and Nimmo (1975), in assessing the effects of several pollutants on estuarine organisms, demonstrated their effects to be additive, each pollutant increasing the toxicity of an effluent by the level of its own toxicity. Chapman and Brinkhurst (1980), have, however, demonstrated that Tubificidae are not able to survive for long periods above a chloride concentration of 56mM, a finding which appears to be supported by laboratory work in the present study (Chapter 4). Levels of chloride approaching and exceeding 56mM are found in some

locations in the Midlands area (Severn Trent Water Report, 1985/86) and it is suggested that at these salinities care should be exercised in the identification of live Oligochaeta, as *Lumbricillus rivalis* is in appearance very similar to a small tubificid, being pink and curling tightly when disturbed, and is only readily separable after live examination under high power magnification.

Of the two gammarid species present in Millwood Brook, *Gammarus pulex* did not appear to be able to adapt to the conditions found below the mine and sewage outfalls as it was not found below these outfalls. Mixed populations of *G. pulex* and *G. tigrinus* have been encountered in the R. Idle and R. Poulter (Section 2:3:2) and also by Holland (1976) in the R. Weaver, but have not been recorded in Millwood Brook, each species occurring there separately. Holland also records *G. duebeni* Liljeborg and *G. zaddachi* Sexton together with *G. tigrinus*, but these have not been recorded in the extended sampling area in the Midlands. *Crangonyx pseudogracilis* (Bousefield) has been recorded from the R. Poulter together with *G. pulex* and in one sample, *G.tigrinus*.

In addition to the effects of increased salnity, the organic matter introduced from the Water Reclamation Works would in itself have a serious effect on the river fauna. Hynes (1960) described the consequences of such discharges, with the reduction in diversity downstream resulting in a fauna similar to that found in Millwood Brook at sites M11, with large populations of *Chironomus* spp., *Potamopyrgus jenkinsi* (Smith), Tubificidae and *Asellus aquaticus* (L.). The major differences between Millwood Brook and this classic description are that, as stated previously, there were no Tubificidae in Millwood

Brook near the sewage works, these being replaced by *L. rivalis*. Also, *Cladophora* spp. (blanket weed) are described by Hynes as forming large growths in the summer below sewage outfalls, often completely covering the river bed. This weed was almost completely absent in Millwood Brook and instead both above and below the sewage works there were large growths of *Enteromorpha* spp. which in summer completely covered the river bed. Furthermore there were no noticeable growths of "sewage fungus" in Millwood Brook, in contrast to many other organicly polluted streams, although most stones and animals below the black sludge zone were coated in organic slime. Hynes (1960) states that *Sphaerotilus* spp, a major constituent of sewage fungus, cannot survive an NaCl concentration greater than $300mgl^{-1}$, which may explain its absence from Millwood Brook.

Millwood Brook is a tributary of the River Poulter, which, up to the winter of 1985, had directly received no mine drainage water, and had mean annual chloride levels of only 2.5mM (Severn Trent Water 1985/86). However, since 1986, between September and April it now receives intermittent discharges of mine drainage water at Nether Langwith. These discharges are almost identical to those at Creswell. This increases the conductivity of the river at Nether Langwith from 0.93mS/cm (1.4mM Cl^-) above the effluent to 4.88mS/cm (42mM Cl^-) directly below. At present this has had little effect on the fauna of the R. Poulter, although at Norton, 2km from the discharge, there was a persistent rise in conductivity during the winter months. *G. tigrinus* was recorded from the summer 1986 samples, having been absent from the 1986 spring and samples, and further changes in fauna are likely.

The persistent changes in the fauna of Millwood Brook due

to increased salinity, as described in this chapter, demonstrate the need to determine accurately the salinity which would occur in a fresh-water body if saline effluent was introduced, in order to predict probable changes in fauna. In particular it appears that such effluents cause *G. pulex* to be replaced by *G. tigrinus* and that vulnerable groups such as stoneflies, mayflies and in extreme cases even tubificids are removed altogether. The rivers in the Midlands appear to be under increasing pressure from saline mine drainage, with new mines being opened in the Vale of Belvoir and existing mines working at greater depths, and in the future there will be a continuing need for monitoring to detect the effects of these discharges.

2:3 Distribution of *Gammarus pulex* and *Gammarus tigrinus* in the River Idle Catchment

2:3:1 Methods

Between March and September 1986, thirty-two sites on the rivers Idle, Maun and Meden, Rainworth Water and Vicar Water were surveyed for the presence of *G. tigrinus* and *G. pulex*. Sample sites on all the rivers were several kilometres apart, and conductivity was measured as an indication of salinity, as this was easily recorded using portable equipment.

Each site was sampled using the kick and search method described in Section 2:2:2, if the water was shallow enough to allow access, or by pushing a net along the river bed if the water was deeper. Rocks and patches of

weed were thoroughly searched for *Gammarus*. The samples were returned to the laboratory, sorted and the animals identified as described in Section 2:2:2, and the resulting species lists used in data analysis (Chapter 3).

2:3:2 Results

The position of the sites and the species of *Gammarus* found at each site is shown in Figure 2:7. There was a clear pattern in the distribution of the two species, with *G. pulex* present in rivers above mine inputs, such as in the River Maun, Rainworth Water and Millwood Brook. Conductivities at these sites were generally below 1mS/cm, while below mine inputs, this rose to above 2mS/cm, and *G. tigrinus* predominated.

An exception to this pattern was the River Maun at Sookholme Bath, where there were no *G. tigrinus* despite high conductivities, and no *G. pulex* despite the area being in the headwaters of the river. Downstream of this area, the River Meden, where the tributaries merge, is large, and dilutes the mine input sufficiently to lower the conductivity of the water to below 1mS/cm, and there were populations of *G. pulex*. In another area of river which did not follow the general pattern, the River Poulter above Clumber Park, the input of mine drainage water was intermittent, flowing only between September and April. Above this input, there were large populations of *G. pulex*, which were therefore available to repopulate the area after discharge ceased in the summer. However, at the junction of Millwood Brook with the River Poulter, there was a population of *G. tigrinus* and it seems likely that this species will be able to move upstream rapidly

FIGURE 2:7

Map showing the position of sites sampled for invertebrates between March and September 1986.

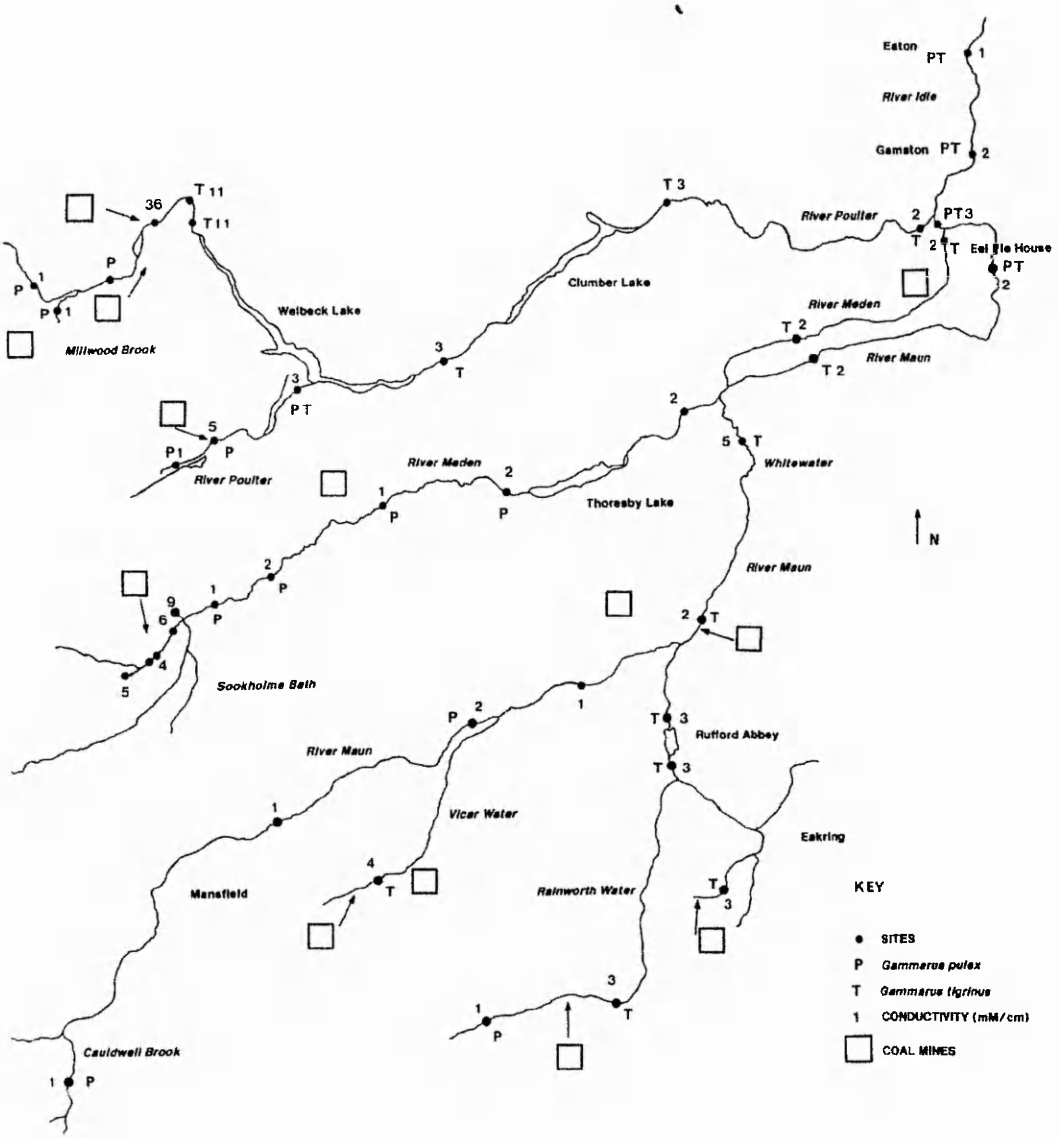
Sites are indicated by a black dot.

P indicates the presence of *Gammarus pulex*

T indicates the presence of *Gammarus tigrinus*

Conductivity (mS/cm) is given to the nearest whole number.

The position of coal mines is indicated by an open square, and the position of known discharges of mine effluents by an arrow.


Eston
PT
1
River Idle
Gamston PT
2
T 3
River Poulter
2
PT3
T 2
T
Eel Pie House
PT
2
36
T 11
T 11
Clumber Lake
Welbeck Lake
River Meden
T 2
River Maun
T 2
Millwood Brook
3
T
3
PT
5
P1
P
River Poulter
River Meden
2
1
P
P
Thoresby Lake
2
5
T
Whitewater
N
River Maun
2
P
1
P
9
6
4
5
Sookholme Beth
2
T
River Maun
P
2
1
T
3
Rufford Abbey
T
3
1
Vicar Water
Eakring
Mansfield
4
T
Rainworth Water
T
3
KEY
SITES
P Gammarus pulex
T Gammarus tigrinus
1 CONDUCTIVITY (mM/cm)
COAL MINES
Caudwell Brook
1
P
1
P
3
T

in future.

The extreme nature of the saline pollution of Millwood Brook was seen in the recorded conductivities, which were higher than in any other river. The only other river to approach the salinity of Millwood Brook was Vicar Water, which was also a small stream continuously receiving mine drainage water.

At three sites on the Rivers Maun and Idle, there were populations of both *G. pulex* and *G. tigrinus*. The rivers at these sites were large (over 15m wide), but not necessarily deep, as at Eaton, for example, the River Idle was less than 20cm in depth at the time of sampling.

2:3:3 Discussion

From the results of this survey, it seems that *G. tigrinus* has indeed been encouraged in the River Idle catchment by the input of saline effluents from the coal mining industry. A typical pattern is for *G. pulex* to remain as the only amphipod in the headwaters of a river, until there is an input of saline effluent. This increases the conductivity of the stream, which in some instances may become too saline for either species to survive, and below this area, *G. tigrinus* is present alone. In the lower reaches of a river, where conductivities have been reduced by dilution, the two species may co-exist if *G. pulex* is re-introduced via a clean tributary. Once *G. tigrinus* is established in an area, it is able to maintain populations at low conductivities.

The two species are often separated by a section of river which contains no *Gammarus* of either species, due either to extreme salinity (as in Millwood Brook), or organic

pollution as in the River Maun below Mansfield, where the fauna at the sampling site consisted of *Asellus aquaticus*, *Chironomus* spp. and Tubificidae. Where *G. pulex* re-colonises a section of river (from clean tributaries) in an area below a saline affected reach, it is able to co-exist with *G. tigrinus* at large river sites, for example at Eaton, where the river was wide and shallow, with a pebbly bottom. This may suggest that it is only the greater salinity tolerance of *G. tigrinus* which allows it to out-compete *G. pulex* in saline areas, and in non-saline areas the two species can co-exist if *G. tigrinus* is introduced.

The Severn-Trent Water Authority report for 1987/88 stated that *G. tigrinus* had been present in the River Idle system for twenty years, after originally being spread from Liverpool docks via the Trent and Mersey Canal into the Cheshire Salt Flats and thence into the River Trent and River Idle via the Chesterfield canal. If the species has been present in the River Idle for only 20 years, its rate of upstream spread in the Mansfield area would be approximately 0.9 km/year, as the most upstream population was recorded 18km from the River Idle confluence. This compares with an upstream migration rate of 0.75km/year achieved by *G. pulex* in the Isle of Man (Hynes, 1970). In Millwood Brook, however, *G. tigrinus* is extending its populations upstream much more slowly, as between 1985 and 1988 the species moved only 1km upstream. This is most likely to be due to the poor conditions of organic pollution and high salinity in this river.

It is difficult to estimate how far upstream *G. tigrinus* will be able to spread in Millwood Brook, as close to the mine input, salinities are higher than those in which the

species is recorded in the rest of the river system, and also in the area of the saline River Weaver (Holland, 1976).

CHAPTER 3

Mathematical Analysis of Field Data and Predictive Modelling

3:1 Introduction

Using the description of the macro-invertebrate fauna associated with saline pollution in Millwood Brook presented in Chapter 2, the effects of saline pollution on benthic macro-invertebrates could be analysed mathematically. The aims of this data analysis were to allocate the sites sampled on Millwood Brook and its tributaries into groups according to the salinity and physical characteristics of the sites, and to discover the species which were characteristic of each salinity group. This would enable a predictive model of saline pollution to be constructed, which would produce a suggested species list for any site in the East Midlands from environmental data which included salinity, and allow the effects of increasing salinity at a site to be predicted.

To provide a numerical analysis of a large raw data matrix of presence/absence records, ordination techniques are generally employed by community ecologists. These methods provide a spread of species (Q-type analysis) or sites (R-type analysis) along one or more mathematically derived axes. The species or sites can then be divided into groups which may correspond to ecological communities. The division of ordination axes can be performed in several ways, the most common being divisive cluster analysis (eg Scullion and Edwards, 1980) or

indicator species analysis (eg Murphy and Edwards, 1982, Wright *et al.*, 1984).

Ecological data sets can be visualised as a swarm of data points, each point reflecting the position of a species in relation to an environmental gradient. Each environmental gradient affecting the species can be thought of as a "dimension", so that the data swarm is spread out over many dimensions. Ordination methods are designed to "project" this multi-dimensional swarm of data points onto two or three calculated axes in such a manner as to reflect the underlying patterns of the data, and allow its easy interpretation.

In addition to the main patterns within the data, which are due to persistent gradients in environmental parameters, ecological data sets also contain a large amount of "noise". This "noise" is caused by the responses of a few individuals to unpredictable environmental changes and often to sampling errors. A successful method of ordination is able to reduce the effects of the "noise" and produce data points which are parallel with two or three principal axes, which can then be related to those environmental variables which most strongly influence the data.

Many commonly used analyses are based on principal component analysis (PCA) (Pielou, 1984). This method assigns a score to each site using the following steps:

The data are centered by dividing every element in the data matrix (X) by its row total. The new matrix is labelled X_R

The matrix $R=X_R X'$ is formed

An eigenanalysis of R is carried out to produce the matrix U

The matrix $Y=UX_R$ is calculated.

Each column of Y gives the new set of co-ordinates for one of the sites, which can be plotted. (The complete mathematics of PCA can be found in many texts, for example Pielou, 1984).

This method suppresses noise in the data since the first few principal components nearly always reflect the concerted responses of groups of species, and when such groupings act "in concert" it is unlikely to be the result of localised, temporary accidents.

The basic method of calculation chosen for the analysis of the data set in the present study is reciprocal averaging (RA). This method produces similar results to a centered PCA, especially when, as in this case, abundance values are not used, but has the advantage that it provides an ordination for both species (Q-type analysis) and for sites (R-type analysis) at the same time, with the species scores derived from the site scores.

The main steps of RA are:

Arbitrary trial scores are assigned to each species

A first set of site scores is calculated from the weighted species scores

A second set of species scores is calculated from the first set of site scores

A second set of site scores is calculated from the second set of species scores.

Steps 3 and 4 are repeated until the vectors (weights) maintain constant relative proportions, that is if one is increased the second increases proportionally.

This method is obviously laborious when calculated by hand, as the number of cycles to be performed before stability is achieved can be quite high, and depends on the accuracy of the first estimate of species weights, but several computer programs are now available to perform this calculation.

Both PCA and RA suffer, however, from two mathematical artefacts, The "arch effect" and the "scale contraction effect". The "arch effect" produces an unnatural curve in the final scores and results from the "non-linear" nature of an ecological data set. In a community occupying an environmental gradient, in this case salinity, the species composition changes along the gradient in response to the increasing value of the variable. Each species, however, responds independently of each other to the gradient (Gauch and Whittaker, 1972) and its response can be drawn as a normal curve (Figure 3:1). If the environmental gradient is sampled at a series of sites, each site will probably contain the peaks of several species and the data structure will be non-linear. When the species scores are added together and plotted, a distinct "arch" is seen in the resulting plot, which distorts the resulting analysis. The size of the arch depends upon the distance between sampling sites, and not on the underlying trends in the data.

The "scale contraction effect" results from the calculating methods of PCA and RA and has the effect of contracting the scales on the principal axes, particularly at either end, so that outlying sites appear to be more closely grouped that they actually are, and it is difficult to separate visually the groups site groupings.

An extended method of reciprocal averaging, called

FIGURE 3:1

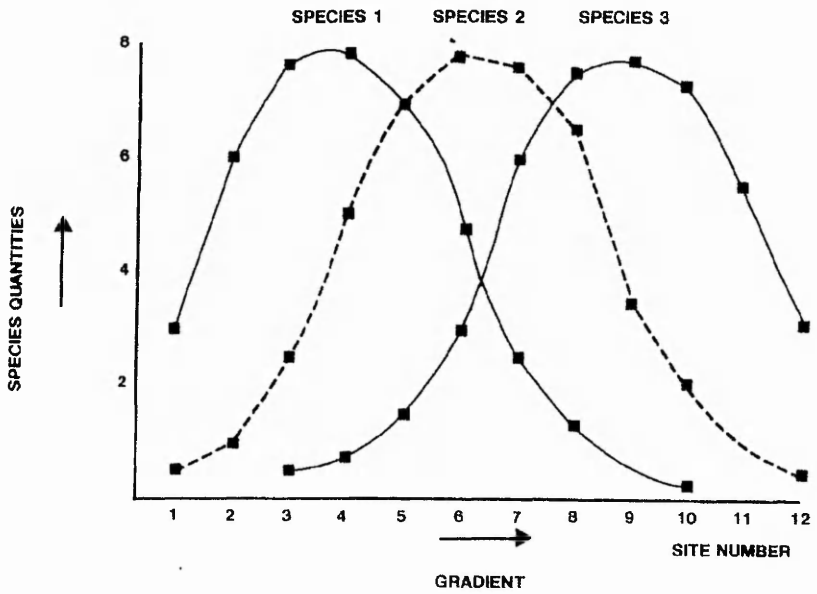
Graphs to show two mathematical artefacts which can occur when using Reciprocal Averageing

Graphs were calculated from a set of artificial data with three species and twelve sites.

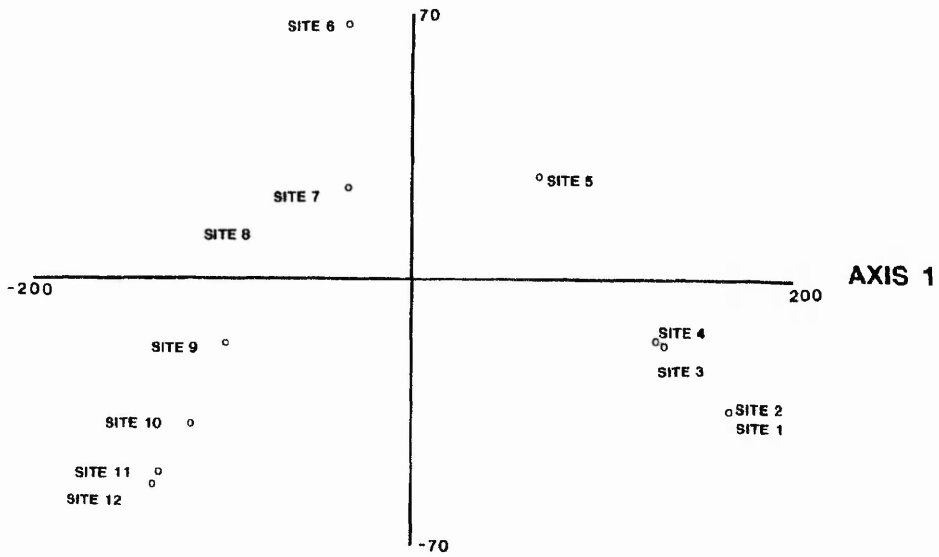
The first graph shows the abundance of each species along the gradient, and the second the resulting Axis 1 and Axis 2 scores resulting from RA ordination.

The second graph shows the "arch" effect, as the data points are distributed in a horse-shoe shape, and the "scale contraction effect", as the points for sites 1-4 and 9-12 are much closer together than the points for sites 3-8.

DISTRIBUTION CURVES OF THREE SPECIES ALONG AN ENVIRONMENTAL GRADIENT



RA ORDINATION OF 12 SITES TO SHOW THE "ARCH" EFFECT



AXIS 2

detrended correspondance analysis (DCA) (Hill and Gauch, 1980) has been developed to overcome these two difficulties. The arch effect is removed by dividing the RA-ordinated data set into several short segments at right angles to the first axis, and then sliding the segments with respect to each other until the arch disappears. To do this, the segments are moved up and down until the average height of the points in each segment is equal. The scale contraction at each end of each segment is removed by rescaling the axes in those areas to produce an even spread of points. This process is obviously mathematically complicated, and a commercial program called DECORANA (DEtrended CORrespondance ANAlysis) has been developed (Hill, 1979a). There are some critics of this method, eg Wartenberg *et al.*(1987) since it involves large amounts of data manipulation, but testing with real and artificial data has demonstrated that the method gives useful results and allows ecologically correct interpretations to be made from non-linear data sets. This is specially true when it is used in conjunction with partitioning techniques such as indicator species analysis and cluster analysis. Detrended correspondance analysis has been used in a range of biological situations including the examination of phytoplankton populations (Levandowsky, 1972), Pinewood communities (Hill *et al.*, 1975), vegetation stuctures (Whitaker, 1987), persistence of communities (Townsend *et al.*, 1987) and freshwater communities (Wright *et al.*, 1984).

A further useful development of RA involves the partitioning of an ordination to produce groups of sites directly, in a form similar to cluster analysis. A program for this called TWINSPAN (TWo-way INdicator

ANalysis) has been developed by Hill (1979). This program produces groups by the following method:

An RA ordination is performed on the whole data set to provide a crude ordination.
The ordination is divided at its centre to produce two groups of samples.
Individual species which are considered "preferential" to each side of the division are identified.
A second ordination of the whole data set is performed using the preferential species as a basis for the species weights.
The second ordination is divided at an appropriate point to produce two groups, not necessarily equal in size.
Steps 1-5 are repeated using the two halves of the division to produce four groups.

This process is repeated until the user is satisfied that no profit would result in dividing the groups further.
The resulting groups can be displayed on the axes produced by DECORANA and used to construct a "key" to the sites, using the "preferential" species as indicators of each group of sites.
The DECORANA axes can then be "scaled":- that is, compared with groups of environmental variables to identify those which are negatively or positively related to the axis scores. This can be done by correlation of chemical and physical variables with the axis scores, followed by a regression using the axis score and the variables which correlate strongly with it. The boundaries between TWINSPAN groups can then be measured in terms of the calculated values of each variable.

An alternative method of calculating the boundaries between groups is multiple discriminant analysis (MDA) (Klecka, 1975).

MDA produces "discriminant functions" using combinations of environmental variables. The version of MDA used here is contained on the SPSSX computer package and its use is described by Norusis (1985). In this method, the environmental factors for each site are read into the computer, together with the TWINSPAN group to which the site belongs. The linear combinations of environmental variables which correctly assign the sites to their groups are then calculated. This method thus recognises that the DECORANA axes are not related to one environmental variable, but rather to a combination of several variables working together, and produces results which are comparable with a stepwise multiple regression.

3:2 Methods

3:2:1 Data Analysis

The data from appendices A and C were coded and entered into the TWINSPAN and DECORANA programs following standard procedures described in the program manuals (DECORANA; Hill, 1979a and TWINSPAN; Hill, 1979b.). In all DECORANA runs, the option to downweight rare species was employed to avoid such species having a disproportionate effect on the analysis. The TWINSPAN program was run in standard settings, using one cut-level for pseudospecies (since the data was presence-absence) and three levels of division.

The following sets of data were analysed:-

1) The 24 sites on Millwood Brook and its inputs, with animals identified to "family" level
2) The 24 sites on Millwood Brook and its inputs, with animals identified to species level or as far as possible (This is referred to as "species level")
3) The 24 sites on Millwood Brook and its inputs, together with 14 other sampled sites identified to "species" level.

The term "family" level was used here to describe convenient taxonomic groupings such as Ephemeroptera and Diptera, as well as true families.
The resulting axis scores from DECORANA were plotted and the TWINSPAN groups drawn over the plots. Environmental data from Appendix 3 were then correlated with DECORANA scores from the two axes which explained most of the variation in the data, and the best correlations used to calculate regression lines and "scale" the DECORANA axes.

3:2:2 Predictive Computer Modelling

Firstly, "Test" sites from which only species lists were available were classified into the TWINSPAN groups produced using Millwood Brook data. This was done using the species lists for the site grouping at each TWINSPAN division, and the method is demonstrated in Figure 3:8. A program was written for the BBC computer (titled "CLASS") which could perform this grouping and so describe the likely salinity of a site from which only a species list was available. The names, codes, grid references and chemical data from these sites are listed in Appendix B,

complete species lists in Appendix C, and a listing of the program "CLASS" is provided in Appendix D.

Secondly, to produce a program which could give a predicted species list for sites with known environmental data, MDA (Multiple Discriminant Analysis) was applied to the TWINSPAN groups of sites and the most important environmental variables identified by regression analysis.

Eight environmental variables which had been shown to be highly correlated with the DECORANA axes (log_{10} concentrations of chloride and calcium, log_{10} conductivity, number of species, flow rate, substrate type, and log_{10} of the width and depth of the main river channel) were initially used to generate discriminant functions. The variables "number of species" and "calcium concentration" were eliminated from later analyses as calcium was correlated very strongly with chloride concentration and actual number of species present was not useful if a predictive program was to be devised. The six remaining environmental variables were flow rate, log_{10} chloride, log_{10} conductivity, log_{10} mean channel width, log_{10} mean channel depth and substrate type and these were used in MDA with equal weighting to produce six discriminant functions.

The discriminant functions were then used to classify new sites using the method decribed by Moss *et al.* (1987). There were five stages to this method, which are described in Appendix E.

A computer program (titled "PREDICT") was written for the BBC computer which assigned sites to TWINSPAN groups using this method, and predicted the species which might occur at them. The program requires values for the six environmental variables used in calculation to be entered

into the computer, and from these the TWINSPAN group and predicted species list is calculated. The program was tested using the 14 "Test" sites from the East Midlands listed in Appendix B, which were not included in the original database, and for which environmental data and species lists were available.

To provide an estimate of the number of species which could be expected at a saline affected site, a regression was calculated between number of species at Millwood Brook sites and $\log_{10}$ chloride concentration (in mM). This was used in the predictive program to provide estimates of the number of species which might occur at a site. Since the Millwood Brook sites had been sampled three times, and many sites, including the "test" sites, are only sampled once, the numbers of species found at the "test" sites was lower than the number found at Millwood Brook. A number of species reduced by one third was suggested as a more likely figure for sites which had been sampled only once.

3:3 Results of TWINSPAN and DECORANA Analysis

3:3:1 Family Level Analysis

The TWINSPAN groups from the family level analysis are shown in Figure 3:2. At Level 1 in this classification the two groups corresponded with the lower salinity sites (conductivity below 4 mS/cm; Group 2) and the higher salinity sites (conductivity above 4mS/cm; Group 3). The indicator groups for the low salinity sites were Ephemeroptera, Planariidae and Glossiphoniidae.

At Level three, the four groups derived from the less

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 the three levels of division, and are enclosed in dotted lines. Species which were identified as "indicators" for a particular division are shown in italics at the relevant division.

CLASSIFICATION OF SITES SHOWING TWINSPAN GROUPS

a) MILLWOOD BROOK SITES classified using 'Family' level identification

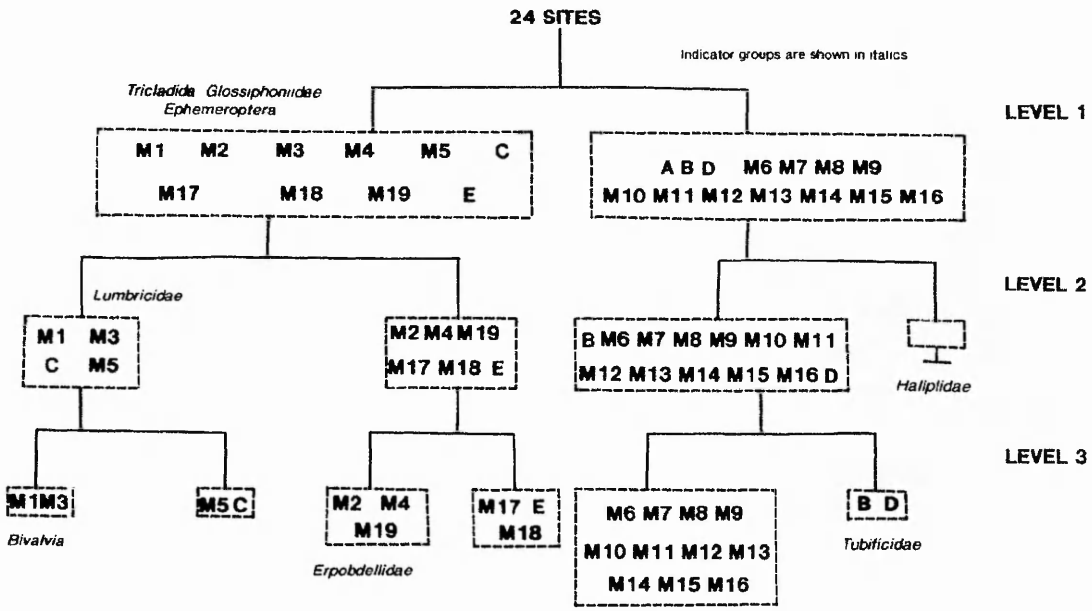


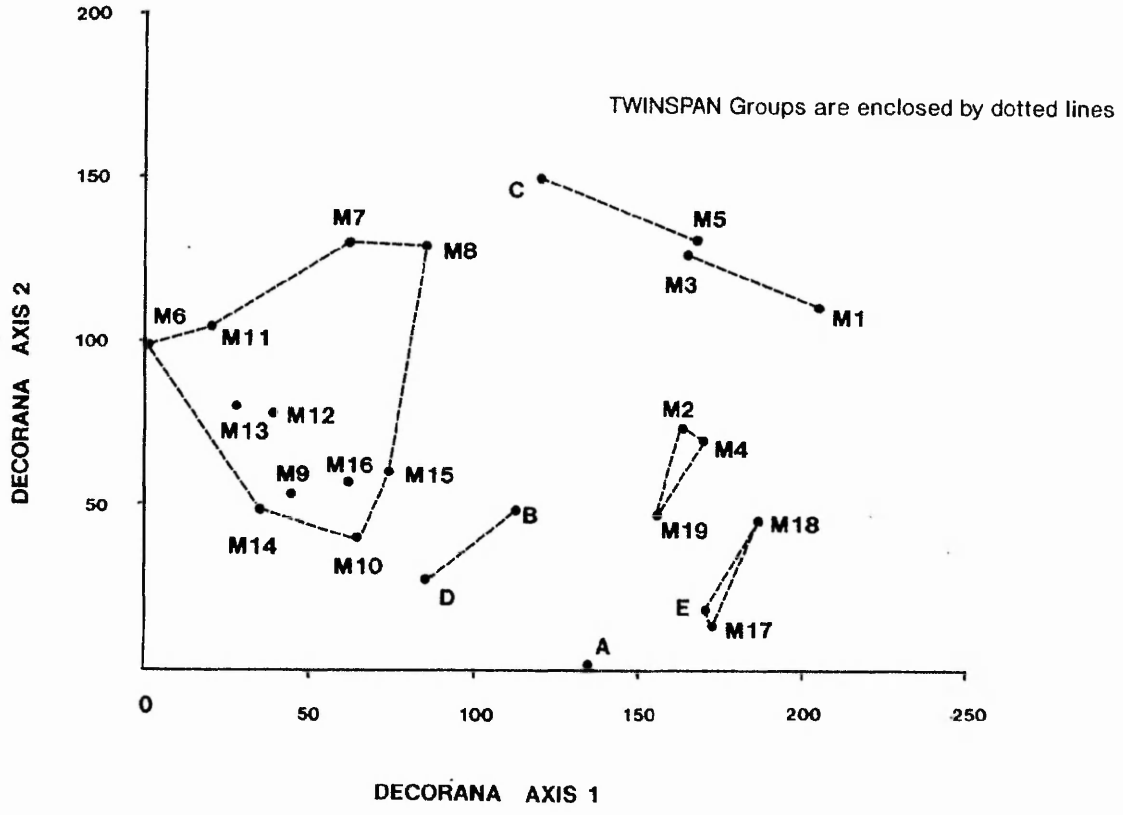
FIGURE 3:3

Ordination Plot of Millwood Brook Sites using DECORANA Axes 1 and 2, with taxa identified to "Family" Level.

Millwood Brook sites are plotted on DECORANA Axes 1 and 2, and their position indicated with the site number. The TWINSPAN Groups of sites calculated using Millwood Brook sites and "Family" level data are enclosed by dotted lines.

Ordination Plot

a) MILLWOOD BROOK SITES classified using "family" identification



saline sites (Groups 8 to 11) were quite even in size, and began with the cleanest sites (M1 and M3) in Group 8. The sites in Group 9 (M5 and C) were occasionally affected by quite high salinity, while those in Group 10 (M4, M2 and M19) were affected by salinity below 2mS/cm and were shallow with a hard substrate. The sites in Group 11 (E, M17 and M18) were very mildly saline and soft-bottomed.

The analysis was less successful in dividing the more saline sites at this level. This was probably due to the low number of families which were found at these sites, most of which very common, and species-level identification would be expected to improve this. The family classification did, however, single out Site A, which received intermittent but very saline effluent and was very species-poor. It also removed, in Group 13, sites B and D, both of which had occurences of Tubificidae, which were not present at the other saline sites. These two sites, although receiving intermittent saline discharges, had spring inputs which reduced the effects of the salinity by creating clean-water areas where more sensitive groups could survive.

When the TWINSPAN groups were drawn on the DECORANA axes (Figure 3:3), sites from the negative side of the division were at the higher end of the ordination on axis 1, which suggested that this axis was negatively correlated with chloride concentration. The apparent correlations with axis 2 were less clear, but sites with shallower water have lower scores on this axis.

3:3:2 Species-level Classification

When all the identified taxa were included in the

FIGURE 3:4

Classification of Millwood Brook Sites using TWINSPAN with "Species" level Identification of taxa.

TWINSPAN Groups of sites are shown from each of the three levels of division, and are enclosed in dotted lines. Species which were identified as "indicators" for a particular division are shown in italics at the relevant division.

CLASSIFICATION OF SITES SHOWING TWINSPAN GROUPS

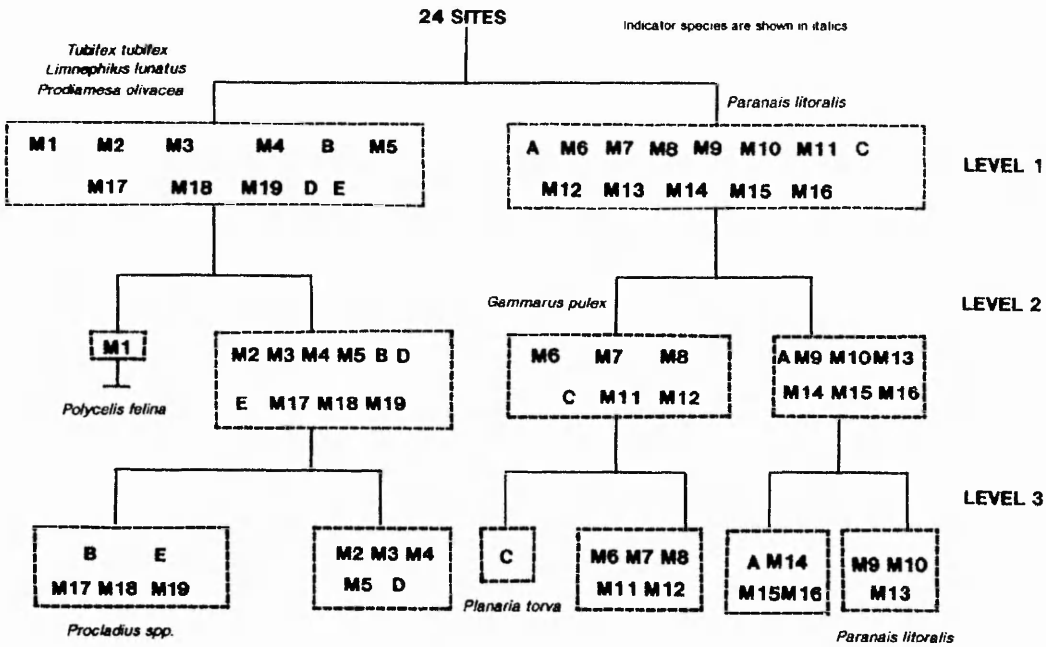


FIGURE 3:5

Ordination Plot of Millwood Brook Sites using DECORANA Axes 1 and 2, with taxa identified to "Species" Level.

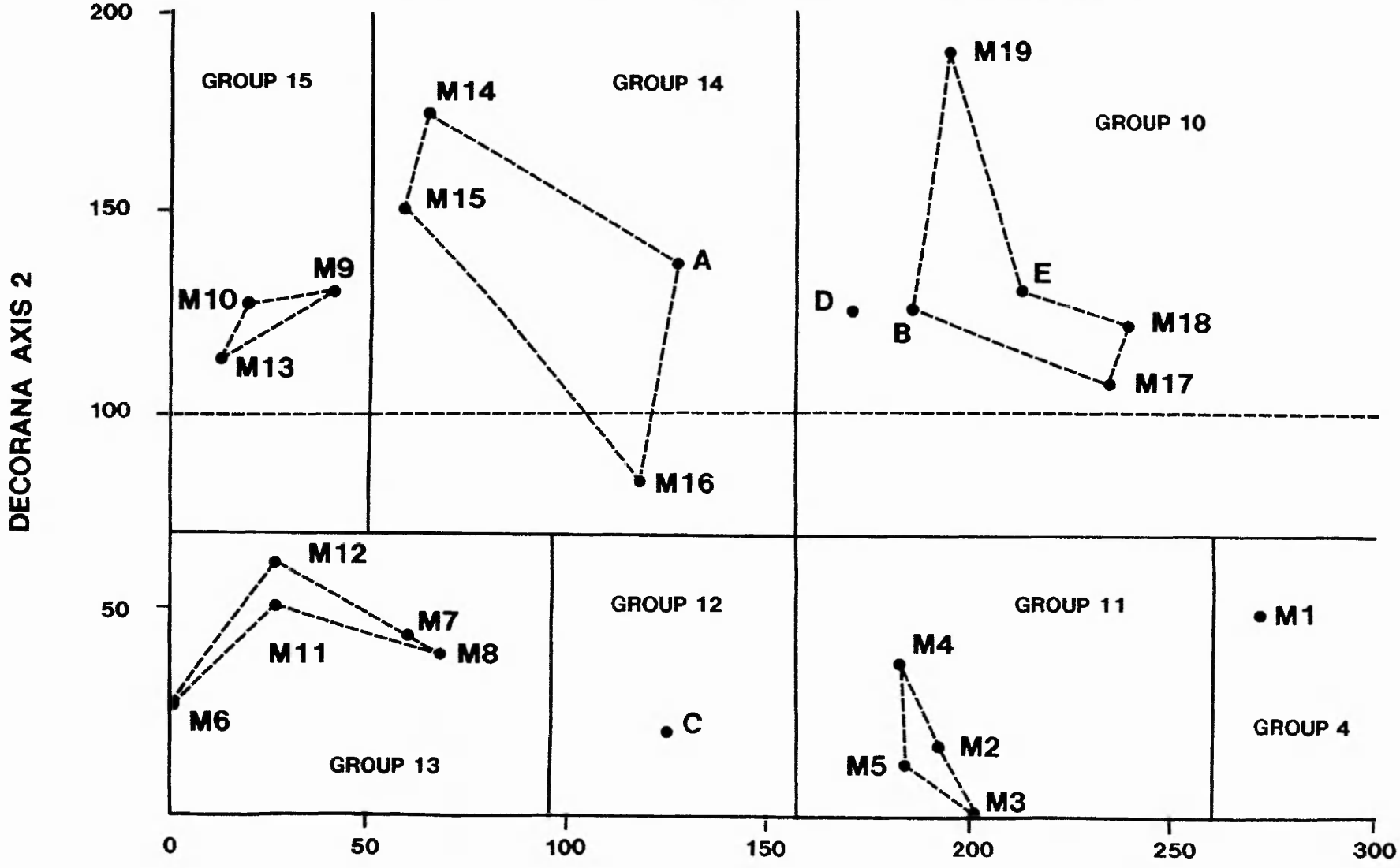
Millwood Brook sites are plotted on DECORANA Axes 1 and 2, and their position indicated with the site number. The TWINSPAN Groups of sites calculated using Millwood Brook sites and "Species" level data are enclosed by dotted lines.

The ranges of each TWINSPAN Group on both axes are enclosed by solid lines.

The dotted horizontal line beginning at 100 on Axis 2 shows the division of groups after MDA analysis, when Site M16 was moved into Group 12

Ordination Plot showing AXIS 1 and AXIS 2

MILLWOOD BROOK SITES showing GROUP NUMBERS and Division of Axes



TWINSPAN classification, (Figure 3:4), most sites remained on the same side of the ordination at level 1, the exceptions being D and B, which moved to group 2 and C, which moved to group 3, although this site was described as "misclassified" by the program. With a larger range of taxa in the ordination, different indicator organisms were selected. The first division used *Tubifex tubifex*, *Limnephilus lunatus* and *Prodiamesa olivacea* to produce Group 2 and *Paranais litoralis* to produce Group 3.

The second division of Group 2 sites divided off site M1. This site was the cleanest and smallest in the classification, with a diverse fauna including stoneflies which were not found elsewhere. The Group 3 sites were divided using *G. pulex* as an indicator, and separated the more saline sites into Group 6 and less saline into Group 7.

The third level of division produced six groups of sites (10-15) in which two sites (B and D) were described as "borderline", and had changed sides in the first division when compared to the family-level classification. Site C was separated out in Group 12, with the most highly saline sites in Group 13 and the less saline in Groups 14 and 15. *Paranais litoralis* was again used as an indicator species for highly saline sites.

When these groups were drawn on DECORANA axes 1 and 2, (Figure 3:5) they could be seen to be clearly separated on both axis 1 and axis 2, with only sites D and C not adhering closely to a group. This set of DECORANA scores was correlated with a range of physical and chemical variables and the calculated correlation coefficients are shown in Table 3:1. It can be seen that the best correlations with axis 1 were number of species and $\log_{10}$

TABLE 3:1

Correlations between DECORANA Scores on Axes 1 to 4 calculated using Millwood Brook Data and Environmental Variables measured between April 1986 and April 1987.

Variable	DECORANA Axis			
	1	2	3	4
Chloride (upper) (mM/l)	-.66	-.09	-.14	-.26
Chloride (lower) (mM/l)	-.69	-.02	-.26	-.02
Chloride (mean) (mM/l)	-.75	-.13	-.25	-.25
Chloride (log mean)	-.87	-.21	-.32	-.17
Calcium (upper) (mM/l)	-.7	-.12	-.26	-.31
Calcium (lower) (mM/l)	-.74	-.05	-.34	-.09
Calcium (mean) (mM/l)	-.75	-.16	-.37	-.23
Calcium (log mean) (mM/l)	-.85	-.01	-.46	-.16
Temperature (upper)(^{0}C)	-.53	.33	-.25	-.196
Temperature (lower) (^{0}C)	-.77	-.20	-.25	-.24
Temperature (mean) (^{0}C)	-.69	.103	-.25	-.32
pH (upper)	.54	.46	-.14	-.09
pH (lower)	.44	-.32	-.02	.28
pH (mean)	.78	.07	.39	.38
Cond. (upper) (mS/cm)	-.65	-.07	-.14	-.32
Cond. (lower) (mS/cm)	-.89	.03	-.22	-.15
Cond. (mean) (mS/cm)	-.77	-.12	-.24	-.25
Cond. (log mean) (mS/cm)	-.89	.13	-.25	-.18
Number of species	.85	-.04	-.12	.24
Distance from source (km)	.29	.43	-.32	.04
Flow (m/sec)	.21	.19	-.27	.08
Width (m)	.24	.49-	.22	.02
Substrate type (1 to 5)	.299	-.77	.13	.25
Depth (log mean) (cm)	-.08	.58	-.03	-.5

* r is significant at the 1% level above 0.487 (+ or -)

of mean conductivity, calcium and chloride concentration, while axis 2 correlated most strongly with $\log_{10}$ mean depth, substrate type, flow rate and mean channel width. Axes 3 and 4 were, as usual, weaker reflections of axes 1 and 2, although axis 3 correlated quite strongly with upper pH. The proportion of the variation which the axes described were 47% (axis 1), 27% (axis 2), 17% (axis 3) and 9% (axis 4).

The regression lines calculated from axis 1 were for $\log_{10}$ chloride, $\log_{10}$ calcium, $\log_{10}$ conductivity and number of species, and for axis 2 were substrate type, $\log_{10}$ depth, $\log_{10}$ width and distance from source. (Table 3:2) The DECORANA axes were then scaled and divided to indicate ranges of environmental factors for the groups (Figure 3:5), which were referred to by their TWINSPAN number, as shown in Table 3:3. The divisions were made halfway between the lowest and highest axis scores for each adjacent group.

The environmental type of the sites in each group could, therefore, be broadly classified as:-

Group 4; small sized streams with low chloride and hard substrate.

Group 10; medium sized streams with slight chloride and soft substrate.

Group 11; small sized streams with slight chloride or other input and hard substrate.

Group 12; small sized streams with moderate chloride and hard substrate.

Group 13; small sized streams with high chloride and hard substrate.

Group 15; medium sized streams with high chloride and soft substrate.

Group 14; medium sized streams with moderate chloride and soft substrate.

3:3:3 Millwood Brook Sites and "Test" Sites

To discover whether these site groupings were stable, TWINSPAN and DECORANA analyses were repeated using the 14 additional "Test" sites from Appendix B, although these sites had only been sampled once, not three times as had those on Millwood Brook. The resulting groups are shown in Figures 3:6 and 3:7.

From Figure 3:7, it can be seen that the original groups 10 and 11 tend to combine, as do groups 12 and 14, while group 15 becomes a group of saline, still water sites. The area of moderate pollution becomes less clear, with a large group of 12 sites closely joined by a smaller group of 4 sites.

Correlations and regressions between environmental measurements and DECORANA scores were calculated as previously. The most strongly correlated variables were the same seven which had the highest correlation coefficients when using Millwood Brook sites alone (Number of species, $\log_{10}$ chloride and calcium concentration, $\log_{10}$ mean channel depth and width, $\log_{10}$ conductivity and substrate type.)

When the axes were scaled and divided, the divisions on axis 2 were much less clear when the extra sites were included, although this axis was still highly correlated with width, depth and substrate type. However, when the divisions were compared with those calculated using Millwood Brook data alone, the range of environmental conditions in groups on axis 1 and axis 2 were similar, differing only in lower axis 1 values for groups 13 and

FIGURE 3:6

Classification of Millwood Brook and "Test" Sites using TWINSPAN with "Species" level Identification of taxa.

TWINSPAN Groups of sites are shown from each of the three levels of division, and are enclosed in dotted lines. Species which were identified as "indicators" for a particular division are shown in italics at the relevant division.

CLASSIFICATION OF SITES SHOWING TWINSPAN GROUPS

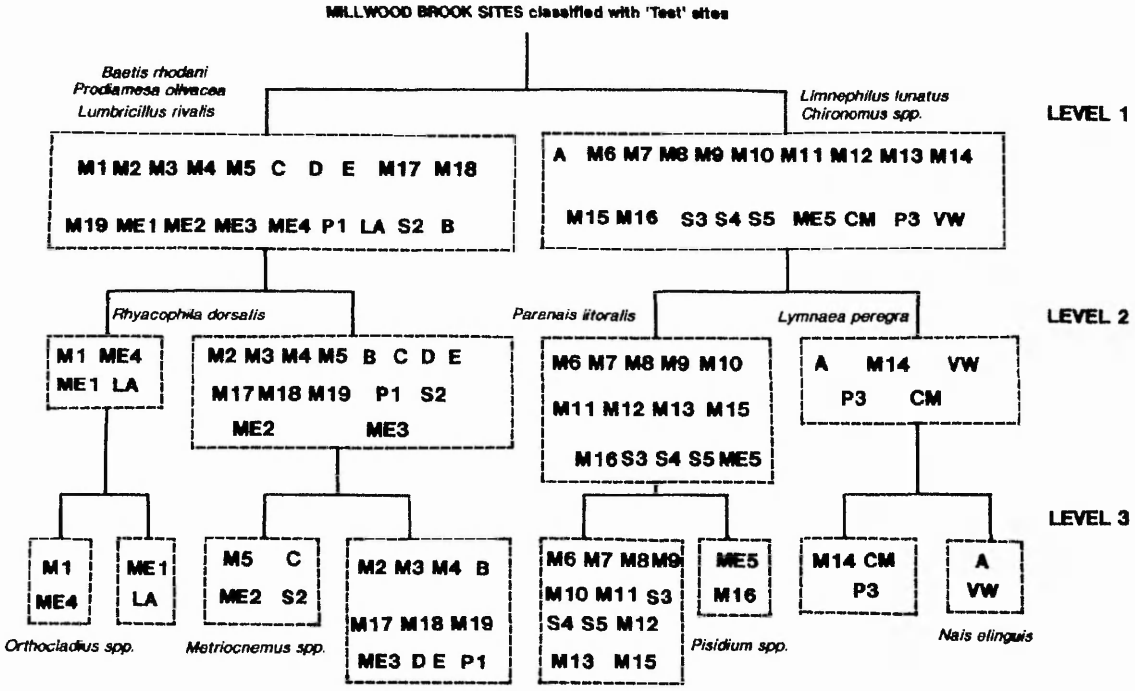


FIGURE 3:7

Ordination Plot of Millwood Brook and "test" Sites using DECORANA Axes 1 and 2, with taxa identified to "Species" Level.

Millwood Brook sites are plotted on DECORANA Axes 1 and 2, and their position indicated with the site number. The TWINSPAN Groups of sites calculated using Millwood Brook sites and "Family" level data are enclosed by dotted lines.

The ranges of each TWINSPAN Group on both axes are enclosed by solid lines.

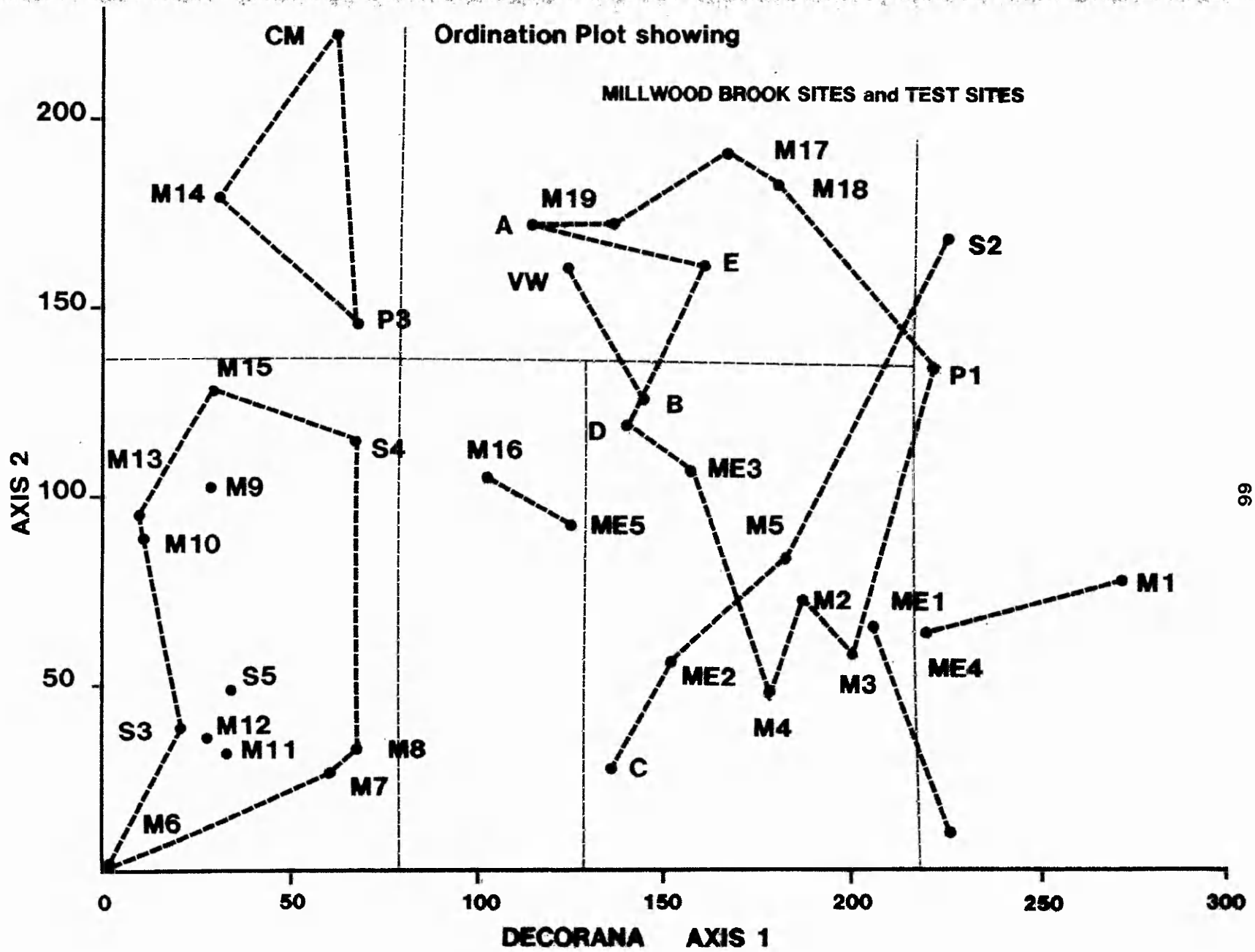

Ordination Plot showing
MILLWOOD BROOK SITES and TEST SITES
CM
M14
P3
M17
M18
M19
A
E
VW
S2
P1
B
D
M15
S4
M13
M9
M10
M16
ME3
ME5
M5
M1
M2
ME1
ME2
M3
ME4
M4
S5
S3
M12
M11
M8
M7
M6
C
AXIS 2
200
150
100
50
0
50
100
150
200
250
300
DECORANA AXIS 1

TABLE 3:2

Regression Equations Calculated from Millwood Brook Environmental data and DECORANA Axis 1 and Axis 2 Scores

Axis 1

(i)	Log chloride (annual mean in mM/l)		
	=2.35-0.0065*axis 1	F=67.4	1%
(ii)	Number of species (total)		
	=4.5+0.1229*axis 1	F=55.39	1%
(iii)	Log calcium (annual means in mM/l)		
	=1.1971-0.00261*axis 1	F=59.71	1%
(iv)	Log conductivity (annual mean in mM/l)		
	=1.2841-0.00466*axis 1	F=88.9	1%

AXIS 2

(i)	Log depth (in cm)		
	=1.187-.0027*axis 2	F=10.98	5%
(ii)	Substrate (1 to 5)		
	=4.363-0.0187*axis 2	F=33.06	1%
(iii)	Width (m)		
	=2.09+0.23*axis 2	F=26.17	5%

F is the variance ratio for the regression and the significance level for F is indicated.

TABLE 3:3

Millwood Brook Sites included in each TWINSPAN Group calculated using Millwood Brook Data alone and "Species" Level Identification

TWINSPAN GROUP	SITE
4	M1
10	M19, B, E, M17, M18
11	M4, M2, M5, M3
12	C
13	M6, M12, M11, M7, M8
14	M14, M15, M16, A
15	M9, M10, M13

14.

3:4 Results of Predictive Computer Modelling

3:4:1 Predicting TWINSPAN Group from Species Lists

The lists of species considered "preferential" to each of the TWINSPAN group generated using the 24 Millwood Brook sites are shown in Figure 3:8. Each species was classed by this method as either "preferential" or "non-preferential" to the groups at each division. A non-preferential species occurred equally in both groups in a division, while a preferential species occurred more often in one group than in the other. For example, at Level 1, *Potamopyrgus jenkinsi* was described as "non-preferential" and would be equally likely to occur at sites in Group 1 and at sites in Group 2. *Paranais litoralis*, however, was listed as "preferential" to Group 2, and would be expected at sites in that group, but rarely at a site from Group 1.

A site from which a species list is available may be "keyed" through Figure 3:8 to arrive at a final TWINSPAN group using the method described in the legend, and it was this method which was followed by the computer program "CLASS". The groups in which the test sites were placed using this program are shown in Table 3:4.

3:4:2 Predicting Species Lists from Environmental Data

The discriminant functions derived from MDA were used in the construction of a key to predict TWINSPAN group and species lists from environmental data. The environmental variables used to derive the MDA functions were used in

TABLE 3:4

Prediction of TWINSPAN Group and Number of Species using "CLASS" and "PREDICT" for Thirteen "Test" Sites

SITE	No of Species	CLASS Group	PREDICT 1ST Group	% Found	PREDICT 2ND Group	% Found
ME1	20	4	10	80	-	-
ME3	11	10	15	45	14	45
ME4	19	11	12	67	-	-
S2	12	4	10	75	15	17
S3	4	12	15	50	14	75
S4	4	13	13	75	-	-
S5	7	12	13	71	-	-
VW2	5	12	12	80	-	-
P3	4	15	15	75	14	100
LA	30	4	10	50	-	-
RW1	7	14	13	43	12	100
RW2	6	14	13	67	12	67
L1	32	10	10	75	15	22
mean				65.6		60.8

different combinations until a set of features which could correctly place all sites was found. The variables used were Log_{10} chloride, conductivity, width and depth, and flow rate. The only site which could not easily be placed by any combination of variables was M16, which originally was in TWINSPAN Group 14. This site was, however, quite widely separated from the other sites in Group 14 on DECORANA axis 2, where it appeared to be halfway between this group and Group 12. When the site was placed in Group 12, and the MDA analysis repeated, the site was correctly placed by all combinations of variables. The site did, in fact, have several features in common with the other site in Group 12, site C, in that it had saline water (from Millwood Brook) and cleaner water from input D. It also received intermittent heavy saline or organic pollution from input D. It was decided, therefore, to include site M16 in Group 12, and to move the division line on axis 2 (shown as a dotted line in Figure 3:5). This change did not move any other site, and allowed 100% success in placing all sites using only six easily measured variables.

The MDA discriminant functions (shown in Table 3:5) were then used to construct a predictive key using the mathematical method described by Moss *et al.*, (1987), and described in Appendix E. This calculation method removes the rigid straight-line boundaries between groups with a varying circular boundary based on the Euclidian distance of a site from the centroid of the group.

This method was used as the basis of a BASIC program ("PREDICT") suitable for BBC computers, which produces a predicted species list for a site when the 5 environmental variables are entered, together with probability values for each species. A listing of the

TABLE 3:5

MDA Functions Calculated using
Environmental Data from
Millwood Brook Sites and TWINSPAN Groups

Environmental Variable	MDA Function Number F1	F2	F3
Flow Rate (A)	0.77958	0.18814	0.31433
$Log_{10} Cl^-$ (B)	0.23317	-0.2705	2.55266
Log_{10} Conductivity (C)	0.45764	1.06	-2.1177
Log_{10} Width (D)	0.48187	-0.46457	-0.21784
Log_{10} Depth (E)	0.45915	-0.09857	1.04736
Substrate (F)	-1.1952	0.42625	0.72261

Environmental Variable	MDA Function Number F4	F5	F6
Flow Rate (A)	0.23285	1.06497	1.06497
Log_{10} Chloride (B)	-0.0938	-1.43383	-.30865
Log_{10} Conductivity (C)	0.11178	1.4523	.34046
Log_{10} Width (D)	0.48187	-0.46457	-0.21784
Log_{10} Depth (E)	-0.80559	0.37034	-0.10349
Substrate (F)	0.14133	0.0992	-0.19946

program is given in Appendix F.

The probability calculation also assigns a score of 1 to each of the 20 most common species in the Millwood Brook data-set. For other species it then divides the number of occurrences of the species in a group by the number of sites in the group. This probability of occurrence for each species is then multiplied by the probability of group membership of the site. The program rejects sites which have significant chi-squared values and produces species lists for two groups if the site is closely related to them both. Figure 3:9 shows a sample print-out from this program.

The "Test" sites from Appendix B were again used to test the accuracy of the program. The numbers of species predicted when compared to the ones actually found, are shown in Table 3:6. From this table, it can be seen that the ratio of species found/species predicted is never less than 50%, and is generally about 75%, which is similar to the correct prediction rates found by Moss *et al.*, (1987)

Table 3:7 shows a comparison of the species actually found at three of the "test" sites with the species predicted. The first site, ME1, was a clean site at the head of the river Meden, ME1. PREDICT suggests 23 species at the site, compared with the 20 actually found, and suggests 13 species present with a probability of 89%, of which 8 were actually found. Six species were predicted at a probability of 71%, of which three were present. The other species found were predicted with lower probabilities, with 35% the lowest. Three species, all Diptera, were not predicted.

The other two sites, VW2 and RW1, were chosen to demonstrate a problem when using a subjectively decided

TABLE 3:6

Prediction of TWINSPAN Group and Number of Species for "Test" Sites using "PREDICT"

SITE	NO OF SPECIES	PREDICT SPECIES	1ST GROUP	NO CORRECT	2ND GROUP	NO CORRECT
ME1	20	14	10	16	-	-
ME3	11	18	15	5	14	5
ME4	19	25	12	13	-	-
S2	12	15	10	9	15	2
S3	4	8	15	2	-	-
S4	4	0	13		3-	-
S5	7	10	13	5	-	-
VW2	5	8	12	4	-	-
P3	4	9	15	3	14	3
LA	30	28	10	15	-	-
RW1	7	14	13	3	12	7
RW2	6	12	13	4	12	4
L1	32	14	10	24	15	7

TABLE 3:7

Actual (A) and Predicted (P) Species Composition of Two "Test" Sites together with the Predicted Probability of Occurence of Each Species Predictions made using PREDICT program

SITE	NO	SPECIES	A	P	PROB(%)
ME1	23	Polycelis nigra/tenuis	+	+	35
		Potamopyrgus jenkinsi	+	+	89
		Lymnaea peregra	+	+	89
		Sphaeriidae	+	+	89
		Nais elinguis	+	+	89
		Asellus aquaticus	+	+	49
		Erpobdella octoculata	+	+	71
		Hydracarina	+	+	71
		Gammarus pulex	+	+	89
		Baetis rhodani	+	+	89
		Elmis aenea	+	+	71
		Rhyacophila dorsalis	+	+	35
		Hydropsyche angustipennis	+	+	35
		Macropelopia spp.	+	+	35
		Cricotopus spp.	+	+	89
		Orthocladius	+	+	89
		Simulium ornatum	+		
		Pericoma sp	+		
		Limnophora	+		
		Dendrocoelum lacteum		+	89
		Tubifex tubifex		+	89
		Limnodrilus hoffmeisteri		+	89
		Procladius spp.		+	89
		Thienemannimyia spp.		+	89
		Prodiamesa olivacea		+	89
		Chironomus spp.		+	89
		Ceratopogondae		+	89

PREDICTED GROUP = GROUP 10

TABLE 3:7 (continued)

SITE	NO	SPECIES	A	P	PROB(%)
RW1	14	Lymnaea peregra	+	+	45
		Lumbricillus rivalis	+	+	45
		Gammarus tigrinus	+	+	22
		Chironomus spp.	+	+	36
		Hydropsyche angustipennis	+		
		Prodiamesa olivacea	+		
		Micropsectra	+		
		Potamopyrgus jenkinsi		+	45
		Paranais litoralis		+	45
		Asellus aquaticus		+	45
		Empididae		+	45

PREDICTED GROUP (FIRST) = GROUP 13

SITE	NO	SPECIES	A	P	PROB(%)
RW1	14	Lymnaea peregra	+	+	41
		Lumbricillus rivalis	+	+	41
		Gammarus tigrinus	+	+	41
		Chironomus spp.	+	+	41
		Hydropsyche angustipennis	+	+	13
		Prodiamesa olivacea	+	+	41
		Micropsectra	+	+	13
		Potamopyrgus jenkinsi		+	41
		Sphaeriidae		+	41
		Nais elinguis		+	41
		Asellus aquaticus		+	41
		Baetis rhodani		+	41
		Cricotopus		+	41
		Eukiefferiella		+	41
		Orthocladius spp.		+	41
		Ceratopogonidae		+	41

PREDICTED GROUP (SECOND) = GROUP 12

TABLE 3:7 (Continued)

SITE	NO	SPECIES	A	P	PROB(%)
VW2	8	Potamopyrgus jenkinsi	+	+	50
		Nais elingius	+	+	50
		Lumbricillus rivalis	+	+	50
		Ceratopogonidae	+	+	50
		Empididae	+		
		Lymnaea peregra		+	50
		Sphaeriidae		+	50
		Asellus aquaticus		+	50
		Gammarus pulex/tigrinus		+	50
		Baetis rhodani		+	50
		Cricotopus spp.		+	50
		Eukiefferiella spp.		+	50
		Orthocladius spp.		+	50
		Chironomus spp.		+	48

PREDICTED GROUP = GROUP 12

environmental variable, in this case substrate type. Site VW2 was placed in Group 12 using a substrate value of 4, despite high chloride and conductivity measurements, and in this group 9 species were predicted which were not found at the site. If, however, the substrate type was reduced to 3, the site was placed in Group 13, and all 5 species present were predicted.

In the case of site RW1, two groups were suggested, Group 13 and Group 12 when the substrate type was entered as 3, of which the second, Group 12, more accurately predicted the fauna at the site. In addition, the chloride and conductivity at the site were within the range of Group 12, rather than Group 13. If, however, the substrate was entered as 4, the site was placed in Group 12 as the first option.

Table 3:4 shows the TWINSPAN Group in which the Test sites were placed by both "CLASS" and "PREDICT". It can be seen that there was little agreement between the placings between the two programs, and in particular that sites which were classed in Group 4 using their fauna tended to appear in Group 10 using PREDICT. This is a result of the small number of sites in Group 4 (Site M1 only), which, although a non-saline site, was also rather specialised in its fauna. When the Test sites were included in the TWINSPAN groupings, site LA, a non-saline site on a the River Lathkill which was much larger, sites M1 and LA were grouped closely with sites ME1 and ME4, which were also non-saline sites from larger rivers (see Figure 3:6). Despite this problem, which also applies to Group 12, the predicted species lists provide good agreement with the actual lists, and this agreement could be improved if the Test sites were sampled three times in a year, as were the Millwood Brook sites, to produce more

FIGURE 3:9

Sample print-out produced by the computer program "PREDICT"

"TEST" Site S4 is used as an example. The environmental data entered into the program were as follows:-

Flow = $0.6msec^{-1}$
Chloride = 636mM
Conductivity = 5.6mS/cm
Width = 1m
Depth = 8cm
Substrate = 3

This placed the site in TWINSPAN Group 13, and a predicted species list together with probabilities of occurence was produced for this group.

THE PREDICTED NUMBER OF SPECIES AT SITE S4

1

OR 0

IF YOU HAVE ONLY SAMPLED ONCE

S4

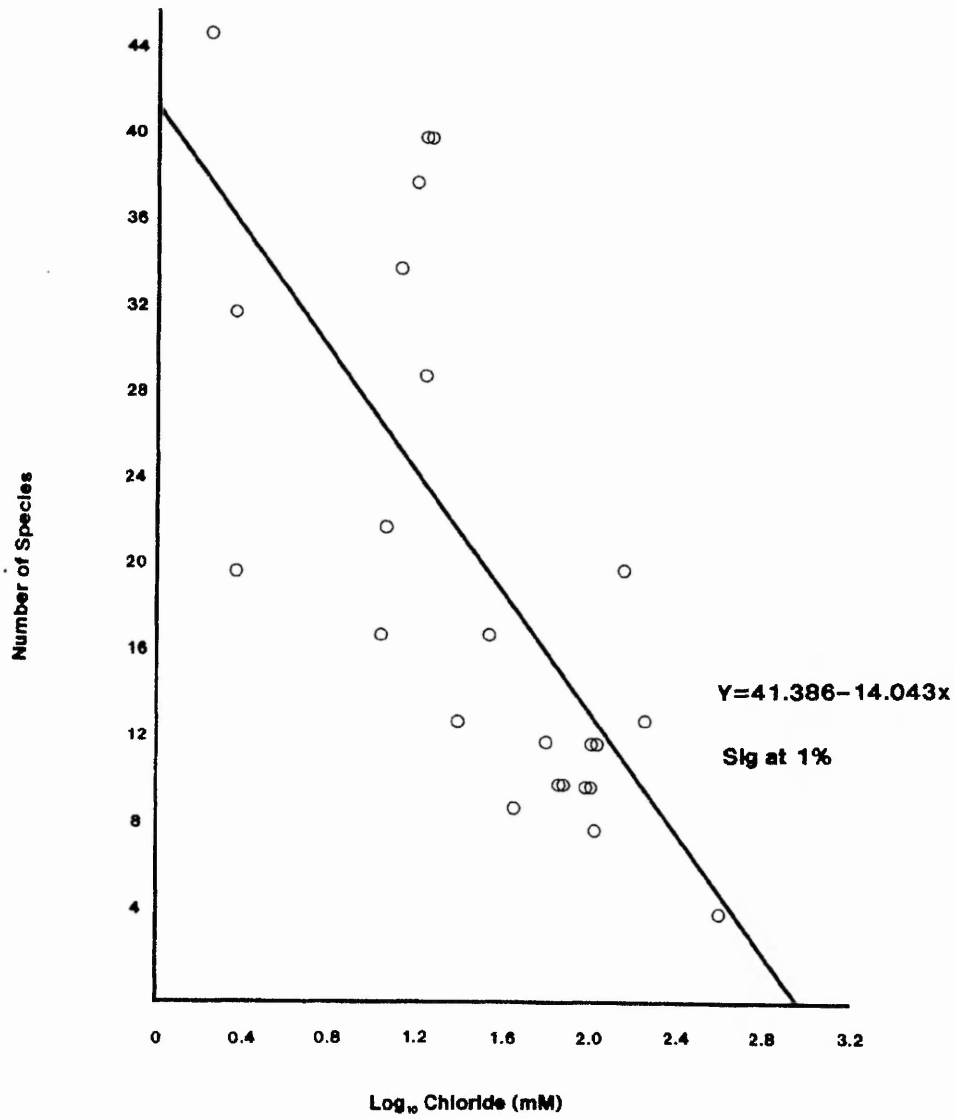
GROUP 13

TAXA	PROBABILITY (%)
Potamopyrgus jenkinsi	91
Lymnaea peregra	91
Paranais litoralis	91
Nais elinguis	73
Lumbricillus rivalis	91
Lumbricidae	18
Glossiphonia complanata	18
Asellus aquaticus	91
Gammarus pulex	45
Gammarus tigrinus	45
Elmis aenea	18
Procladius spp.	73
Cricotopus spp.	54
Orthocladius spp.	73
Chironomus spp.	73
Empididae	91
Psychoda spp.	36
Ceratopogonidae	18

FIGURE 3:10

Regression Line of number of species recorded at a site against Log_{10} mean chloride concentration

Original data points are shown as open circles.



REGRESSION LINE OF NUMBER OF SPECIES AGAINST LOG CHLORIDE

complete species lists.

PREDICT also provides an estimate of the number of species which would be expected to occur at a site. This figure is based on the regression of $\log_{10}$ chloride (in mM) against species was significant at the 1% level ($r = 0.727$, $F = 24.671$ with 1 and 23 D.F.), and is shown in Figure 3:10. The number of species predicted from this line is, however, almost always higher than the number of species found at the test sites, reaching close agreement only at the extreme ends of the chloride concentration range. This is partly due to the slight curvature of the relationship between the two variables, but also a result of the lack of sampling of the test sites, which were sampled only once, while the Millwood Brook sites, from which the line was calculated, were sampled three times. The predicted values were between 0.3 and 0.5 times greater then those found, and a compensation factor is suggested in the programme for sites sampled only once. Table 3:6 shows the numbers of species predicted and found at the "Test" sites.

3:5 Discussion

The aim of this analysis was to produce a predictive model of saline pollution which could be used to determine what would happen to sites which became polluted by saline discharges and so to recommend maximum salinity levels for a stretch of river which was to receive new saline discharges or in which the salinity was to be reduced.

Predictive models have been produced by other authors, in

particular Wright *et al.*, (1984), who used faunal and environmental data from a large number of unpolluted river sites (370) in all areas of mainland Britain, and produced a preliminary classification of freshwater sites based on their fauna. This has recently (Moss *et al.*, 1987) been updated to provide predictive modelling of clean-water sites from environmental data, and uses mathematical methods similar to those employed in this study. The classification produced by Wright *et al.*, however, does not include many rivers from the Midlands area, with only the Dove in Derbyshire and the Smite in Leicestershire as representatives of the area. This omission was due to the difficulty of finding rivers in the East Midlands which were not significantly affected by pollution of any kind. This classification cannot, therefore, be used to predict the fauna characteristic of polluted rivers in the Midlands, many of which have elevated salinity and most most some organic enrichment. Modelling of smaller numbers of polluted sites has also been undertaken by Townsend *et al.* (1983), (34 acid sites in Southern England) and in Wales by Ormerod (1988), both using TWINSPAN and DECORANA. Both these surveys, however, were concerned with the effects of acidity, and used sites of pH between 4 and 7, below the pH range of nearly all sites in this survey. The extension of river classification into the polluted streams and rivers of the English East Midlands is therefore useful, as these rivers are very heavily used as disposal channels for industrial wastes, (Millwood Brook, for example, has 30 separate discharges of industrial and domestic waste in the 15km from its source). These rivers are likely to remain heavily used for such purposes in the near future. With the opening of new coal mines in the Vale of

Belvoir, a method to predict the change in fauna which could occur when saline water is discharged into a river will become particularly useful.

The sites on Millwood Brook, which were used as a basis for this classification, are more extreme than most in the area, but the most polluted sites give an indication of the probable consequences for a water-course in the immediate vicinity of a saline discharge. The sites below the Creswell Water Treatment Works also suffer from high levels of organic pollution. This introduces a complicating factor into the classification, as many sites are both saline and organically polluted. The organic pollution tends to produce soft, silty substrates close to its input, and this is the difference which is used on DECORANA axis 2 to separate groups 13 and 15, both of which have high salinity, but at the sites in Group 15 there were persistent organic inputs.

DECORANA Axis 2 is likely, therefore, to be related to measures of organic pollution such as BOD and total nitrate. The measurement of these factors in highly saline conditions, however, is more difficult than in a fresh water. The salinity of the water interferes with the action of the ion-specific electrodes now commonly used to measure nitrate levels (Orion Research Inc., 1981), and chemical methods are complex and time consuming (Department of the Environment, 1972). The measurement of ammonia by ion-specific electrodes is not affected by salinity and could be used as a measurement of organic pollution, although this is only useful close to an input. Other methods of measuring organic pollution such as a measurement of Total Suspended Solids (TSS) or total carbon might also be of use.

There are also problems with methods which rely on

bacterial activity such as BOD and *Escherischia coli* counts as these are also affected by salinity. The number and diversity of bacterial species in saline water is reduced and changed (Bishop, 1988), making these less comparable with normal rivers, and the COD (Chemical Oxygen Demand) of the water is also affected by the presence of iron salts.

In view of the difficulty of using these normally efficient methods of detecting organic pollution, they have not been included in the environmental variables used in the classification, and organic pollution is recognised only by the effect which it has on river substrate, and on the change in species composition which it produces. It was felt, however, that the inclusion of some factors such as TSS or ammonia levels in the MDA analysis would improve the accuracy of placing sites into TWINSPAN groups.

The classification was very successful, despite the above difficulties, in predicting the fauna of the test sites, with agreements in the species list which compare well with those found by Moss *et al.*, (1988). Many East Midlands rivers have a reduced fauna when compared to sites in the rest of the country, particularly in groups such as stoneflies, and this reduction makes prediction of the fauna at any one site an easier task than it would be at more diverse sites.

The predictive model would benefit from further refinement, in particular by the inclusion of more clean-water and moderately saline sites, sampled with the same intensity as the Millwood Brook sites, as the data on which the model is based is limited in these areas. The model is able, however, to define communities which would result from the introduction of saline effluents

into small rivers.

An increase in salinity at a previously fresh water site would produce a fauna which changed from that of Group 4 to that of either Group 10 or 11. A further increase would produce a fauna similar to that of Group 12, where species such as *Gammarus tigrinus* and *Lumbricillus rivalis* are found replacing freshwater animals. Any additional salinity would reduce the fauna to that of Group 13, which contains only species extremely tolerant of salinity, such as *L. rivalis*, *Potamopyrgus jenkinsi* and Diptera such as Empididae.

CHAPTER FOUR

Field and Laboratory Population Studies on *Gammarus pulex* and *Gammarus tigrinus*

4:1 Introduction

A preliminary analysis of the results from the investigation of saline affected benthic communities in the River Idle catchment suggested that there were several species which were "indicators" of either saline or non-saline areas. The former included the amphipod *Gammarus tigrinus* and the oligochaete *Lumbricillus rivalis* and the latter *Gammarus pulex* and *Tubifex tubifex*. The distribution of these species has been described in Chapter 2, but, although this indicated that the relative frequency of these species could be related to salinity, the success of *G. tigrinus* and *L. rivalis* in saline areas was not proven to be due to a superior ability to withstand salinity, rather than to other factors such as lack of competition at polluted sites. Population studies on the four species were therefore undertaken to compare the reproductive cycles and success of these species in saline and non-saline conditions. *Gammarus pulex* and *G. tigrinus* were studied both at field sites and in the laboratory, *L. rivalis* and *T. tubifex* solely in the laboratory (Chapter 5).

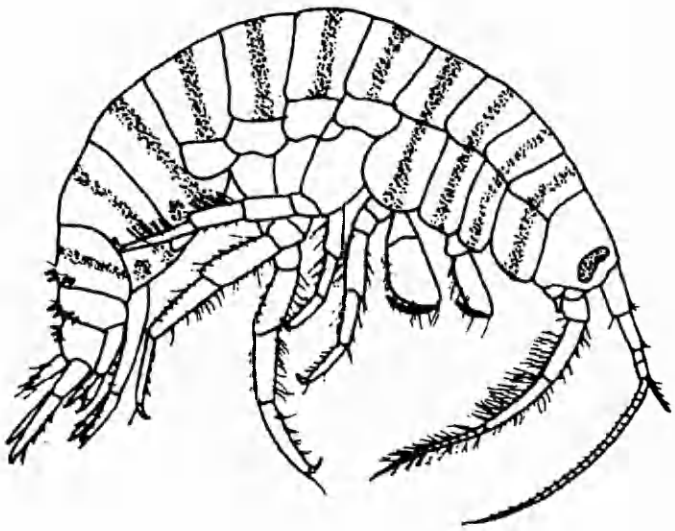
Gammarus tigrinus has been observed extending its range in England by several authors (eg Holland, 1976; Savage, 1981), and during the course of the present study it has

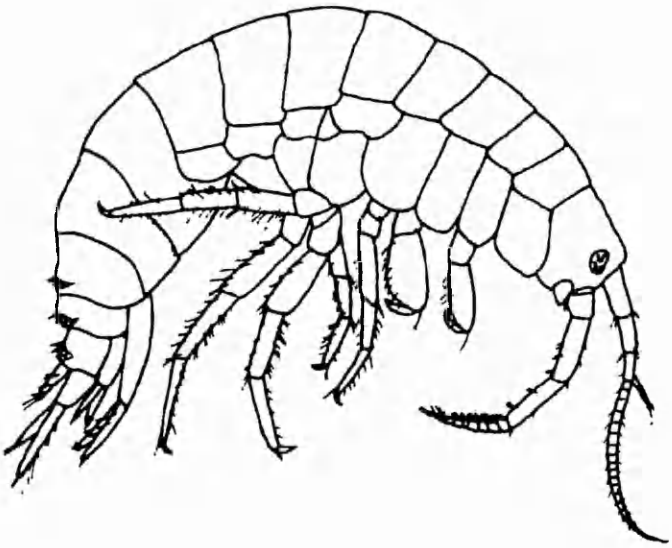
FIGURE 4:1

Drawings of *Gammarus pulex* and *Gammarus tigrinus*

With thanks to Dr. G.J. Bird for permission to reproduce his original drawings

Gammarus tigrinus





Gammarus pulex

been seen to spread upstream in Millwood Brook (Chapter 2). A drawing of this species and the native *G. pulex* is shown in Figure 4:1. The reasons why *G. tigrinus* is replacing *G. pulex* in England and other gammarid species such as *G. fossarum* on the Continent, are generally given as a combination of greater tolerance to salinity and an ability to produce large numbers of offspring (Ruoff, 1968; Savage, 1982; Severn-Trent Water Authority Report, 1987/88). The life history studies described in this chapter was undertaken to determine if these were the reasons for the success of *G. tigrinus* in the East Midlands.

There have been many studies of the life history of *G. pulex* in Britain, (eg Welton, 1979; Sutcliffe, 1981), but little attention has been paid to this aspect of *G. tigrinus*. It has been investigated by Savage (1981) in saline Cheshire meres, and Hynes (1954), in drainage dykes below high-tide level, but there has been no published account of its life-history in inland running waters in this country. The species has been described on the continent by Ruoff (1968) and Pinkster *et al.*, (1977), but these studies relate to lakes and very large rivers.

All the investigations, however, show that both species follow the gammarid reproductive cycle which was described by Hynes (1954), and can be briefly summarised as follows:

> In mating, the male grasps the female with his gnathopods by pushing the claw of each leg between the dorsal tergites of the female's pereon. The pair remain together in this pre-copula position for a few days (depending on temperature), until the

female moults. The male then turns the female round and places his sperm on her ventral surface. The female subsequently breaks away and before her cuticle hardens, eggs are deposited in a brood pouch made up of four pairs of plates (oostegites) and situated between the first four pairs of pereopods. The eggs are fertilised in this pouch and develop and hatch there, the young leaving a few days after hatching. A male may attach himself to a female a few days before the young are released.

Each female is capable of producing several broods and moults with each mating. The number of eggs, broods and development time depends both on the species and on the temperature of the water, as does the timing of breeding seasons and there may be several generations in a year. *Gammarus* moult continually throughout their life, and require up to ten moults before becoming mature (Hynes, 1954).

The field studies were designed to follow the life history of cohorts of each species and to estimate the time taken to reach maturity, mean fecundity and mortality at each stage. From these data, the "k" values for separate stages in the life history could be calculated (Haldane, 1949; Varley and Gradwell, 1970). The "k" value is calculated by subtracting the $\log_{10}$ of numbers alive at stage n+1 from the $\log_{10}$ of those alive at stage n. A "k" value can be seen as a measure of the "killing-power" between each stage in the life of a species, and can be used to determine in which stage of the life cycle most mortality occurs. The size of the k values is often related to "r" or "k" selection (MacArthur and Wilson, 1967; Pianka, 1970). In this

method of classifying the life-history of different species, a typical *r*-selected species would have a life-history characterised by early maturity, many small young, small size, large reproductive effort and a short life, while a *K*-selected species would have late maturity, few large young with some element of parental care, large size, small reproductive effort, and long life. An *r*-selected species would be characteristic of empty, disturbed sites, which it would be able to colonise rapidly, while a *K*-selected species would be found in more stable, crowded habitats.

It is necessary, however, when comparing the life-history of several species for *r* or *K* selection, to compare species of the same type, as a comparison of brood size and the size of young between an amphipod and a bird would clearly provide no information about *r* and *K* selection, since the difference in size of the two species renders the environment in which they live very different. Many authors now prefer to refer to the *r-K*-continuum, (Begon and Mortimer, 1981) in which the species under investigation are compared only with each other. This approach has been adopted with the comparison of *G. pulex* and *G. tigrinus* using *k* factor analysis.

The laboratory studies were designed to complement the field studies by providing information on egg hatching success and growth rates of both species in relation to salinity. This was necessary as there was no completely fresh-water population of *G. tigrinus*, and no saline population of *G. pulex* in the field. The methods and results sections of this Chapter are therefore divided into two sections, the first describing field studies and the second laboratory experiments. The two sections are discussed together, however, since the two studies are

complementary.

4:2 Population Analysis and Mortality of *Gammarus pulex* and *Gammarus tigrinus*

4:2:1 Methods

Four sites were chosen for the study: sites M1 and M3 with only *G. pulex* present and M19 and OLL with only *G. tigrinus*. Sites M1, M3 and M19 were regular sampling sites on Millwood Brook, and site OLL was situated on Rainworth Water near Ollerton, just below Rufford Abbey Lake. (Grid reference SK 647 668). Its position can be seen on Figure 2:7. All four sites were shallow (less than 50cm deep) and had stones and gravel as substrate. Photographs of the sites are shown on Plate 3.4 Site OLL was chosen in preference to sites on Millwood Brook such as M16 and M14, as these had either a soft substrate (M14) or were near major inputs (M16) which made flow and conductivity unstable. The four sites chosen had healthy populations of *Gammarus* that had been present throughout 1986.

These sites were sampled once every two weeks between March 19 1987 and April 20 1988, by kick sampling, as described in Chapter 2. In addition, the temperature, conductivity and chloride concentration of the water at each site were measured each month, using the methods described in Chapter 2. Kick samples were taken from both weedy and stony areas of the river, within a 1m wide transect across the river width, and the underside of stones in the sample area was searched for *Gammarus*. This was especially important in the sampling of *G. tigrinus*, as the adults of this species frequently aggregated on

PLATE 3

Views of Millwood Brook at Markland Grips (site M1, photograph 1) and Creswell Crags (site M3, photograph 2)

1

2

PLATE 4

View of the River Poulter just after crossing the A614 (site M19, photograph 1) and Rainworth Water at Ollerton (site OLL, photograph 2)

1

2

the underside of stones, particularly in late winter, and could be missed during kick sampling. *G. pulex* did not aggregate under stones to the same extent, but tended to be found in large numbers in patches of aquatic vegetation. Once collected, the kick and search samples were washed into large plastic buckets and the animals returned to the laboratory alive. Kick samples were taken until it was judged that there were at least 100 animals in the plastic bucket.

The samples were sorted live in the laboratory as soon as possible after their return to avoid deaths and cannibalism. The sorting procedure was as follows:

The bucket containing the sample was vigorously stirred and a portion poured into a white plastic tray, and all the *Gammarus* in the tray removed and killed. This was done by placing the animals in methanol in a petri dish. Pairs in pre-copula were kept in a separate dish to avoid confusion.

The process was repeated until approximately 100 individuals had been removed. To avoid any size bias, all the animals poured into a tray were removed, even if this took the total number of animals in the sample over 100.

The animals were then sorted according to sex and the number of eggs in the first five ovigerous females (if present) counted. Females were recorded as ovigerous if there were eggs or young in the brood pouch, (these could easily be seen under a binocular microscope) or if the brood pouches were well-developed with interlocking hairs on the edge of the oostegites. Mature males were recognised under the binocular microscope by examining the

ventrum between the last pair of pereopods. If the animal was a mature male, there was a pair of processes (the penes) which project inwards along the ventral surface from the base of the pereopod.

The sorted animals were then allowed to dry at room temperature (approximately 20^0C) for one week, then weighed on a Cahn model 500 micro-balance. The animals were considered dry if the balance reading remained completely stable once it had come to rest. (This type of balance is very sensitive, weighing to an accuracy of 0.001μg and any moisture remaining in the animals showed as a steadily decreasing weight reading.) Initially, a sample of dried animals was weighed every day until a stable weight was reached, but this point was always reached within a week.

The dry weights of the animals were recorded on data files using a BBC computer, and analysed using a program written to calculate the numbers of males, ovigerous females and juveniles (<1mg weight) and frequency of each size class. The frequency and mean weight of mature males and females was also calculated.

The *Gammarus* weights were grouped into frequency classes. Animals below 1mg in weight were grouped in 10 classes of 0.1mg intervals, and animals above 1mg in classes of 1mg.

To test whether there was any significant variation in size range between the *Gammarus* in separate aliquots taken from the same sample bucket, two samples of approximately 100 animals were taken from the same kick sample from site M1 in May 1987. The aliquots were sorted

and weighed using the methods described above, and the reulting figures compared using "t" tests. Table 4:1 shows the results of the replicates. No mean weight in the replicate is significantly different from the original sample, indicating that the stirring of the sample in the bucket produced an even distribution of animals.

In April 1987, the relationship between wet and dry weight of individuals of both species was checked. To do this, forty animals of each species, selected to represent as wide a range of sizes as possible, were first weighed wet (alive but dried for 30 seconds on a tissue). The weight of the animal was taken when the balance reached its maximum reading, before it began to decrease as moisture evapourated. The animals were then killed in methanol following the method described above, allowed to dry and weighed again.

Figure 4:2 shows the regression lines for both *G. pulex* and *G. tigrinus*, together with 10 original data points for both species. Both lines were significant at the 1% level and were not significantly different from each other, indicating that the method of drying chosen produced weights which were directly related to the wet weight of the animal.

In order to relate the frequencies of size classes in the population to actual abundance, the population density was estimated four times in the year, in June, September, December 1987 and April 1988, following an initial estimation of population density in April 1987.

Population size estimates of benthic invertebrates are liable to be imprecise due to the aggregated distribution of the individuals (Elliot, 1977). To gain a reasonable estimate of population density, large numbers of samples

TABLE 4:1

Mean weights of Gammarus from two sub-samples taken from the same kick sample, together with the values of "t" calculated for the means of the two samples.

	Sample 1			Sample 2				
Section	mean	se	size	mean	se	size	t	sig
Whole sample	1.19	1.91	112	1.62	2.3	115	1.5	NS
Juveniles	0.49	0.52	92	0.65	0.67	89	1.8	NS
Males	4.69	2.86	17	5.93	2.59	19	1.4	NS
Females	3.79	0.78	4	3.51	0.5	3	-0.6	NS
Mated male	8.3	0.72	2	7.16	3.13	3	-0.5	NS
Mated fem.	4.44	0.38	2	3.5	0.5	3	-2.2	NS

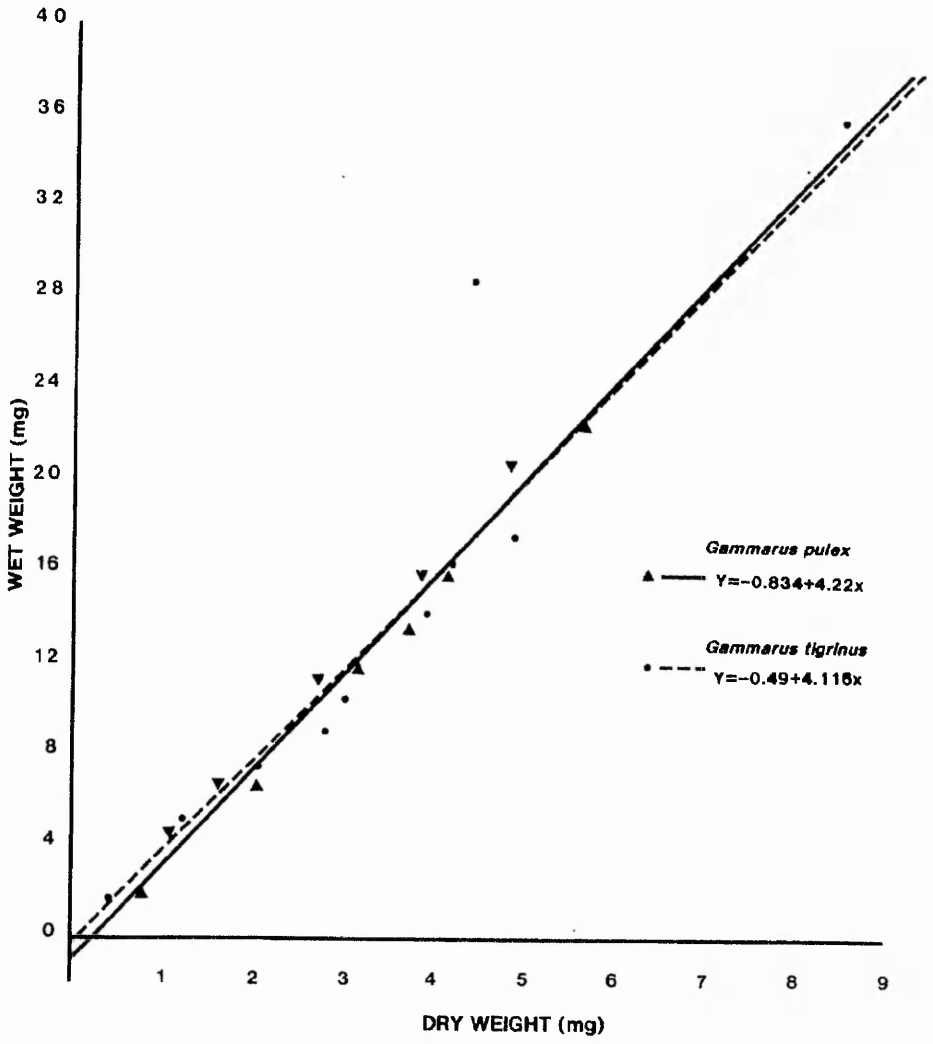
All weights are in mg

FIGURE 4:2

Regression lines of wet and dry weight of *Gammarus pulex* and *Gammarus tigrinus*.

The original data points for *G. pulex* are shown as solid triangles, and the regression line as a solid black line.
The original data points for *G. tigrinus* are shown as circles, and the regression line as a dotted line.

Both lines are significant at the 1% level.


40
36
32
28
24
20
16
12
8
4
0
WET WEIGHT (mg)
1
2
3
4
5
6
7
8
9
DRY WEIGHT (mg)
Gammarus pulex
Y=-0.834+4.22x
Gammarus tigrinus
Y=-0.49+4.116x

must be taken. It was decided not to estimate population density every fortnight, as it was felt that the large numbers of samples which would have to be taken would be very destructive of the sites, particularly the small site M1. It was decided, therefore, to estimate population density at each sampling occasion from the five detailed estimates. To do this, a graph of the numbers m^{-2} against time was plotted, and the points for each site joined by a straight line (Figure 4:3). The numbers present at any date could then be read from the graph. The figures from the graph were estimates of population density only, as the density of *Gammarus* could fluctuate irregularly between two sampling dates.

To provide population estimates samples of $0.25m^2$ were taken every metre across the complete width of the river at each site. At site M1 there were four samples in September when the river was very low, and five in the other seasons. The samples at site M1 had no gap between them, as the river was very narrow at this site. Sites OLL and M3 required 5 samples to cover the width of the river, and site M19 eight, except for September, when the river was very low and less wide. To take a sample, $0.25m^2$ quadrat was placed on the stream-bed, with a standard pond net downstream, and the substrate within the quadrat stirred so that the animals were washed into the net. All stones within the quadrat were also washed into the net. The samples were preserved at the site in 5% formalin, carefully sorted by hand in the laboratory and the *Gammarus* counted. The numbers of ovigerous females were recorded separately.

The proportions of individuals in each weight class, mature males and ovigerous females and the numbers of eggs carried by each ovigerous female at each sampling

date were used to calculate different aspects of the life history of both *Gammarus* species.

4:2:2 Results

At three of the four sites sampled, (M1, M3 and M19) water-flow was maintained throughout the year, with sites M1 and M19 having particularly stable discharge rates. At site OLL, however, the river completely ceased to flow between May 27 and June 1 1987, when the lake at Rufford Abbey, which feeds the river, was drained to repair mining subsidence. The river was reduced to a series of damp patches in which no live *Gammarus* were found, although there were large numbers of dead and dried animals. By June 10, when flow had been restored for about a week, *G. tigrinus* was recolonising the site, and the population density was approximately half that seen on May 27. Quantitative samples were taken at OLL on June 10 to determine the population density. By June 22, numbers of *Gammarus* appeared to have returned to the level found in May. The river flowed normally throughout the rest of the sampling period.

4:2:2:1 Water chemistry

The conductivities, chloride concentrations and temperatures of the four sites, taken once per month are shown in Appendix G. The most saline of the four sites was OLL, with a maximum value of 35mM chloride, and the least saline was M1, with a maximum chloride concentration of 2.5mM.

The highest summer water temperatures (22.5°C and 22°C) were found at sites OLL and M19. The temperatures at these sites were in general 1 - 2 °C higher than at sites M1 and M3 throughout the year.

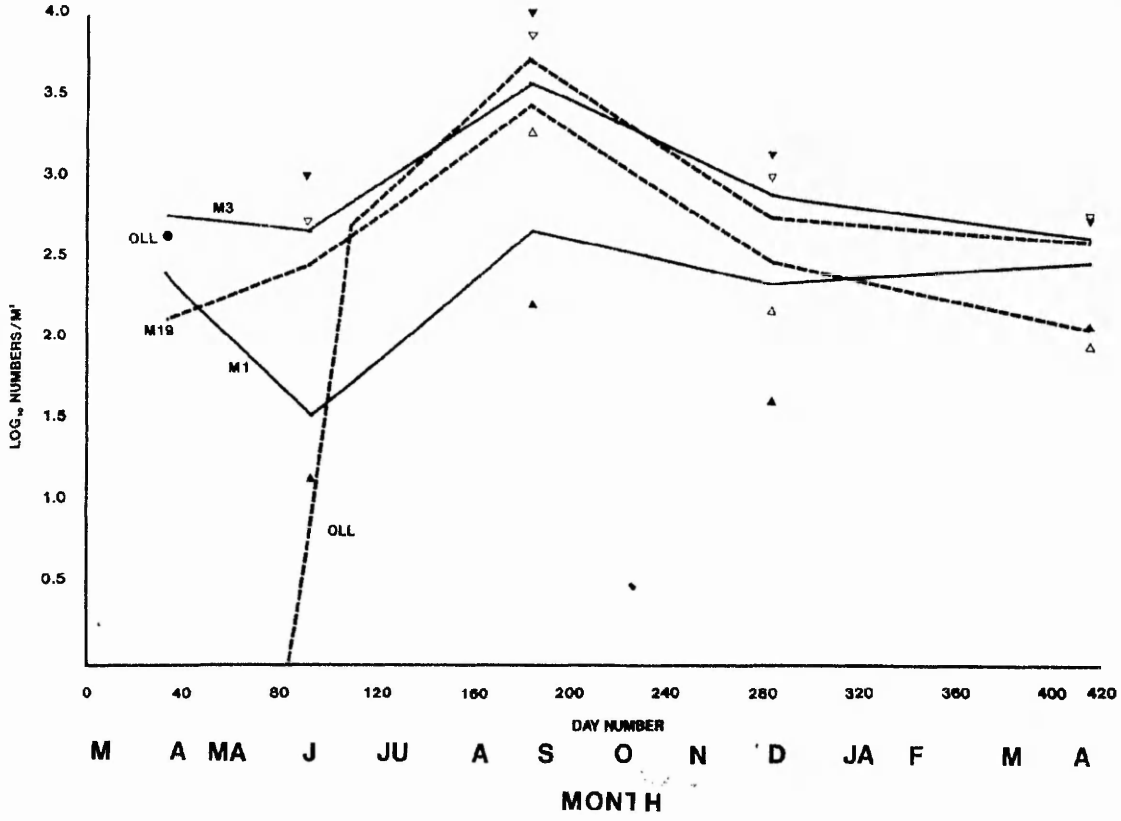
4:2:2:2 Population Density

Figure 4:3 shows the mean estimated population densities for all sites in the four months when they were sampled, (June, September, December, April), together with extra samples taken at OLL in June.
In the winter and spring samples, the population size of both species was similar, with between 400 and 800m^{-2}. These numbers declined in the June sample and increased to a peak in the September sample. At site M1, there were double the numbers of *Gammarus pulex* in September (996m^{-2}) when compared to December, while at site M3 this species increased 10-fold to 6000m^{-2}. The population density of *G. tigrinus* increased 18-fold from April to early September at both sites. The density of animals at all sites declined between the September and December samples.
The numbers of *G. pulex* at site M1 were always lower than those at site M3. At site OLL, *G. tigrinus* did not follow the same pattern as the other species in early spring, as at this time the river dried and the population was destroyed. However, the site was rapidly recolonised, as can be seen in Figure 4:3, and by September had a higher population density than *G. tigrinus* at site M19.

FIGURE 4:3

Mean Numbers m^{-2} of *G. pulex* and *G. tigrinus* at four sites, M1, M3, M19 and OLL on five sampling occasions between April 1987 and April 1988

Site names are marked adjacent to the lines to which they refer.
Solid lines represent *G. tigrinus*, dotted lines *G. pulex*.
Ranges of values for each species are shown by triangles, open triangles for *G. tigrinus* and solid triangles for *G. pulex*


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
LOG10 NUMBERS/M3
M3
OLL
M19
M1
OLL
0
40
80
120
160
200
240
280
320
360
400
420
DAY NUMBER
M A MA J JU A S O N D JA F M A
MONTH

4:2:2:3 Population Structure

Table 4:2 shows the summary statisics for both species. The mean weight of mature *G. tigrinus* was lower than that of *G. pulex* at both sites, although the maximum mean size of the two species was similar. There were mature males in samples of both species on all occasions, although mated males were not present in winter samples of *G. tigrinus*.

Juveniles of *G. tigrinus* appeared in the population at a minimum size of 0.013mg (dry weight), while juveniles of *G. pulex* appeared at a minimum weight nearly three times this value, 0.034mg.

The smallest mature males and ovigerous females found (of both species) were between 0.9 and 1.0mg, although the mean weight of mature and mated animals was always higher than this. The majority of animals, however, matured at about 1mg in both species, and ovigerous females of this weight could also be found, although most ovigerous females were above 2mg. It was decided to use the 1-2mg size class as an indication of maturity in calculations, as this was the size range in which most animals became mature, although many, particularly females, did not mate until they were slightly larger. The minimum size of mature males and females was lowest in late summer and highest in May and June.

The largest *G. tigrinus*, weighing 12.647mg and found in May, was from site OLL, and the largest *G. pulex*, weighing 13.97mg and also found in May, was from site M3. At site M1 the largest animal weighed 12.198mg (February) and at site M19 the largest (found in May), weighed 12.647mg. In all cases the largest animals in a sample

TABLE 4:2

Summary statistics of *Gammarus* spp. Collected from Four Sites Between April 1987 and April 1988

Sites underlined support *G. tigrinus*

Category	Site	Max	Min	Med	Mean	SE
Whole population	M1	4.98	0.77	2.22	2.5	1.19
Weight (mg)	M3	4.69	0.66	2.18	2.2	1.15
	M19	3.5	0.31	1.43	1.75	0.99
	OLL	5.96	0.29	1.97	2.2	1.53
Mature males	M1	7.01	3.61	5.43	5.39	0.92
Weight	M3	9.51	2.19	4.64	5.44	2.13
(mg)	M19	7.04	0	3.98	3.75	1.35
	OLL	13.1	0	4.41	4.99	2.43
Ovigerous females	M1	6.14	2.35	4.45	4.48	0.85
mean weight	M3	6.97	0	5.02	4.53	1.91
(mg)	M19	4.86	0	2.23	1.97	1.74
	OLL	7.04	0	2.61	2.75	2.44
Pre-copula males	M1	10.81	0	8.11	6.11	4.01
weight	M3	11.54	0	7.2	5.29	4.35
(mg)	M19	5.31	0	2.04	1.82	1.8
	OLL	10.16	0	4.69	4.26	3.87
Pre-copula females	M1	6.5	0	4.24	3.58	2.38
weight	M3	6.62	0	4.28	3.17	2.64
(mg)	M19	5.31	0	2.04	1.82	1.8
	OLL	6.9	0	3.35	2.54	2.32
% Mature males	M1	36.5	7.3	22.1	21.2	7.72
	M3	42.5	5.3	14.3	16.17	8.2
	M19	54.7	0	13.3	18.2	13.2
	OLL	67.6	0	16.7	21.2	16.3
% Ovigerous females	M1	37.5	0.8	11	11.6	9.25
	M3	24	0	5.3	8.91	7.82
	M19	52.1	0	5.55	11.5	14.2
% juveniles	M1	94	9.6	44	45.8	22.1
(<1 mg)	M3	86.9	11.4	51.2	53.1	23.5
	M19	91.9	3.36	47.1	45.2	29.7
	OLL	94	0	50.9	49.7	29.8

were males.

Figures 4:4a, b, c and d show the percentage of each weight group for the four sites on the dates when population size was measured directly.

At both sites with *G. tigrinus* the most immediately apparent change during the year was the presence of juveniles between June and September, and their absence between December and April. In *G. pulex* there were juveniles present throughout the year, although the highest percentages occured in April and the lowest in December.

The large adult *G. tigrinus* which were present at the beginning of June were nearly all absent by September, and by the end of June the bulk of the population was made up of juveniles. In December high percentages of the populations consisted of small adults (between 1 and 4mg weight), which grew through the winter so that by April of the following year there were again large adults (9-10mg) present.

The similarity between the population size in April 1987 and April 1988 indicated that the decline in numbers after the summer breeding activity was a regular occurence and not the result of poor conditions in one year. The composition of the population was also very similar on 20 April 1987 and 29 April 1988, as can be seen when Figures 4:4 and 4:5 are compared. The difference between the populations of *G. tigrinus* in April 1988 when compared to the April 1987 samples was due to the appearance of new juveniles, which were not present in the 1987 samples, taken one week earlier. There were also new juvenile *G. pulex* in April at site M3, although there were few at M1.

FIGURE 4:4a

Percentage of the population of *Gammarus pulex* at Site M1 in each weight class on June 10 1987, September 2 1987, December 9 1987 and April 20 1988.

The range of weights (in mg) in each size class is as follows:

Class 1	0.0-0.1
Class 2	0.1-0.2
Class 3	0.2-0.3
Class 4	0.3-0.4
Class 5	0.4-0.5
Class 6	0.5-0.6
Class 7	0.7-0.8
Class 8	0.8-0.9
Class 9	0.9-1.0
Class 10	1-2
Class 11	2-2
Class 12	2-3
Class 13	3-4
Class 14	4-5
Class 15	5-6
Class 16	6-7
Class 17	7-8
Class 18	8-9
Class 19	9-10
Class 20	10-11
Class 21	11-12

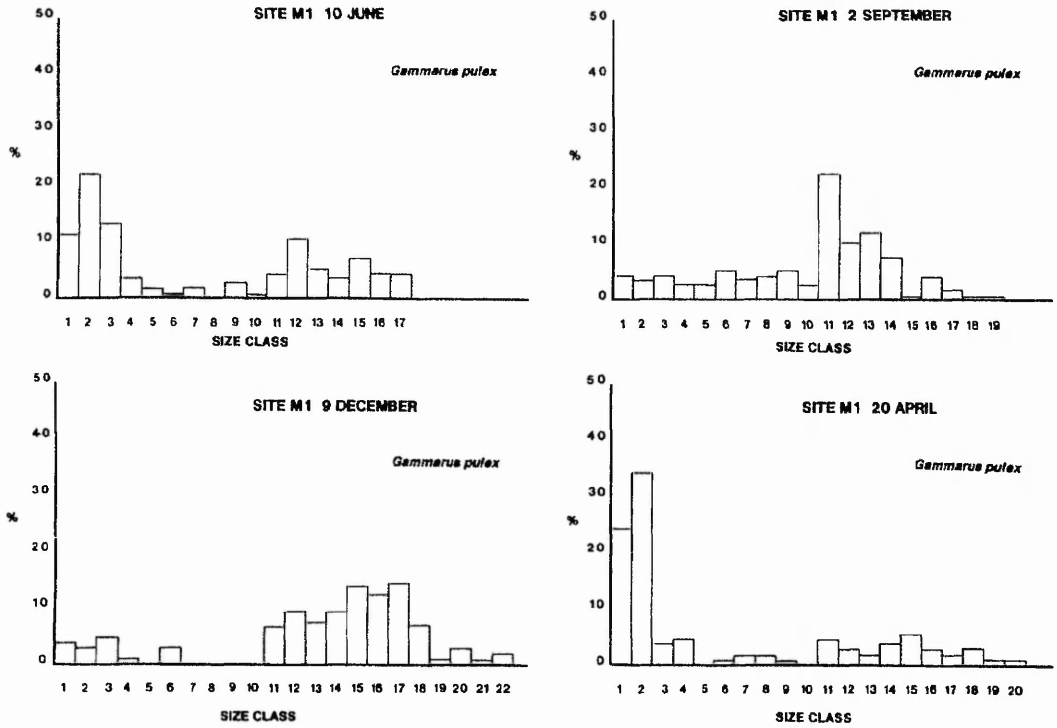

SITE M1 10 JUNE
Gammarus pulex
%
SIZE CLASS
SITE M1 2 SEPTEMBER
Gammarus pulex
%
SIZE CLASS
SITE M1 9 DECEMBER
Gammarus pulex
%
SIZE CLASS
SITE M1 20 APRIL
Gammarus pulex
%
SIZE CLASS

FIGURE 4:4b

Percentage of the population of *Gammarus pulex* at Site M3 in each weight class on June 10 1987, September 2 1987, December 9 1987 and April 20 1988.

The range of weights (in mg) in each size class is as follows:

Class 1	0.0-0.1
Class 2	0.1-0.2
Class 3	0.2-0.3
Class 4	0.3-0.4
Class 5	0.4-0.5
Class 6	0.5-0.6
Class 7	0.7-0.8
Class 8	0.8-0.9
Class 9	0.9-1.0
Class 10	1-2
Class 11	2-2
Class 12	2-3
Class 13	3-4
Class 14	4-5
Class 15	5-6
Class 16	6-7
Class 17	7-8
Class 18	8-9
Class 19	9-10

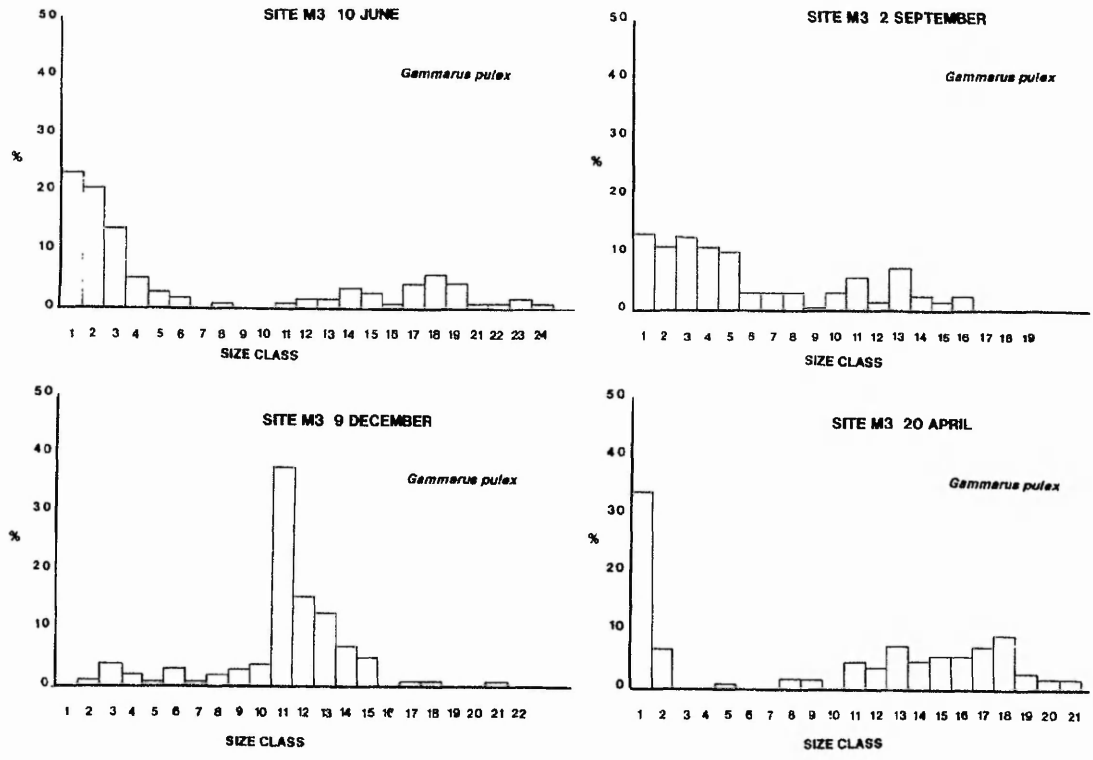
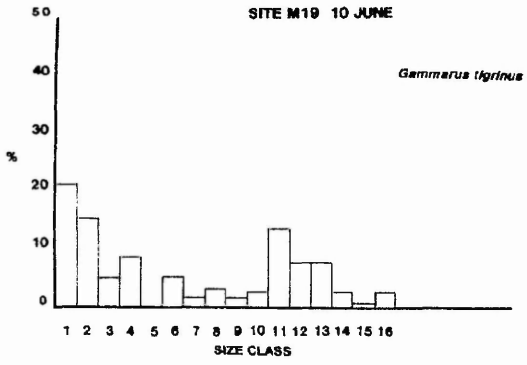

SITE M3 10 JUNE
Gammarus pulex
%
SIZE CLASS
SITE M3 2 SEPTEMBER
Gammarus pulex
%
SIZE CLASS
SITE M3 9 DECEMBER
Gammarus pulex
%
SIZE CLASS
SITE M3 20 APRIL
Gammarus pulex
%
SIZE CLASS

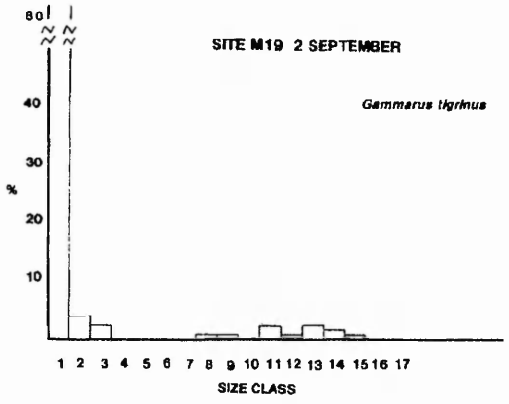
FIGURE 4:4c

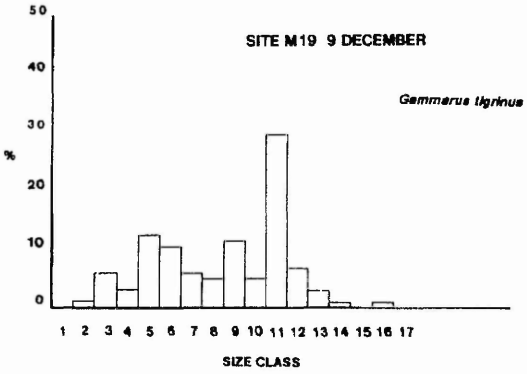
Percentage of the population of *Gammarus tigrinus* at Site M19 in each weight class on June 10 1987, September 2 1987, December 9 1987 and April 20 1988.

The range of weights (in mg) in each size class is as follows:

Class 1	0.0-0.1
Class 2	0.1-0.2
Class 3	0.2-0.3
Class 4	0.3-0.4
Class 5	0.4-0.5
Class 6	0.5-0.6
Class 7	0.7-0.8
Class 8	0.8-0.9
Class 9	0.9-1.0
Class 10	1-2
Class 11	2-2
Class 12	2-3
Class 13	3-4
Class 14	4-5
Class 15	5-6
Class 16	6-7
Class 17	7-8
Class 18	8-9
Class 19	9-10
Class 20	10-11
Class 21	11-12


SITE M19 10 JUNE
Gammarus tigrinus
%
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
SIZE CLASS


SITE M19 2 SEPTEMBER
Gammarus tigrinus
%
80
40
30
20
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
SIZE CLASS


SITE M19 9 DECEMBER
Gammarus tigrinus
%
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
SIZE CLASS

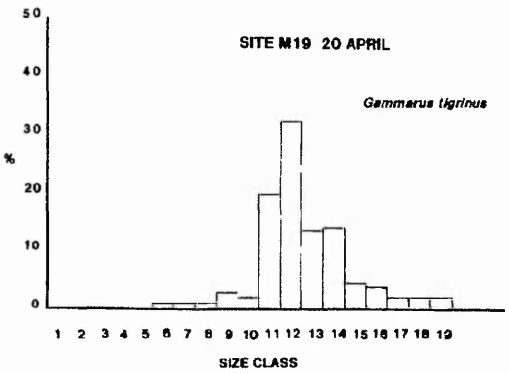
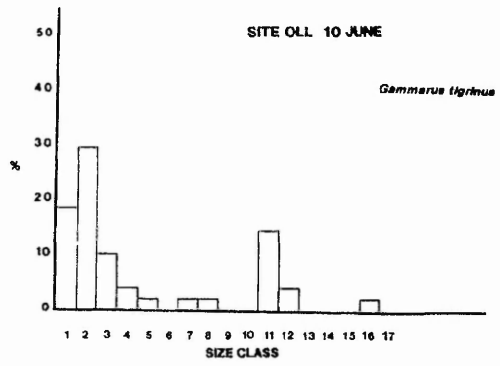

SITE M19 20 APRIL
Gammarus tigrinus
%
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
SIZE CLASS

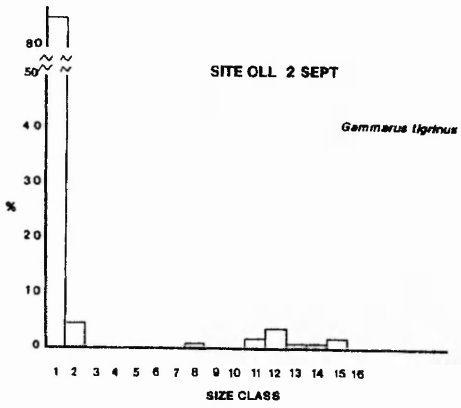
FIGURE 4:4d

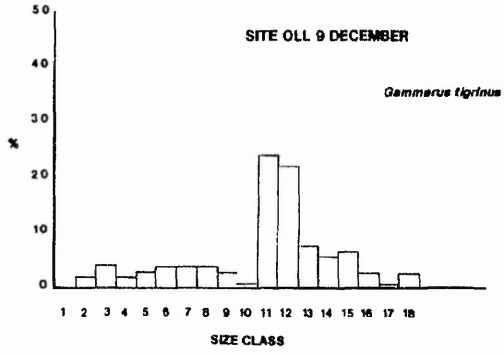
Percentage of the population of *Gammarus tigrinus* at Site OLL in each weight class on June 10 1987, September 2 1987, December 9 1987 and April 20 1988.

The range of weights (in mg) in each size class is as follows:

Class 1	0.0-0.1
Class 2	0.1-0.2
Class 3	0.2-0.3
Class 4	0.3-0.4
Class 5	0.4-0.5
Class 6	0.5-0.6
Class 7	0.7-0.8
Class 8	0.8-0.9
Class 9	0.9-1.0
Class 10	1-2
Class 11	2-2
Class 12	2-3
Class 13	3-4
Class 14	4-5
Class 15	5-6
Class 16	6-7
Class 17	7-8
Class 18	8-9
Class 19	9-10
Class 20	10-11
Class 21	11-12


SITE OLL 10 JUNE
Gammarus tigrinus
%
SIZE CLASS


SITE OLL 2 SEPT
Gammarus tigrinus
%
SIZE CLASS


SITE OLL 9 DECEMBER
Gammarus tigrinus
%
SIZE CLASS

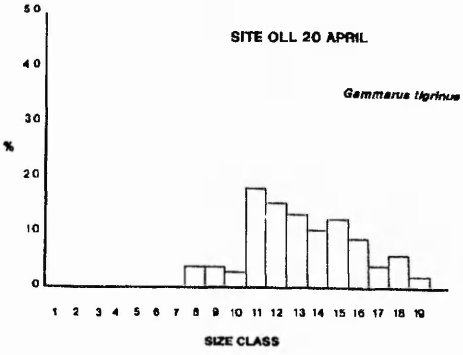
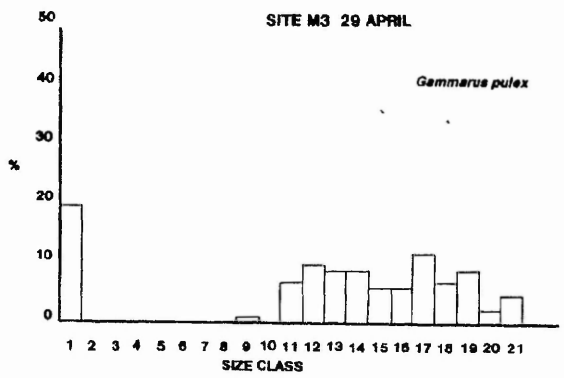

SITE OLL 20 APRIL
Gammarus tigrinus
%
SIZE CLASS

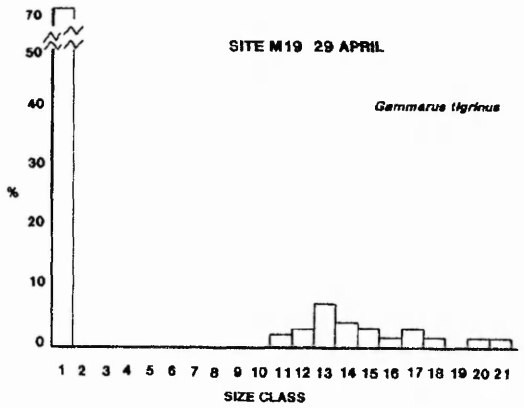
FIGURE 4:5

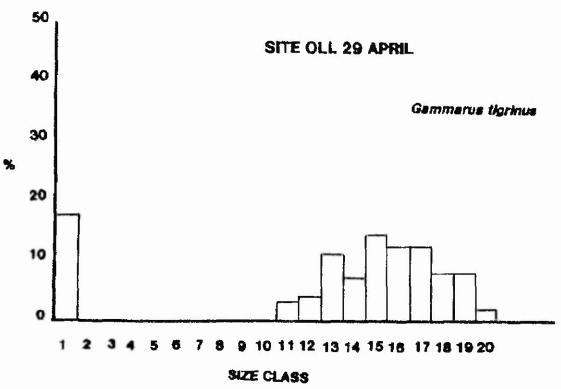
Percentage of the population of *Gammarus pulex* and *Gammarus tigrinus*at Sites M1, M3, M19 and OLL in April 1988

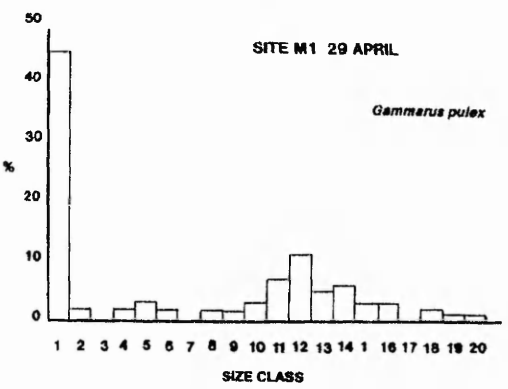
The range of weights (in mg) in each size class is as follows:

Class 1	0.0-0.1
Class 2	0.1-0.2
Class 3	0.2-0.3
Class 4	0.3-0.4
Class 5	0.4-0.5
Class 6	0.5-0.6
Class 7	0.7-0.8
Class 8	0.8-0.9
Class 9	0.9-1.0
Class 10	1-2
Class 11	2-2
Class 12	2-3
Class 13	3-4
Class 14	4-5
Class 15	5-6
Class 16	6-7
Class 17	7-8
Class 18	8-9
Class 19	9-10
Class 20	10-11
Class 21	11-12


SITE M3 29 APRIL
Gammarus pulex
%
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
SIZE CLASS


SITE M19 29 APRIL
Gammarus tigrinus
%
70
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
SIZE CLASS


SITE OLL 29 APRIL
Gammarus tigrinus
%
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
SIZE CLASS


SITE M1 29 APRIL
Gammarus pulex
%
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 1 16 17 18 19 20
SIZE CLASS

4:2:2:4 Reproductive cycles

Figures 4:6a, b, c and d show the percentages of juveniles, ovigerous females and 1-2mg adults at all sites against sample number (1-29). From these graphs, the day when peak numbers of each of these groups occurred could be determined. These times were used to estimate the number of days taken to grow from egg to juvenile and then to 1-2mg adult, as in Table 4:3. The time between successive maxima of ovigerous females and juveniles was calculated for each species. However, from laboratory experiments (see later results) and published literature, (Hynes, 1955; Nilsson, 1977; Welton, 1979; Steele and Steele, 1972; Savage, 1982), it was known that although the time taken for ovigerous females to deposit eggs was roughly the same in each species, and dependent on temperature, the time taken for juveniles to grow to small adults was much longer in *G. pulex* than in *G. tigrinus* at the same temperature.

To calculate the time taken for newly-released juveniles to reach the 1-2mg size class, therefore, the first maxima following a peak of juveniles was used in *G. tigrinus*, and the second in *G. pulex*. This method of calculation gave results which were similar to results from laboratory experiments and those in the published literature (eg Hynes, 1955; Welton, 1977; Sutcliffe, 1981; Savage, 1982).

The data in Table 4:3 show that the retention time for young was similar in both species, with the lowest values being around 14 days in July, August and September, and the longest of about 28 days in April, May and September. The time taken to grow from the 0.01mg juvenile class to the 1-2mg adult class (development time) for the two

FIGURE 4:6a

Percentage of ovigerous females, juveniles under 1mg dry weight and 1-2mg adults at sites M1 and M3

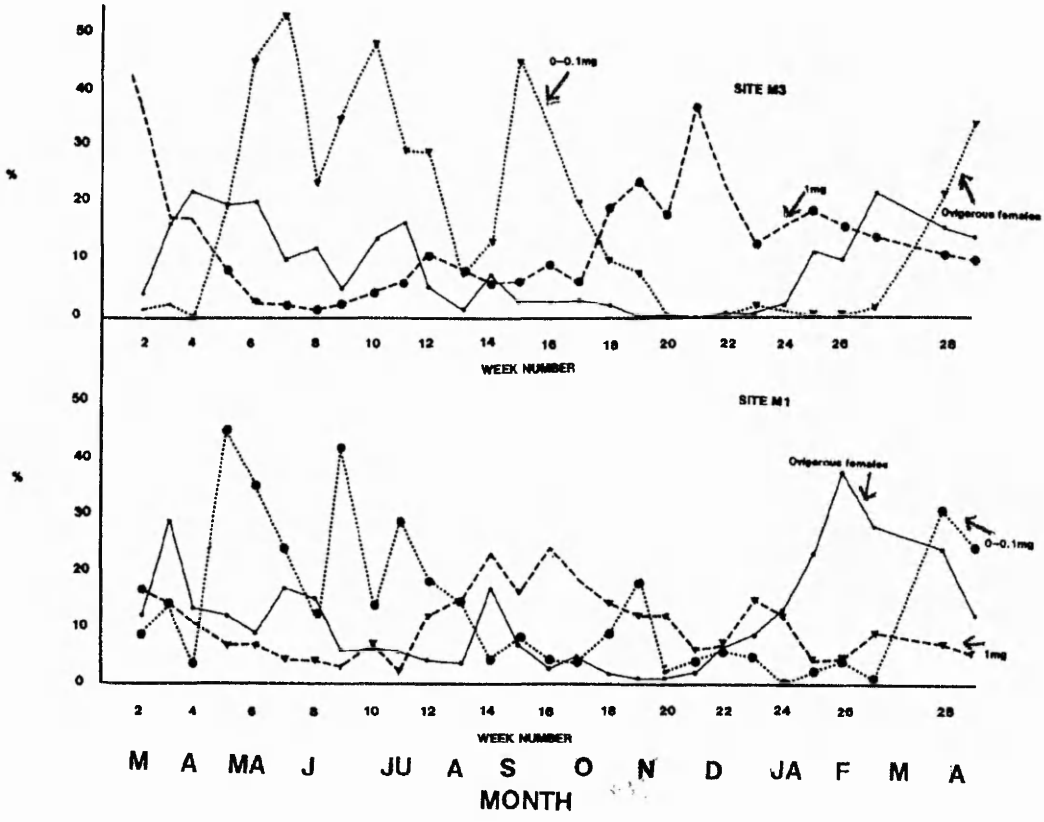

SITE M3
0–0.1mg
1mg
Ovigerous females
%
WEEK NUMBER
SITE M1
Ovigerous females
0–0.1mg
1mg
%
WEEK NUMBER
M A MA J JU A S O N D JA F M A
MONTH

FIGURE 4:6b

Percentage of ovigerous females, juveniles under 1mg dry weight and 1-2mg adults at sites M19 and OLL

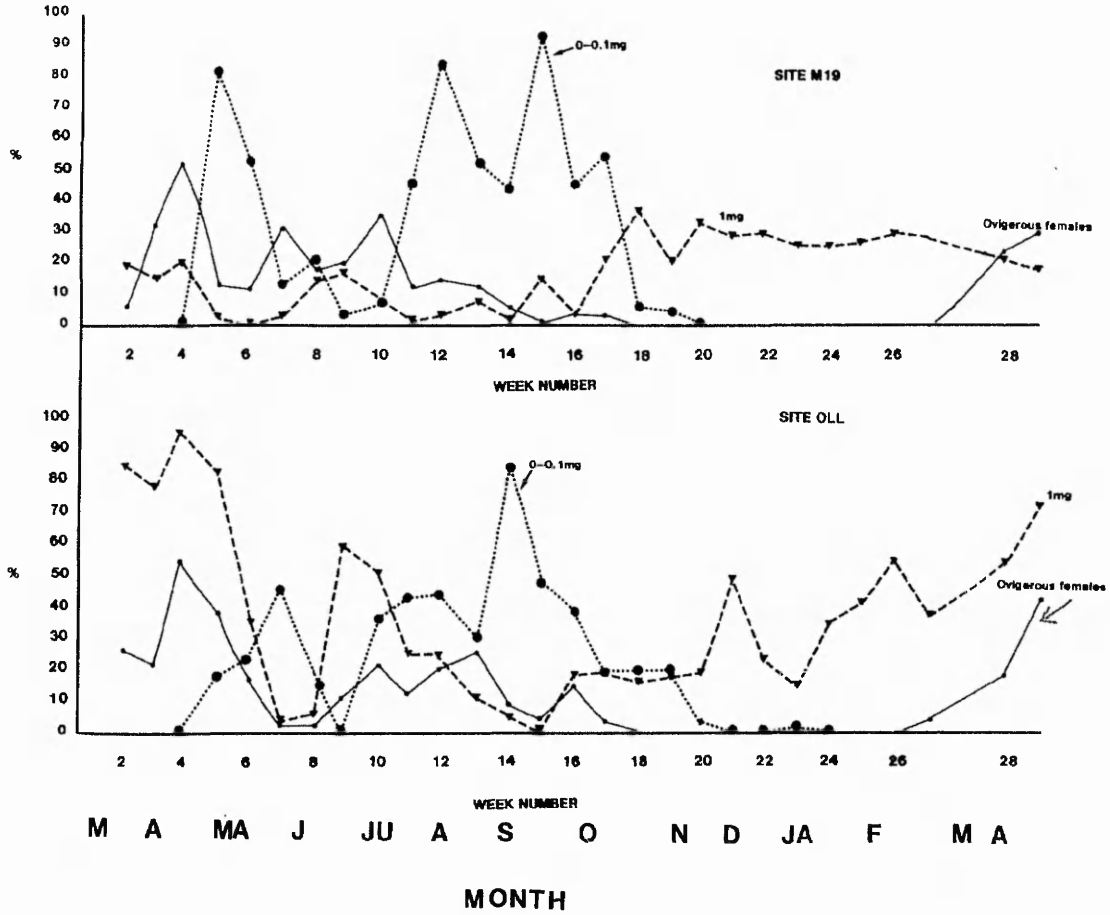

SITE M19
0–0.1mg
1mg
Ovigerous females
%
WEEK NUMBER
SITE OLL
0–0.1mg
1mg
Ovigerous females
%
WEEK NUMBER
M A MA J JU A S O N D JA F M A
MONTH

TABLE 4:3

Development times (in Days) of

Gammarus pulex and *Gammarus tigrinus*

Between Egg (E), Juvenile (J) and Adult(A)

Measured at Sites M1, M3, M19 and OLL

Site	Day (E)	Day (J)	Day (A)	J-E	A-J
M1	32	60	186	28	26
M1	88	114	214	26	100
M1	130	143	270	13	127
M1	186	200	312	14	112
M1	228	257	369	29	112
mean				22	115.4
M3	44	60	156	16	96
M3	102	130	214	28	84
M3	143	156	257	13	101
M3	186	200	284	14	84
M3	228	257	340	29	83
mean				20	89.6
M19	44	60	114	16	54
M19	88	102	172	14	70
M19	130	156	200	26	44
M19	156	186	243	30	57
M19	214	228	270	14	42
mean				20	53.4
OLL	44	88	114	44	26
OLL	130	156	186	26	30
OLL	172	186	214	14	28
OLL	214	257	284	43	27
mean				31.8	27.8

species was different at all four sites, although *G. pulex* always had a longer development time than *G. tigrinus*, with the longest being 160 days and 70 days respectively. At site M3, *G. pulex* had a mean development time of 89.6 days compared with 122.2 at site M1.

At site OLL *G. tigrinus* produced only 4 cohorts of juveniles compared with 5 at the other three sites, due to the drying of the river bed in early June. (A cohort is defined throughout as a group of individuals which were born (released from the brood pouch) at about the same time. This drought destroyed the cohort which was released in April, and the animals present after June were immigrants from other areas, presumably from upstream of Rufford Park and downstream of Ollerton where the river did not dry out. The development time of later generations at OLL was much shorter than at M19, perhaps due to the continued immigration of young adults from other areas obscuring the recruitment of smaller numbers from the original cohort released at the sample site.

Both species had the potential to produce 5 cohorts in a year, and in *G. pulex*, these were produced throughout the year, with the last group of juveniles which were released in November or December reaching maturity in February or March. *Gammarus tigrinus*, in contrast, produced all its offspring between April and September, with the last juveniles reaching maturity in November or December. These young adults then grew over the winter and began to breed in April the following year.

The number of generations per year which the two species could produce was also estimated using the development times calculated from Table 4:3. ("Generation" time is defined here as the time from release to first breeding cycle.) In *G. pulex*, the juveniles born between

April and July bred in late August to late September, while those born later than this, in August and September, matured in the following January to March. If most of the *G. pulex* which began to breed in early spring died in June, as the drop in the size of ovigerous females around this time suggests, then each female would be able to produce three broods before death. *Gammarus pulex*, therefore, appeared to produce three generations per year at sites M1 and M3, one which was born in spring and matured in late summer, one which was born in mid-summer and matured in January-February, and one which was born in September and matured in March-April.

In *G. tigrinus*, the juveniles born in April began to breed in June-July, and those born in July-August in September. If the females which bred in April died in June (as in *G. pulex*), as suggested by the drop in size of ovigerous females in this month, then each female could produce 3-4 broods before death. The juveniles born in April and maturing in June-July, could produce 6 broods before September. Those maturing in September might or might not (depending on temperature) breed before the winter, producing one further brood. This species also seemed able to produce three generations per year, the first born in April and maturing in June-July, the second born in June-July and maturing in September, and the last born in September and overwintering to mature in March -April.

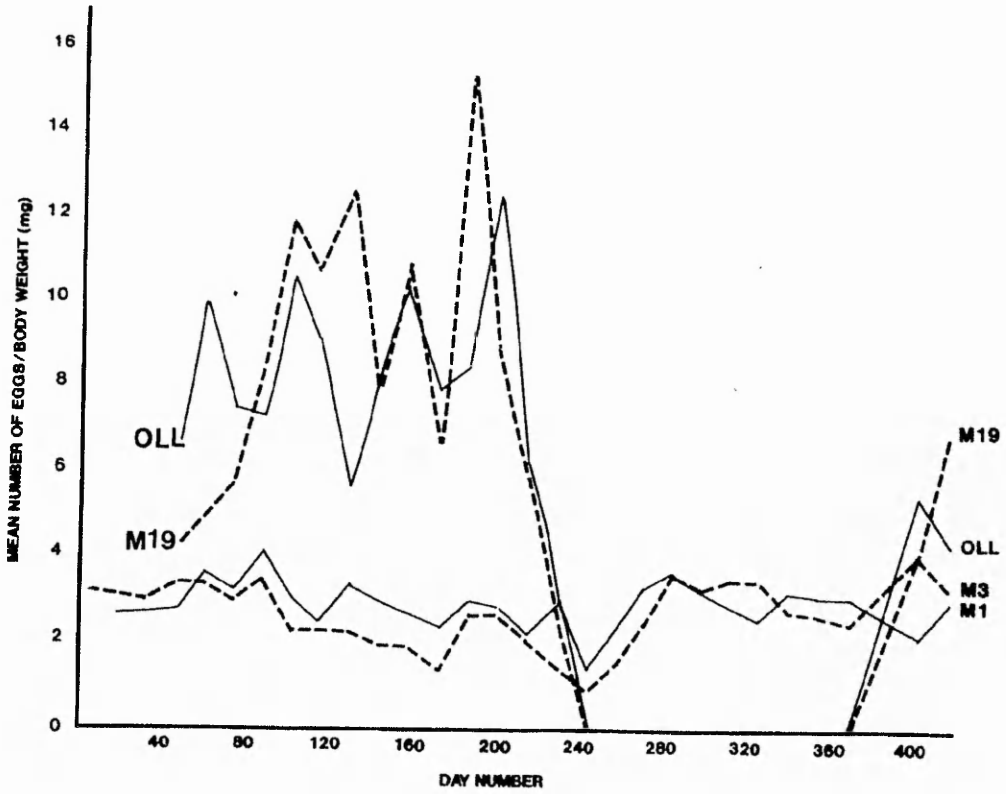
4:2:2:5 Fecundity

Figure 4:7 shows the number of eggs per mg of body weight of ovigerous females against day number at all sites. The figures are the means of numbers of eggs and body weight

FIGURE 4:7

Graph showing the number of eggs per mg body weight found in *Gammarus pulex* at sites M1 and M3, and *Gammarus tigrinus* at sites M19 and OLL against day number

In *G. pulex*, figures from site M1 are shown as a solid line and from M3 as a dotted line
In *G. tigrinus*, figures from site M19 are shown as a solid line and from OLL as a dotted line


MEAN NUMBER OF EGGS/BODY WEIGHT (mg)
16
14
12
10
8
6
4
2
0
40
80
120
160
200
240
280
320
360
400
DAY NUMBER
OLL
M19
M19
OLL
M3
M1

measured for five females from each site on every sampling occasion when such females were present. It is clear that *G. tigrinus* females produced more eggs per mg than *G. pulex* except at the end of their breeding season, when numbers of eggs carried per female in this species declined sharply. The highest mean value was recorded at site M19, where a mean of 15.5 eggs/mg was recorded in September. All the females examined on this date were between 1.5 and 3.5 mg in weight, and carried between 26 and 49 eggs. The maximum number of eggs was found at site OLL, where in May a female weighing 4.84mg was found carrying 95 eggs. The maximum number of eggs carried by a *G. pulex* female was 34, from an individual weighing 4.77mg in April.

The ratio of eggs/mg body weight in *G. pulex* varied between 1.01 and 4.18 over the sampling period, and in *G. tigrinus* between 2.99 and 15.5. The pattern of peaks in the ratio of eggs to body weight was similar at sites M19 and OLL, with animals from both sites showing 4 peaks of eggs/mg body weight, in April, June, August and September.

The variation in the egg/body weight ratio suggests that in *Gammarus* spp. it is too simplistic to draw a correlation or regression between number of eggs and body weight. There was a significant relationship between number of eggs and body weight in both species, but since this varied with season it was not considered to be very meaningful. The mean values of the ratio for the two species were, however, significant when compared using a "t" test, which indicates that *G. tigrinus* was significantly more fecund than *G. pulex*.

The pattern of peaks in number of eggs carried was less clear for *G. pulex* than for *G. tigrinus*, although there

were small peaks in April, May, September, December and again in April.

4:2:2:6 Numbers of Juveniles Released

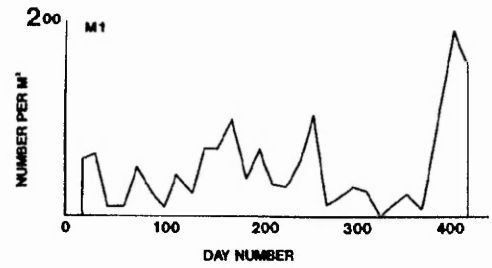
The numbers of juvenile *Gammarus* (<0.1mg) present on each sampling occasion were claculated from the population size and percentage of 0-0.1mg individuals on that date. The resulting numbers were plotted against day number and are shown in Figure 4:8. The maximum number of juveniles present at any one sampling occasion are shown in Table 4:4. The figures for site OLL were begun in July, after the population had apparently recovered from the drought. *Gammarus pulex* at site M1 produced the lowest numbers of offspring, although this species was capable of much higher rates of reproduction, as at site M3, where 10 times as many juveniles were released. *Gammarus tigrinus*, although having a breeding season of only about 180 days, produced the largest numbers of offspring. At site OLL, the largest numbers of juveniles were produced despite the shortened breeding season.

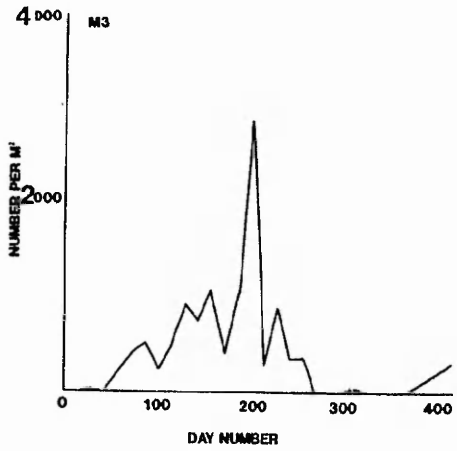
4:2:2:7 Survival from Release to Adult

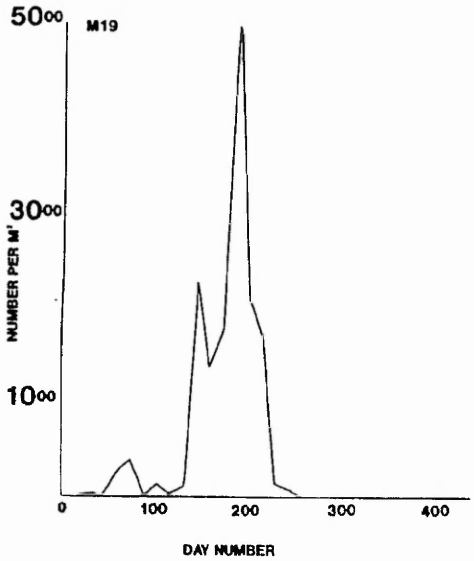
From the dates of maximum frequency of ovigerous females, juveniles and 1-2mg adults, the percentage survival of both species was estimated. The population size at each relevant date was estimated from Figure 4:3, the percentage of animals in each category at that date was obtained and the two figures used to calculate the number of animals in each size class. The number of eggs per ovigerous female on each date was used to estimate the

FIGURE 4:8

Number of Juveniles m^{-2} at Sites M1, M3, M19 and OLL between April 1987 and April 1988


M1
200
NUMBER PER M²
0
100
200
300
400
DAY NUMBER


M3
4000
2000
NUMBER PER M²
0
100
200
300
400
DAY NUMBER


M19
5000
3000
1000
NUMBER PER M²
0
100
200
300
400
DAY NUMBER

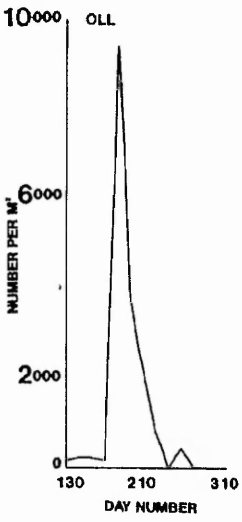

OLL
10000
6000
2000
0
NUMBER PER M²
130
210
310
DAY NUMBER

TABLE 4:4

Maximum Numbers of Class 1 Juveniles m^{-2}
and Length of Breeding season

Species	Site	Length of Season (Days)	Maximum No of Juveniles
G. pulex	M1	398	198
G. pulex	M3	398	2,950
G. tigrinus	M19	183	5000
G. tigrinus	OLL	140	9,500

potential number of juveniles in the population. The percentage survival could then be calculated from the resulting data.

Table 4:5 shows the resulting survival rates from egg to 1-2mg adult. These rates were very variable, although those for *G. pulex* were generally higher than those of *G. tigrinus* with means of 39.8% and 14.8% compared with 7.7% and 13.5% for *G. tigrinus*. The higher survival rate of *G. tigrinus* at site OLL was perhaps the result of a depleted population due to the mass mortality in June and the resulting immigration. The survival rates from juvenile to adult produced numbers of adults which were often higher or only slightly less than the numbers of juveniles, particularly in *G. pulex*. This was probably due both to inaccuracies in the estimation of population size and also to immigration of animals from areas of high population to areas with lower population size.

It is apparent from the table that, in both species, the survival from egg to juvenile was reduced when there were more eggs present. This might have been due both to increased mortality and emigration.

When the mean number of eggs per female was plotted against the number of surviving 1-2mg adults, there was a clear positive relationship, that is, the more eggs m^{-2} were produced, the more 1-2mg adults were recruited. However, when the ratio of number of 1-2mg adults m^{-2} to number of eggs m^{-2} was plotted against number of eggs m^{-2}, as in Figure 4:9, the effects of density on survival could be seen, as the relationship became negative, with a high number of eggs m^{-2} resulting in a low ratio of surviving adults to eggs. If the numbers of eggs m^{-2} produced had no effect on the percentage of adults recruited, the graphs would be straight positive diagonal

TABLE 4:5

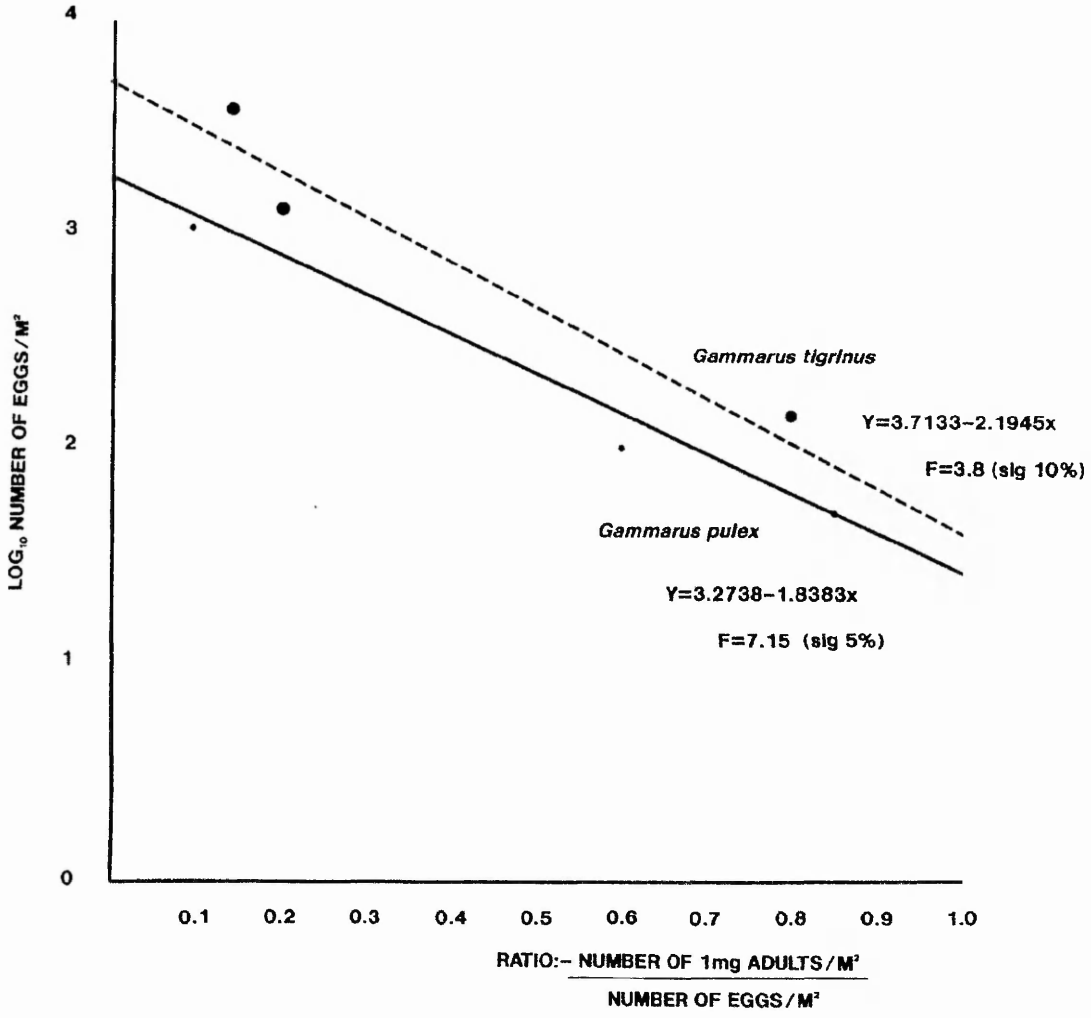
Survival Rates of *Gammarus pulex* and *Gammarus tigrinus* From Egg to 1-2mg Adult

	Number per m^2			%
Day	Eggs	Juveniles	Adults	Survival
Site M1 *Gammarus pulex*				
88	319	42	120	37.6
130	74	70	63	85
186	1702	140	8.8	0.5
228	237	87.5	44.4	19
Site M3 *Gammarus pulex*				
44	2213	260	207	9.4
102	1684	938	484	28.7
143	6274	1093	1100	17.5
186	6666	2878	629	9.4
340	1612	192	227	14
Site M19 *Gammarus tigrinus*				
44	4431	281	136	3.1
88	4431	281	322	7.3
130	7524	1350	626	8.3
156	11,944	4982	574	4.8
214	1644	135	264	16
Site OLL *Gammarus tigrinus*				
130	2692	237	53	2.0
172	7622	9400	102	1.3
214	1052	325	11	1.0

Each line represents one cohort's survival from eggs present on the day indicated.

FIGURE 4:9

Graph showing the regression lines of Log_{10} number of eggs per square metre against the ratio of number of 1mg adults per square metre to number of eggs per square metre for *Gammarus pulex* and *Gammarus tigrinus*.


4
3
2
1
0
LOG10 NUMBER OF EGGS/M²
Gammarus tigrinus
Y=3.7133-2.1945x
F=3.8 (sig 10%)
Gammarus pulex
Y=3.2738-1.8383x
F=7.15 (sig 5%)
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
RATIO:- NUMBER OF 1mg ADULTS/M²
NUMBER OF EGGS/M²

lines, as the ratio of recruited adults to eggs produced would be unchanged with initial egg density. The negative relationship was significant in both species, although the lines are not significantly different from each other.

4:2:2:8 *k*-factor Analysis of Mortality Data

Using the method described by Putman and Wratten (1984) (also described by Varley and Gradwell, 1960; Begon and Mortimer, 1981), the *k* factors for each species were calculated. The number of eggs present was calculated from the number of ovigerous females present per square metre in the population multiplied by the mean number of eggs carried by the ovigerous females. Numbers of juveniles and adults were estimated from the percentage present in the population at the relevant date, multiplied by the numbersm $^{-2}$ present in the population. Table 4:6 shows the results of the "*k*- factor analysis. The total *k* values for *G. pulex* were very variable, but at site M1 the average value was 0.66, while at site M3 the average was 0.84. *Gammarus tigrinus* at site M19 had average values of 1.06, but at site OLL, two of the three values were negative, showing the effects of immigration to that site following the drought.

TABLE 4:6

Log10 maximum Natality. Numbers of Eggs, Juveniles and Adults per square metre and k values For *Gammarus pulex*

SITE M1

Cohort	1	2	3	4
Log no of eggs	2.50	1.87	3.23	2.37
k_1	0.88	1.03	1.08	0.36
Log no juvs	1.62	0.84	2.15	2.01
k_2	-0.66	-0.96	0.27	0.65
Log no adults	2.28	1.8	1.88	1.36
Total k	0.22	0.07	1.35	1.01

SITE M3

Cohort	1	2	3	4
Log no of eggs	3.65	3.22	3.80	3.82
k_1	0.95	0.25	0.76	0.36
Log no juvs	2.70	2.97	3.04	3.46
k_2	0.08	0.29	0	0.66
Log no adults	2.62	2.68	3.04	2.80
Total k	1.03	0.54	0.76	1.02

TABLE 4:6 (Continued)

Log10 maximum Natality, Numbers of Eggs, Juveniles and Adults per square metre and *k* values For *Gammarus tigrinus*

SITE M19 *Gammarus tigrinus*

Cohort	1		2		3		4	
Log no of eggs	3.65		3.75		3.87		4.08	
k_1		1.2		1.66		0.74		0.38
Log no juvs	2.45		2.09		3.13		3.70	
k_2		0.32		-0.41		0.33		0
Log no adults	2.13		2.50		2.80		3.70	
Total *k*		1.52		1.25		1.07		0.38

SITE OLL

Cohort	1		2		3	
Log no of eggs	3.43		2.37		2.74	
k_1		1.06		-1.6		0.23
Log no juvs	2.37		3.97		2.51	
k_2		-0.37		0.95		-0.25
Log no adults	2.74		3.02		2.76	
Total *k*		0.69		-0.65		-0.02

The increase in *k*- values for *G. pulex* between sites M1 and M3 indicated the increasing *r*- selection on this species at a less stable site, while the further increase in *G. tigrinus* indicates that this species is more *r*-selected than *G. pulex*.

4:3 Laboratory Studies on the Survival and Growth of *Gammarus pulex* and *Gammarus tigrinus*

4:3:1 Introduction

The experiments described in this section were designed to complement the field studies by providing information on the survival and growth of the two species in salinities at which they were not found in the field. Laboratory studies on the growth of *G. tigrinus* and *G. pulex* were begun on several occasions during this study, but rarely produced statistically significant results owing to the poor survival rates of *G. tigrinus*. Thsi species is noted to be a 'fragile' laboratory organism by both Dorgelo (1974) and Savage (1982), who found that handling, particularly of juveniles and animals near to moulting, caused either death or a reduction in growth rate. This was found to be the case in the laboratory with *G. tigrinus* during the present study. However, some animals were raised to maturity using methods similar to

those of Savage (1982), where the animals were kept in shallow trays of unaerated water. The *G. tigrinus* in the experiments of Savage were kept at room temperature, but this was considered impractical for the present study, as at room temperature survival of *G. pulex* was very poor, even with aeration. In addition, room temperature fluctuated greatly, especially over weekends when the laboratory building was unheated. A constant temperature room at 10^0C (+ or - 1^0C) was therefore used in all experiments, although survival of *G. tigrinus* was lower under these conditions than at room temperature.

4:3:2 Methods

All experiments were housed in a constant temperature room at 10^0C (+/- 1^0C). The lighting in the constant temperature room provided 12 hours light and 12 hours dark each day.

All solutions for experiments were prepared using carbon-filtered tap water, since this was less toxic to *Gammarus* than non filtered tap water. An analysis of the composition of this water is supplied in Appendix H. Artificial sea water was prepared using sea-salt supplied by Griffin and George, made up to the manufacturers specification. Since an analysis of the product was not available from the manufacturer, the Na^+, Cl^-, Ca^{2+} and K^+ concentrations of each set of solutions was tested before use, in addition to pH. The mean concentration of each measured ion is shown in Table 4:7. The sodium and chloride concentrations in these solutions was lower than those given for standard sea water (Nicol, 1967), but compared more closely with mine drainage water. All NaCl solutions were made up using Analar sodium chloride.

TABLE 4:7

Concentration of some ions (in mM) in
Griffin and George sea salt
Dissolved in filtered tap water

Ion	Concentration (mM)
Na^+	380
Cl^-	465
Ca^{2+}	10
K^+	10

There were three separate sets of experiments, to study the success of egg hatching in increasing salinities, time taken to reach maturity at two different temperatures, and growth rates in two salinities.

Firstly, the hatching of eggs of *G. tigrinus* and *G. pulex* in varying concentrations of sodium chloride and seawater solutions was tested. Eggs used in the experiment were removed from ovigerous females by gently opening the brood pouch with a needle and flushing out the eggs with solution. This was done as quickly as possible and the eggs examined to determine their stage of development. Only eggs containing embryos with a developed eye were used as it was known from previous experiments that eggs would not continue to develop when removed from the female if they were not already advanced to this stage.

The eggs were placed in the wells of sterile tissue-culture containers (each well was 5mm deep and contained 2ml of solution) with 5 or 10 eggs to a compartment, and incubated at 10^0C in the dark. The containers were examined daily and any hatched juveniles and debris removed to avoid contamination.

An egg was considered to have 'hatched' when the juvenile *Gammarus* was uncurled and able to swim and feed.

There were three replicates of each solution, and the experiment was terminated after one month, as it had been found previously that any eggs which did not hatch within one month were no longer viable.

Secondly, 50 juveniles of *G. pulex* and 50 juveniles of *G. tigrinus* (taken from isolated ovigerous females) were placed in separate 2l plastic beakers containing washed river sediment and 500ml of carbon-filtered \ tap water. The animals were left at the appropriate temperature (10^0C and 20^0C in two constant temperature rooms) with as

little disturbance as possible. They were fed with small amounts of trout-fry pellets, provided with leached sycamore leaves, and carefully examined every month. The experimental period was between April and September.

Lastly, two hundred *G. pulex* and 200 *G. tigrinus* of less than 3mm length were placed in separate 5l tanks containing sand and leached sycamore leaves, and 2.5l of a solution made up of carbon-filtered tap water and ANALAR sodium chloride where appropriate. One tank per species contained no additional salt, while the other contained sufficient NaCl to make the solution up to 28mM. The solutions were changed every month. The tanks were maintained at 10^0C in a constant temperature room, and aerated.

The animals were all initially weighed wet on the Cahn micro-balance, with as little handling as possible, to obtain an mean starting weight.

The animals were placed in the tanks in October, and 20 individuals (if available) were removed for weighing in November, December, January, February and April.

4:3:3 Results

As can be seen from Table 4:8, the hatching rate of *Gammarus pulex* eggs was highest in the 1mM concentration, when up to 66% of the eggs successfully hatched. In NaCl solutions, hatching rate declined (from 66% in tap water) as the salinity increased, until at 56mM the hatching rate was 36%, similar to the rate in the 20% seawater dilution (76mM). In increasing strength sea water, hatching rate declined from the control level, until at 80% sea water none of the eggs hatched successfully. The levels of sodium and chloride were almost the same in

TABLE 4:8

Percentage hatching success of isolated *Gammarus pulex* and *Gammarus tigrinus* eggs in sea water dilutions and NaCl solutions

	%Sea-water					
Species	0	20	40	60	80	100
Na^+ (mM)	1	76	152	228	304	380
G. pulex	55	4	25	15	0	0
SE	3	16	1	1	-	-
G. tigrinus	60	70	65	60	60	50
SE	0	25	12	19	28	20

	mM NaCl			
Species	1	14	28	56
G. pulex	66	40	46	36
SE	15	17	6	6
G. tigrinus	40	33	66	66
SE	23	30	20	19

these two solutions, and the salt concentration in NaCl solution and sea water produced the same reduction in hatching success in this species.

In *G. tigrinus* the best hatching success was found in 20% sea water and in sodium chloride solutions of 28mM and above. However, the hatching rate of *G. tigrinus* did not decline in higher seawater concentrations and was less affected by either sea water or NaCl than *G. pulex*.

Table 4:9 shows the percentage survival of both species and the number of days taken to reach maturity at both temperature. Over the 25-32 days which *G. tigrinus* took to reach maturity there was a good survival rate of this species. *Gammarus pulex* took 4 times longer to reach maturity, and the survival rates in this species were correspondingly lower. All animals of a species were found to be mature on the same examination day, and all the animals which survived, matured.

In the growth rate experiment, 61 of the original 200 *G. pulex* were still alive in April in the control tank. Of these, 27 were mature males and 9 ovigerous females, with the others still immature. The *G. tigrinus* in these conditions were all dead by December, although some growth was obtained between October and November.

In the 28mM tanks, all the *G. pulex* were dead by January, but a few *G. tigrinus* were still alive in February and April. The growth rates are shown in Figure 4:9, together with the fitted regression lines for growth rates of *G. pulex* in 1mM, and *G. tigrinus* in 28mM. Both these lines were significant at the 1% level, with *G. pulex* showing a much higher rate of growth than *G. tigrinus*.

TABLE 4:9

Percentage survival and number of days to reach maturity of 50 juvenile *Gammarus pulex* and *Gammarus tigrinus* at two temperatures

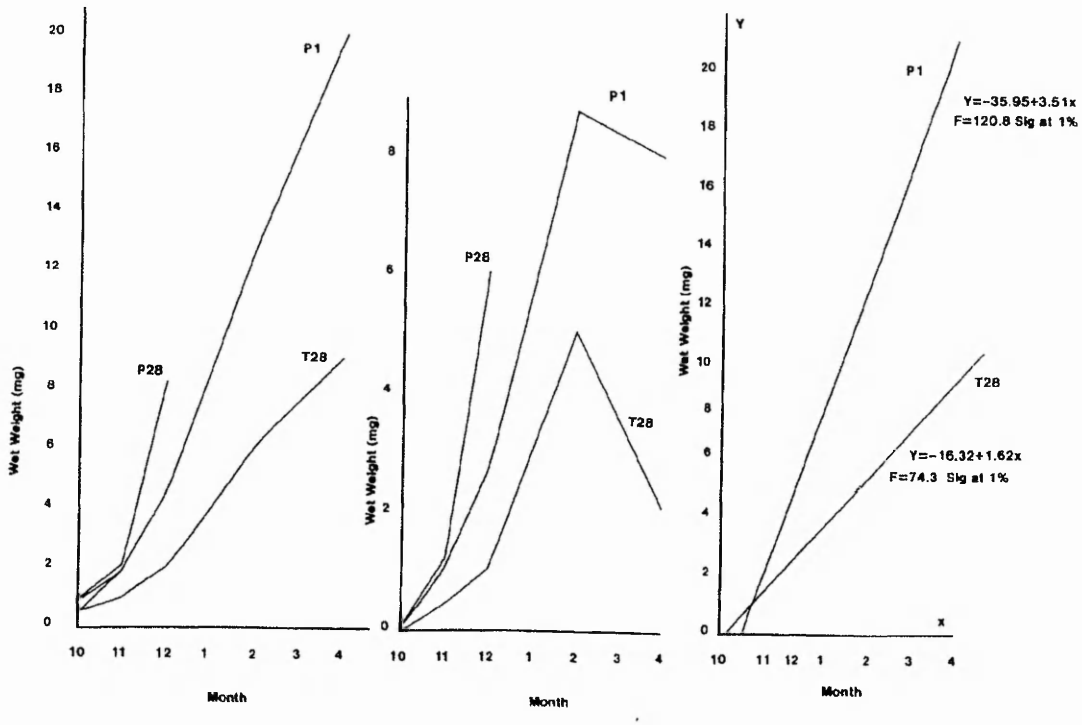
		Gammarus pulex		*Gammarus tigrinus*	
Temp °C	No	Days to maturity	%survive	Days to maturity	% survive
10	50	135	28	32	70
20	50		0	25	50

FIGURE 4:10

Three graphs relating wet weight (mg) of *Gammrus pulex* and *Gammarus tigrinus* to time in months in two salinities 1mM and 28mM NaCl.

Month is referred to by number (10 = October, 11 = November etc)

P1 = *G. pulex* growing in 1mM NaCl
P28 = *G. pulex* growing in 28mM NaCl
T28 = *G. tigrinus* growing in 28mM NaCl


Wet Weight (mg)
P1
P28
T28
Month
Y
X
Y=-35.95+3.51x
F=120.8 Sig at 1%
Y=-16.32+1.62x
F=74.3 Sig at 1%

4:4 Discussion

The life-histories of *G. pulex* and *G. tigrinus* as investigated in the field show clear differences which are summarised in the Table 4:10.

The results for *G. tigrinus* suggest a species which prefers more saline water and higher summer temperatures than *G. pulex*, and which also has a high intrinsic rate of increase. The results of the *r*-and *K*- selection were clear only in that they indicated that *G. tigrinus* was a more *r*- selected species than *G. pulex*, with higher egg and juvenile production, a higher mortality of juveniles and maturity at a smaller size than *G. pulex*. This would give *G. tigrinus* an advantage at sites where there were repeated pollution incidents, particularly in the summer, when it has the ability to reproduce and re-colonise very quickly, as was seen at site OLL after the population was wiped out in early summer. *Gammarus pulex*, however, appears to be more *r*-selected at site M3 than at site M1, where the population size is small, and there are comparatively fewer young, of which larger numbers survive.

Site M1 is exceptionally stable in flow rate and chemistry, being spring-fed, and this environmental stability leads to a reduction in the *r*-selection pressure on *G. pulex* at this site. Site M3 is a larger site and suffers frequent spates in the winter, coupled with some organic discharges from Water Reclamation Works and chemical factories upstream at Creswell. This reduction in stability produced a more *r*-selected population, which had a size and structure similar to that of *G. tigrinus* at site M19. Calow (1981) described differences in the growth and reproduction in the

TABLE 4:10

Comparison of Some Life-Cycle Characteristics of *G. pulex* and *G. tigrinus* in Millwood Brook

	Gammarus pulex	*Gammarus tigrinus*
Breeding season	All year	April-October
Brood size	up to 34	up to 95
Mean numbersm^{-2}	68-7836	652-11060
Mean egg development time (days)	22-25	20-32
Mean development time (days)	84-115	28-53
Chloride distribution (mM)	0.8-1.1	20-100
Max summer Temp(^{0}C)	16	22.5

gastropod *Lymnaea peregra* from exposed and sheltered habitats, which resulted in a more *r*- selected population in the exposed habitats, and a more *K*- selected population in the sheltered habitats.

It was, however, notable how well "synchronised" the onset of breeding and generation times were in both species. It might be expected that the onset of maturity in spring would occur at the same time in populations of *G. tigrinus*, if this were triggered by environmental cues, but later in the season, when generation time was rapid, animals still appeared to be breeding at specific times and these showed clearly in the data.

The winter population size for both species is similar, between 3000 and 1000 m^{-2}, which is comparable with the figure of 481 in *G. tigrinus* found by Chambers (1977) in Holland, and the 820 *G. pulex* per square metre found by Welton (1979) in Dorset chalk streams. The 10 fold increase in summer which he describes, is reflected at both sites M1 and M3. At site M3, a greater increase is found, and at sites M19 and OLL *G. tigrinus* reaches very high summer population densities.

Laboratory experiments support the development times derived from field data, with time to maturity at 10^0C of 135 days for *G. pulex* and 32 days for *G. tigrinus*. At 20^0C the survival rate of *G. pulex* was too poor for development time to be estimated, but that of *G. tigrinus* was 25 days.

The eggs of *G. tigrinus* hatched well in saline water up to full sea water, while those of *G. pulex* did not hatch above 80% sea water. In low salt concentrations, the hatching rate of *G. tigrinus* did not appear to be as high as in moderate salt concentrations, although the results were variable, with 60% hatching in one set of 1mM

solutions, and 40% in another.

The growth rate experiments, conducted at 10°C, showed again that *G. pulex* grows to maturity in around 140 days at this temperature. The survival and growth rate of *G. pulex* was lower in 28mM than in 1.4mM NaCl. In *G. tigrinus*, however, the animals which survived handling grew faster in 28mM and also survived longer at this concentration. *G. tigrinus* appeared to have a slower growth rate than *G. pulex* at both concentrations, but it is difficult to determine whether this was a genuine rate at this temperature, or a reaction due to handling, which in *G. tigrinus* is known to reduce growth rates (Dorgelo, 1974; Savage, 1982).

Gammarus tigrinus was originally a native of the Eastern sea-coast of Northern America. In these areas, it inhabits waters described as brackish or "oligohaline", that is, waters with a chloride concentration of between 10 and 100mM, although in common with many estuarine animals, it is able to withstand full sea water (Bousefield, 1958). In the southern part of its range, in Florida, it is also exposed to summer temperatures of up to 30°C, although in the Northern areas the summer temperatures average 20°C. Ginn *et al.* (1976), reported no reduction in the fecundity of *G. tigrinus* when adults were exposed to temperatures of 26.5°C. Above this temperature there was some reduction in fecundity, although juveniles were still able to survive up to 35°C. In countries where *G. tigrinus* has been introduced, such as England, Holland and Germany, this species would therefore be expected to inhabit areas of similar salinity and temperature range to its native habitat and sites M19 and OLL appear to fall within this range. In the area of the Mersey and Weaver River Authority, in

TABLE 4:11

Comparison of Published figures of development rates in *Gammarus pulex* and *Gammarus tigrinus* with those collected in this study

Gammarus pulex

Author	Temp (°C) °C	Brood time Days	Time to mature Days
Hynes (1955)	15-20		120
Nilsson (1977)	5	65	
	10	36	
Welton (1979)	10	36.5	-
	15	23.9	87.5
Sutcliffe(1981)	21		73
	15		100-120
present study	10-11	26-28	100
	13	14	112-127
	10	30	96
	14-16	13	84
	14	14	84

TABLE 4:11 (Continued)

Comparison of Published figures of development rates in *Gammarus pulex* and *Gammarus tigrinus* with those collected in this study

Gammarus tigrinus

Chambers (1977)	12		35
	14	15.3	33
	16	14	31
	18	9.6	30
	25	9.1	29
Pinkster *et al.* (1977)	10		40-42
	15		32-34
	20		27-29
Brandse de Jong (1978)	15		27-29
present study	8-13	15	54
	16-22	26	44
	9-18	14	42
	11-12	16	54
	16-22	14-26	18-22

Cheshire and Lancashire, *G. tigrinus* was found by Holland (1976) breeding in flashes (small ponds), rivers and estuaries at chloride concentrations up to 100mM, although he states that it was favoured by chloride concentrations in the lower part of the range. Although Holland does not give temperature ranges, the saline flashes from this area where *G. tigrinus* was also described by Savage (1982) have summer temperatures of around 24^{0}C.

In the Netherlands *G. tigrinus* was described as living in oligohaline water with summer temperatures of 20^{0}C by Pinkster *et al.* (1977). These authors also noted a preference for still waters.

In contrast, *G. pulex* was described by authors such as Hynes (1955) as living in waters with a chloride concentration of less than 1mM, and with summer temperatures of between 15-20^{0}C. Sutcliffe (1981), found that juveniles of *G. pulex* did not survive at temperatures above 25^{0}C, and that maximum growth was obtained between 15-20^{0}C.

Previous studies on the life histories of *G. pulex* and *G. tigrinus* provide details of brood time and development time for comparison with the present study. Some of these are summarised in the Table 4:11. The results from the present study can be seen to be in accordance with the findings of other authors, although the development time for *G. pulex* from site M3 appears to be shorter than expected. Both species appear capable of producing five cohorts of juveniles in a year, which were released between April and September in *G. tigrinus* and March and November in *G. pulex*.

Gammarus tigrinus is described from the Netherlands by Pinkser *et al.* (1977) in a period of rapid range

extension. It was first recorded from Holland in 1964 in the Northern part of the Ijselmeer (formerly the Zuiderzee), and in 1965 was found to be the most common amphipod in almost every part of the lake and invading adjacent inland waters. The species initially increased its range at a rapid rate, but by 1971 the rate of spread slowed down and the species disappeared from some localities. By 1973 it appeared to have stopped expanding its range and was living in an area bounded by running freshwaters in the East and South and saline waters at the coast. *Gammarus tigrinus* originally occured at sites together with the three endemic species, *G. pulex*, *G. zaddachi* and *G. duebeni*, but by 1974, these endemic species had disappeared from the areas invaded by *G. tigrinus*.

In England, *G. tigrinus* is still expanding its range. It was recorded by Holland (1976) in the area of the River Weaver in Lancashire and Cheshire, where it occured together with *G. duebeni* and *G. zaddachi*, and was reported by Savage (1981) to have been introduced into the Cheshire Meres in 1974, where it rapidly increased in numbers at the expense of *G. duebeni*, although when these ponds were sampled by myself in 1987, there were *G. duebeni* present together with *G. tigrinus*.

In Germany, *G. tigrinus* was deliberately introduced into several rivers such as the Weser (Ruoff, 1968) to replace the native species which had been eliminated due to pollution, and to provide food for fish. This introduction was so successful that the large numbers of *G. tigrinus* present in the river were at one time able to engulf and consume small fish which were trapped or injured.

Gammarus tigrinus is generally found in the field at

saline sites, but in the laboratory it is able to breed and grow at low salinities (approximately 1.4mM) and should be able to survive in river sites at these chloride levels. It does, however, appear to favour sites which are close to lakes or ponds, and which often have silty sediments, although it is found in running-water sites. Both the sites in this study, M19 and OLL, are below quite large lakes, althought the flow at the sites is fast and the substrate pebbly. Populations of *G. tigrinus* have been found in Rufford Park Lake which is directly upstream of site OLL. It was the draining of this lake, due to mining subsidence, which caused the site to dry out in June.

It is also notable that the sites at which *G. tigrinus* was found often have very little aquatic vegetation, due to increased salinity, or have only *Enteromorpha* spp. present. There are, however, often large populations of oligochaetes, both Tubificidae and Enchytraeidae, and often chironomids such as *Chironomus* spp. *Gammarus tigrinus* has been observed in the laboratory to be an active feeder on small invertebrates such as worms and chironomids, a habit which has also been noted by Savage (1981) and Ruoff (1968). In the laboratory, groups of *G. tigrinus* have been observed by the author holding parts of worms and chironomids in their gnathopods and breaking the prey into parts to consume the soft inner tissue. *Gammarus tigrinus* appears also to "attack" other animals which attempt to feed on the same prey by "kicking" out with their urosome while holding onto the prey with their front legs. This feeding behaviour is shown by males, females and mated pairs of any size, but the larger animals are more successful in capturing prey. *Gammarus pulex* will feed on live prey, but only after a prolonged

period of starvation, while *G. tigrinus* will attack small invertebrates even when other food is available. It is possible, therefore, that *G. tigrinus* has a food source readily available at saline sites where vegetation is absent that is unavailable to *G. pulex*.

The short but prolific breeding season of *G. tigrinus* may also help this species to survive pollution episodes which are often most severe in summer when rivers are low. The rapid recovery of the population at site OLL after destruction in June demonstrates the potential which *G. tigrinus* has for increase. Of the large numbers of juveniles produced, however, very few are left after the winter. It is impossible to determine from this type of study what happens to these juveniles, and whether the lack of recruitment is due to mortality from disease, starvation, predation, or to emigration. The "survival" rates calculated in this chapter are therefore not survival rates in the exact meaning of the term, but can be used as an indication of the recruitment into the adult population of juveniles from a particular cohort.

The sites at which *G. tigrinus* occurs have higher summer temperatures than those at which *G. pulex* is found. It is not possible in this case to separate the effects of salinity and temperature, as all the sites which have the higher levels of salinity also have higher summer temperatures. Several workers (eg Steele and Steele, 1973) have, however, suggested that higher temperatures favour the rapid breeding of *G. tigrinus*. In the laboratory, this species survives well at room temperatures without airation, while *G. pulex* is quickly killed at 20^0C if aeration is not supplied.

From these results there seems to be no reason to suspect that *G. tigrinus* will not continue to increase its range

in the East Midlands. Its spread in England appears to be slow compared to the rapid rate of spread on the Continent, which is perhaps a result of raised salinity being found in rivers rather than lakes, which do not in other areas appear to be the favoured habitat for *G. tigrinus*. It is possible, however, that *G. pulex* may find a refuge in the colder and faster-flowing waters in the upland areas of Britain, or near to stream sources, as is the case in the East Midlands.

In Millwood Brook, *G. tigrinus* is gradually moving upsream, and would appear to have no real check in its progress apart from the area of very high salinity and temperature around the mine outfall. *Gammarus tigrinus* can, however, withstand full sea water, and could perhaps overcome even this barrier. It would then be in a saline pond area, where it would be in direct competition with *G. pulex*.

CHAPTER 5

Laboratory studies on the Reproductive Biology of the oligochaetes *Tubifex tubifex* and *Lumbricillus rivalis*

5:1 Introduction

One of the most striking changes in the fauna of Millwood Brook at the saline-polluted sites examined in Chapter 2 was the elimination of Tubificidae and their replacement by the enchytraeid *Lumbricillus rivalis*. Laboratory studies were therefore devised to study the effects of salinity on the survival and reproduction of two species of worm, one tubificid and one enchytraeid, to seek to explain the field observations.

Tubifex tubifex was selected as a representative tubificid for these experiments as it is a common species which was found at most of those sites on Millwood Brook which supported Tubificidae. Also, it is a well-researched species with a considerable literature, (eg Timm, 1967; Poddubnaya, 1973 and 1984; Adreani *et al.*, 1984 and Lazim and Learner, 1986). In contrast, there are few published studies on the life-history of Enchytraeidae in rivers, although Bird (1982) studied the clean-water species *Propappus volki* in Dorset chalk streams and Learner (1972) studied the life-history of *L. rivalis*, among others, in sewage percolating filters. The shore-line distribution in relation to salinity of several species of *Lumbricillus* (but not including *L. rivalis*) has been studied by Tynen (1969), and Reynoldson (1943) described the life-cycle of *L. lineatus*, which is

probably a synonym of *L. rivalis* (Tynen, 1966).

The reproductive cycle of both oligochaete families is complex and probably varies both between sites and between years at the same site, (Kennedy, 1966; Brinkhurst and Jamieson, 1971). However, both *T. tubifex* and *L. rivalis* have similar reproductive systems. Oligochaete worms are hermaphrodite, with 1 or 2 pairs of testes associated with male funnels and vasa deferentia, with pores situated ventro-laterally (on segment 11 in *T. tubifex*). Posterior to the male system are a pair of ovaries and small, ventral female funnels. The female openings are usually on the segment immediately posterior to the male pores, or very close. In both groups, there are ectodermal pouches (spermathecae), which store sperm after copulation, and the openings to these are found in the segment preceding the male pore. In *L. rivalis* the spermathecae are large and are used as a diagnostic identification feature for this species (Nielsen and Christensen, 1959).

Around the region of the sexual pores is a region of thickened, glandular body wall, known as the clitellum, which produces the cocoons in which eggs are laid. Sexual reproduction is usual in both Tubificidae and Enchytraeidae, but both groups can lay parthenogenetic eggs (observed in Tubificidae by Poddubnaya, 1984). There are some Enchytraeidae (eg *Lumbricillus lineatus*) which have polyploid forms which, although they mate with diploid forms, require only the presence of the diploid sperm for eggs to develop (Brinkhurst and Jamieson, 1971).

The eggs, laid in a cocoon which is either left loose in the sediment or attached to a solid object, have a large yolk sac which supports the development of the young worm

until it is fully formed. Small worms can be seen moving in the cocoon for several days before they emerge and begin to feed.

The number of cocoons laid by worms and the number of breeding cycles through which they pass are highly variable. *Tubifex tubifex* has the ability to re-absorb its sexual organs once it has used up its store of eggs and sperm, to become an "immature" worm which can later grow a new set of reproductive organs and mate again. It is not known how many times worms can repeat this cycle of regression and maturation, or whether *L. rivalis* has this ability.

For this reason, Timm (1967) suggests a four-stage description of stages in the lives of worms, and stresses that a stage cannot be equated with the age of a worm. This system has been modified for the present study, and each stage named as follows:

1:"Juvenile 1" - an individual with no reproductive organs

2:"Juvenile 2" - an individual with reproductive organs beginning to develop

3:"Mature 1" - an individual with a well developed genital system but no clitellum

4:-"Mature 2" - an individual with a developed reproductive system and a clitellum

For these experiments, the "Juvenile 1 and 2" stages have been combined to produce one group (Juveniles) and the "Adult 1 and 2" stages to a second group (Adults). This grouping was necessary as it was difficult without very close examination of each worm to detect when the reproductive organs were beginning to develop, but easy

to see the white masses of eggs and sperm in the reproductive tract before clitellum formation. The Juvenile stage could, therefore, include both small, newly hatched individuals, and larger ones becoming mature or regressing from maturity, while the Mature stage could contain worms which were reproducing for a second or third time.

5:2 Methods

5:2:1 Culture Methods

Tubifex tubifex was cultured in the laboratory using a development of the technique described by Aston (1984). In this method, worms are maintained in a cellulose substrate on which bacteria and other micro-organisms can grow. The worms feed either directly on the micro-organisms themselves or on their products. In these experiments the cellulose substrate was prepared by pulping ashless filter papers, wetted with the required solution, in a mechanical grinder. The cellulose was mixed with sterilised fine sand, and water levels were maintained 1 to 2cm above the level of the cellulose. All cultures were aerated. It was found that each culture needed to begin either with adult worms with an established gut fauna, or with a "starter" portion of cellulose from an established culture which already had microbial colonies. Cultures which were begun using only cocoons had very slow hatching and growth rates, and the cellulose did not become discoloured as in rapidly growing and breeding cultures. Since a regular supply of

nitrate and other nutrients was required for rapid bacterial growth, trout-fry pellets were added each month.

To initiate a breeding stock culture of *Tubifex tubifex*, worms from site M4 on Millwood Brook were placed in a 10*l* aquarium with large amounts of cellulose substrate, a bottom layer of sterilised aquarium sand and river water from site M3, and maintained in low light in a 10^0C constant temperature room. The worms were obtained in January, and were examined under a binocular microscope to select mature *T. tubifex* for the culture. *T. tubifex* worms were recognised under the binocular microscope by their hair chaetae, as they were the only oligochaete species at this site to possess such chaetae. A proportion of the selected worms was killed and examined under a compound microscope to confirm identification (Brinkhurst, 1971). Every two weeks for two months, the culture was examined and all cocoons inspected to ensure that they were those of *T. tubifex*. In addition, five juveniles and one adult were removed, killed and identified, to ensure that the culture contained only *T. tubifex*. The population of worms quickly became established and after the first month, no cocoons or worms of species other than *T. tubifex* were found. Cocoons were laid freely in the cellulose substrate, and the worms were found both in the cellulose and in the bottom layer of sand. The stock culture was maintained as a source of *T. tubifex* worms for the next two years.

Lumbricillus rivalis did not establish a satisfactory breeding colony under the conditions used for *T. tubifex*. These worms have been cultured in the laboratory in autoclaved horse manure (Kirk, 1971) and activated sewage sludge (Learner, 1972), but neither media was readily

available for use in experiments. It was decided to mimic the conditions in Millwood Brook where the worms were found, and to do this filaments of *Enteromorpha* spp. were collected from site M8, and placed in a 10l aquarium tank with a 2cm layer of sterilised fine sand on the bottom. The culture solution was tap water with NaCl added to raise salinity to levels at which the worms were collected in the field. Worms were obtained from Millwood Brook at site M9 and mature *L. rivalis* added to the culture, which was maintained at 10^0C in low light, with aeration. Under these conditions, the worms established a successful breeding colony and many cocoons were laid, either stuck to the walls of the aquarium or within the fronds of *Enteromorpha*. The culture was supplied with *Enteromorpha* every month, which was deep-frozen and thawed before use, to ensure that no new worms were introduced into the colony.

Worms from both of these cultures were used in all the following experiments, which were carried out in low light at 10^0C, with aeration. The conductivity and pH of all solutions was checked before they were added to the experimental cultures, to ensure that they were the same as the original solutions. All solutions used were between pH 7 and pH 8.

5:2:2 Methods to Determine the Survival of *Tubifex tubifex* and *Lumbricillus rivalis* in the Laboratory

Plastic containers (80mm x 80mm) with closely-fitting lids were prepared for culture of both *T. tubifex* and *L. rivalis* using the methods described above, with increasing amounts of ANALAR NaCl added to filtered tap

water to the required concentrations. Thirty small juvenile worms from the stock culture were added to each container, and the cultures aerated using porous air tubing which was introduced through a small hole in the lid. Every week, the worms were counted and the date on which the first worms matured recorded. Worms were considered mature when the white egg masses could be seen around segment 10, whether or not a clitellum was present.

The NaCl concentrations (in mM) used for each species were:

T. tubifex - 1 28 56 85 113 141 (equivalent to 30% sea water)

L. rivalis - 1 56 141 226 280 (equivalent to 80% sea water)

To count the *T. tubifex* worms, a petri dish was marked into segments, and a small portion of substrate poured onto it. Each section was searched carefully under a binocular microscope for worms, and any which were found placed in fresh solution at 10^0C. Fine watchmakers forceps were used at all times to handle the worms so that the delicate juveniles were not damaged. The searched substrate was quickly filtered through a 150micron sieve and returned to the experimental container. All the cellulose and sand from the culture vessel was searched in this way. After counting, all the worms were returned to the culure vessel and fresh substrate added.

To count *L. rivalis*, some of the *Enteromorpha* was poured into the marked petri dish, and the worms counted and removed as before. Care was taken to search inside the

filaments of *Enteromorpha*, as there were often groups of worms and cocoons inside, and also the underside of the container lid, to which large numbers of cocoons adhered. The fragments of *Enteromorpha* were filtered through the fine mesh sieve and returned to the culture pot. The sand was carefully searched for worms, rinsed and replaced. Thawed *Enteromorpha* was added to the culture after each count to provide food and substrate for the worms.
The experiment was terminated after 18 weeks.

5:2:3 Effects of Salinity on Cocoon Laying in Mature *Tubifex tubifex*

Thirty mature T. tubifex from the stock culture were placed in each of six 8cm x 8cm containers with cellulose substrate and solutions containing NaCl concentrations of 1, 28, 85, 113 and 141mM. The cultures were incubated in standard conditions and examined each week for five weeks. The number of adults, juveniles and cocoons was recorded, and also the number of embryos in each cocoon. The length of time taken for the first cocoons to hatch was calculated.

5:2:4 Numbers of Eggs per Cocoon Produced by *Tubifex tubifex* and *Lumbricillus rivalis* in Increasing Salinity

All the cocoons laid in experiment 1 for *L. rivalis* and experiment 2 for *T. tubifex* were removed and placed in petri dishes in the appropriate salt solution and labelled with the date on which they were removed. They were maintained without aeration in low light at 10^0C, and inspected daily. The date on which young worms

emerged was noted and also the number.

5:2:5 Reproductive success of *Tubifex tubifex* in Increasing Salinity

Two plastic containers were prepared as in the first experiment with sand, cellulose substrate and river water (1.1mM Cl^-) from site M3. One container had sufficient ANALAR sodium chloride added to the river water to produce a salinity concentration of 28mM, the other having no additional NaCl. The concentrations of NaCl were chosen using the results from the previous experiment, and were those at which *T. tubifex* was shown to produce fertile cocoons. There was 200ml of solution in each container.

Approximately thirty small juvenile worms from the tubificid stock culture were placed in each pot, and the containers incubated.

Every two weeks for one year, then every four weeks for a second year, the worms in each culture were counted as previously described, and the numbers of mature worms, juveniles and cocoons recorded. Fresh solution was added to each pot when the worms were counted, made up for the first six months using river-water from site M3, and subsequently using filtered tap water when this more constant medium became available. Every other month, additional substrate was added to maintain original levels, and once the solution was changed to tap water, one trout-fry pellet was also added every other month.

After 750 days, the cultures were terminated and all the worms removed for counting. Approximately 100 from each container were killed in methanol and allowed to air-dry before weighing on the micro-balance.

5:2:6 Reproductive success of *Lumbricillus rivalis* in Increasing Salinity

In 20mm x 20mm plastic tissue-culture containers, cultures of *L. rivalis* were established using frozen *Enteromorpha* spp. as described above. In this experiment, adult *L. rivalis* were used to initiate the cultures, as previous tests with juveniles had found that they did not easily establish breeding cultures.

Twenty adult worms were placed in each container at 0, 56, 141 and 226mM NaCl, and there were two replicates of each concentration. The worms were counted every month as in the *T. tubifex* experiments, great care being taken in handling the small *L. rivalis* juveniles. To avoid the chance of missing cocoons inside the strands of *Enteromorpha*, the filaments were not discarded after each month, but a new portion added to each pot. The original sand was also retained, due to the habit of small *L. rivalis* of burrowing into the sand and becoming coated with small sand grains, which often made them difficult to observe. The experiment was ended after one year.

5:3 Results

5:3:1 Culture Methods

The culture methods used allowed thriving populations of worms to become established and the stock cultures were used to provide worms for all laboratory experiments.

FIGURE 5:1

Survival times of adult *Tubifex tubifex* in cultures of increasing salinity

Thirty adult worms were added to the cultures at the beginning of the experiment, and graph points represent the numbers still alive at each count.

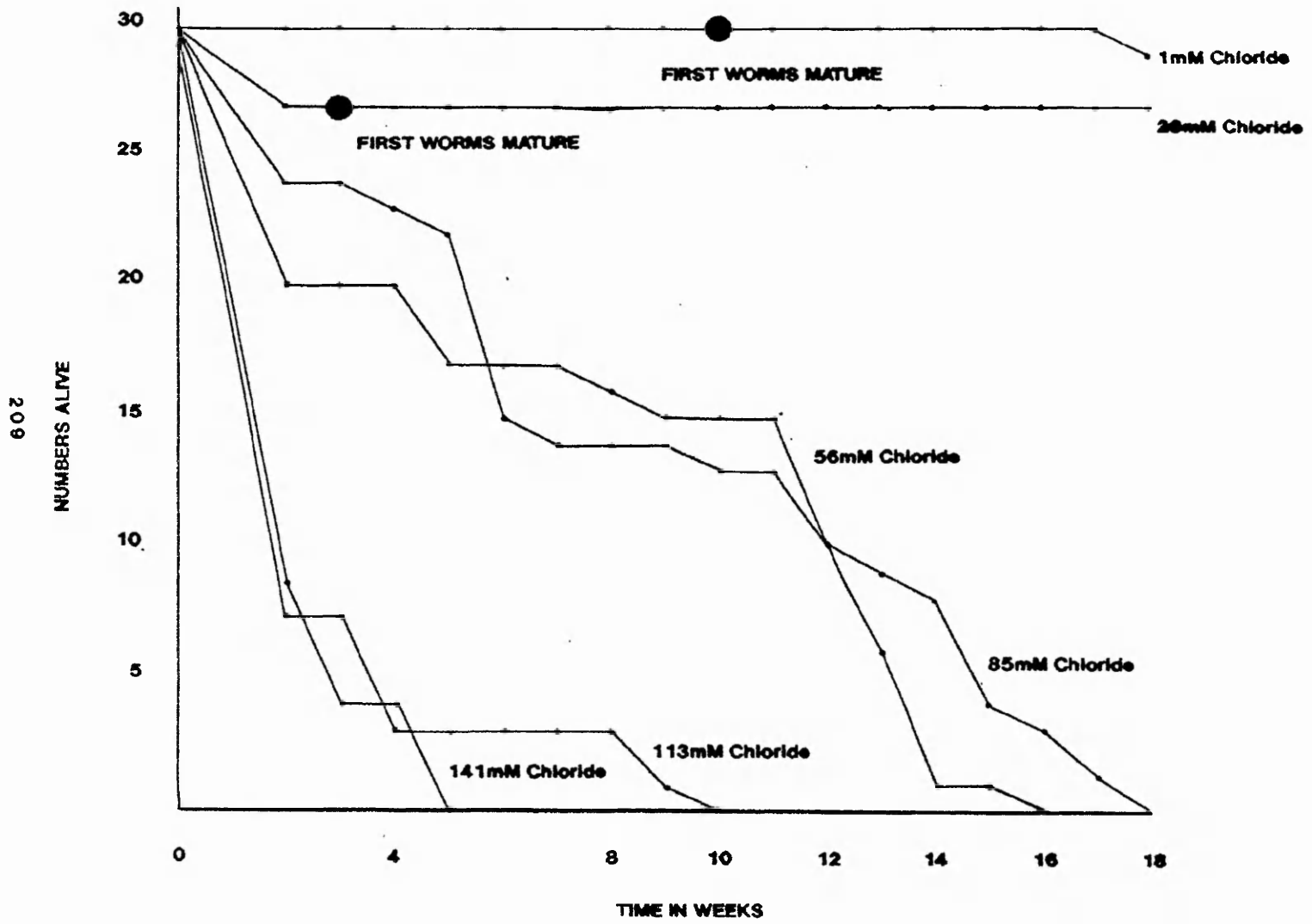
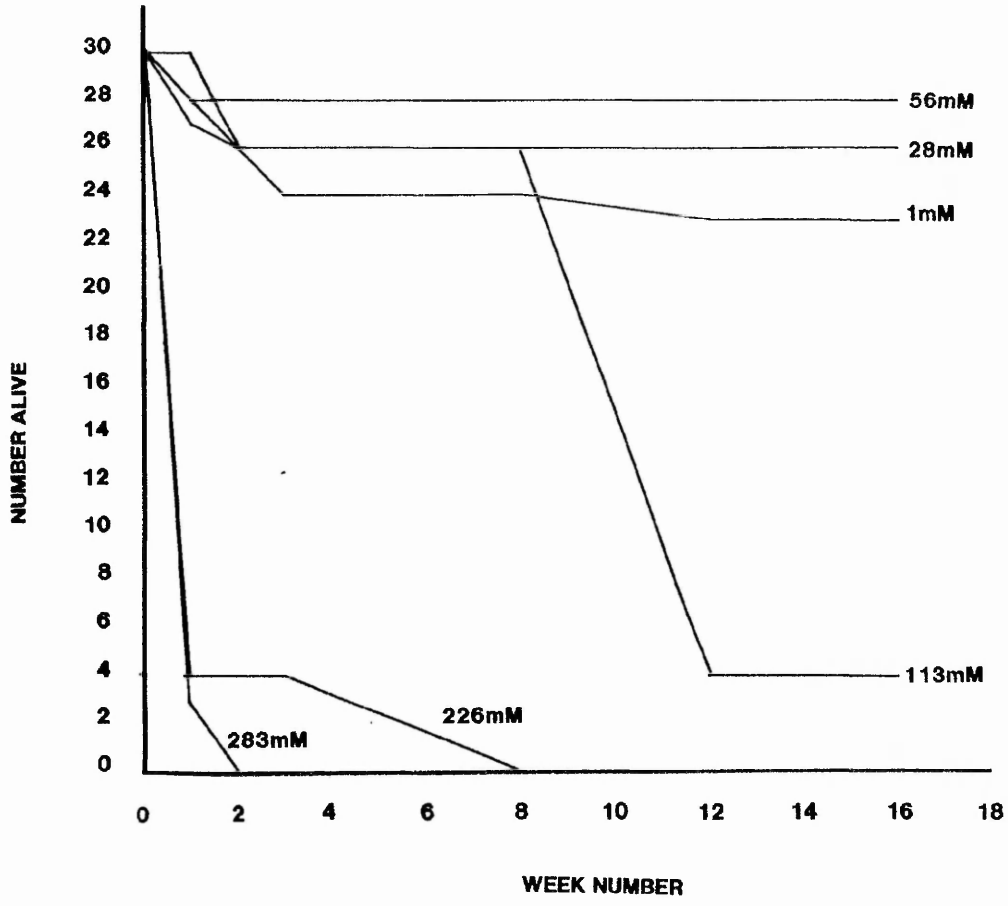

30
25
20
15
10
5
0
4
8
10
12
14
16
18
NUMBERS ALIVE
TIME IN WEEKS
FIRST WORMS MATURE
FIRST WORMS MATURE
1mM Chloride
28mM Chloride
56mM Chloride
85mM Chloride
113mM Chloride
141mM Chloride

FIGURE 5:2

Survival times of adult *Lumbricillus rivalis* in cultures of increasing salinity

Thirty adult worms were added to the cultures at the beginning of the experiment, and graph points represent the numbers still alive at each count.


NUMBER ALIVE
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
WEEK NUMBER
56mM
28mM
1mM
113mM
226mM
283mM

5:3:2 Methods to Determine the Survival of *Tubifex tubifex* and *Lumbricillus rivalis* in the Laboratory

Figures 5:1 and 5:2 show the survival times of *T. tubifex* and *L. rivalis* respectively. For *T. tubifex* it can be seen that the worms in the 1mM and 28mM cultures survived for 18 weeks, with only one death in the 1mM culture and 3 in the 28mM. The worms in the 1 and 28mM cultures grew to maturity, the first worm becoming mature at 10 weeks in the 1mM culture, and at only 3 weeks in the 28mM cuture. At NaCl concentrations above this, the worms did not mature, although in the 56mM culture several worms had reached a large size by week 10. In this culture, there was a steady mortality rate between weeks 2 and 5, after 4 initial deaths, then an increase between weeks 5 and 6, when seven worms died. There was little further mortality until after week 11, when the remaining worms began to die, until all were dead by week 16. The mortality in the 85mM culture followed a similar pattern, with the last worm dying at week 18. In this culture, however, the worms remained small, not growing to the size at which they would normally become mature.

In the 113 and 141mM cultures, initial deaths were rapid, with 87% of the worms dead in 4 weeks, and the remainder dead in 5 weeks in the 141mM culture. In the 113mM culture, 3 worms survived to week 8 before dying by week 10, although once again these worms did not grow well.

Lumbricillus rivalis worms grew equally well in solutions up to 113mM NaCl, but above this concentration did not survive for longer than 2 weeks. The NaCl concentration did not, in this species, alter the onset of maturity,

and all worms became mature after 4 weeks.

5:3:3 Effects of Salinity on Cocoon Laying in mature *Tubifex tubifex*

Mature *T. tubifex* continued to lay cocoons in all the experimental cultures, with a decrease in the numbers of cocoons laid as salinity increased (Table 5:1). In the 1mM and 28mM cultures, 27 and 28 cocoons respectively were laid by worms in one month. This was approximately one cocoon per worm. There were slightly fewer embryos per cocoon in the 1mM than in the 28mM concentration, although this was not significant.

The greatest difference between the 1mM and 28mM cultures was in the number of juveniles which emerged from each cocoon. In the 1mM culture 80% of the eggs which were laid developed and emerged, while in the 28mM culture, only 39% of the eggs developed and emerged.

In 56mM solutions, 15 cocoons were laid, with the same number of eggs per cocoon as in the lower salinities. However, in contrast to the 1mM and 28mM cultures, none of the embryos developed to emerge from the cocoon, although some eggs did hatch within the cocoon and underwent a small amount of development before death. In the 85mM culture, 11 cocoons were laid in the first month, although none of the eggs hatched in the cocoons. Above 85mM NaCl, the worms laid few cocoons, and none of the eggs within these cocoons developed into embryos.

TABLE 5:1

Numbers of Cocoons laid by Adult *Tubifex tubifex* in increasing salinity together with Numbers of Embryos in Cocoons and Number of Emerging Juveniles

NaCl (mM)	Date	No. Adults	No. Cocoons	No. Embryos	No. Juvs	No. eggs/cocoon
1	18.2	30	0	0	0	0
	25.2	28	5	19	0	3.8
	4.3	28	12	40	4	3.3
	14.3	28	13	20	4	1.6
	21.3	28	27	119		4.07
	24.3	31	31	115		3.7
	MEAN					3.3
28	18.2	30	0	0	0	0
	25.2	30	17	65	0	3.8
	4.3	30	22	79	0	3.6
	14.3	30	28	82	1	2.93
	21.3	30	15	70	64	4.7
	24.3	24	50	178	64	3.6
	MEAN					3.7
56	18.2	30	0	0	0	0
	25.2	27	7	28	0	3.4
	4.3	27	11	37	0	3.4
	14.3	27	6	21	0	3.5
	21.3	26	15	55	0	3.7
	24.3	17	12	52	0	4.3
	MEAN					3.7

TABLE 5:1 (Continued)

Numbers of Cocoons laid by Adult *Tubifex tubifex* in increasing salinity together with Numbers of Embryos in Cocoons and Number of Emerging Juveniles

NaCl (mM)	Date	No. Adults	No. Cocoons	No. Embryos	No. Juvs	No. eggs/cocoon
85	18.2	30	0	0	0	0
	25.2	24	1	6	0	6
	4.3	24	3	10	0	3.3
	14.3	24	7	20	0	2.86
	21.3	24	11	31	0	2.82
	24.3	24	17	44	0	2.6
	MEAN					3.5
113	18.2	30	0	0	0	0
	25.2	31	1	4	0	4
	4.3	31	2	8	0	4
	14.3	31	2	8	0	4
	21.3	30	4	10	0	2.5
	24.3	17	6	10	0	1.7
	MEAN					3.24
141	18.2	30	0	0	0	0
	25.2	30	1	6	0	6
	4.3	30	1	6	0	6
	21.3	30	1	6	0	6
	24.3	8	0	0	0	0
	MEAN					6

5:3:4 Number of Eggs per Cocoon Produced by *Tubifex tubifex* and *Lumbricillus rivalis* in increasing Salinity

The numbers of eggs per cocoon, time for worms to emerge, and number of worms emerging are shown in Table 5:2.3 *Tubifex tubifex* cocoons contained between 2 and 6 eggs, which hatched in 14 to 40 days, with mean values of 2 to 3.9 juveniles emerging from each cocoon. *L. rivalis* cocoons contained between 6 and 35 eggs, and hatched between day 19 and day 45. The numbers of *L. rivalis* which emerged from these isolated cocoons was low, only between 4 and 6. Kirk (1971) also found low rates of emergence from isolated *L. rivalis* cocoons when compared to field populations.

5:3:5 Reproductive success of *Tubifex tubifex* in increasing NaCl concentrations

Figure 5:3 shows the numbers of mature and juvenile worms and cocoons in the cultures for each occasion on which they were counted.

In the 1mM culture, *T. tubifex* worms matured in between 79 and 178 days, while in the 28mM culture worms matured more quickly, between 27 and 58 days. In both cultures, the new adults initially produced small numbers of cocoons from which few juveniles survived, but subsequently began a longer period of cocoon laying which produced more juveniles.

In the 1mM culture, there were two maxima of cocoon production, between days 161 and 365 (September to March, maximum in January) and days 638 to 750 (November to

Table 5:2

Summary of Numbers of Embryos per cocoon, emergence times and Juvenile survival rate from cocoons produced by mature *Tubifex tubifex* in increasing salinity

NaCl (mM)	1	28	56	85	113	141
Total No cocoons	27	28	15	11	4	1
Mean embryos per cocoon	3.3	3.7	3.7	3.5	3.24	6
Mean No emerging per cocoon	3.55	1.14	0	0	0	0
Total juveniles	96	32	0	0	0	0
% emerging	80.7	39	0	0	0	0
emergence time (days)	7-24	14-24	0	0	0	0

TABLE 5:3

Number of Embryos per Cocoon, Time taken to emerge, and Number of Emerging Juveniles per Cocoon Produced by *Tubifex tubifex* and *Lumbricillus rivalis* in Increasing Salinity

i) Numbers of eggs per cocoon

	T. tubifex				*L. rivalis*			
NaCl (mM)	mean	max	min	no	mean	max	min	no
1	3.82	6	2	30	15.6	31	6	15
28	3.65	6	2	50	18.7	29	10	7
56	4.05	6	1	15	16.5	35	7	1
85	3.68	5	2	17	16.9	30	9	10
113	2.78	6	1	6	15.4	25	8	12

ii) Time to Emergence (in Days)

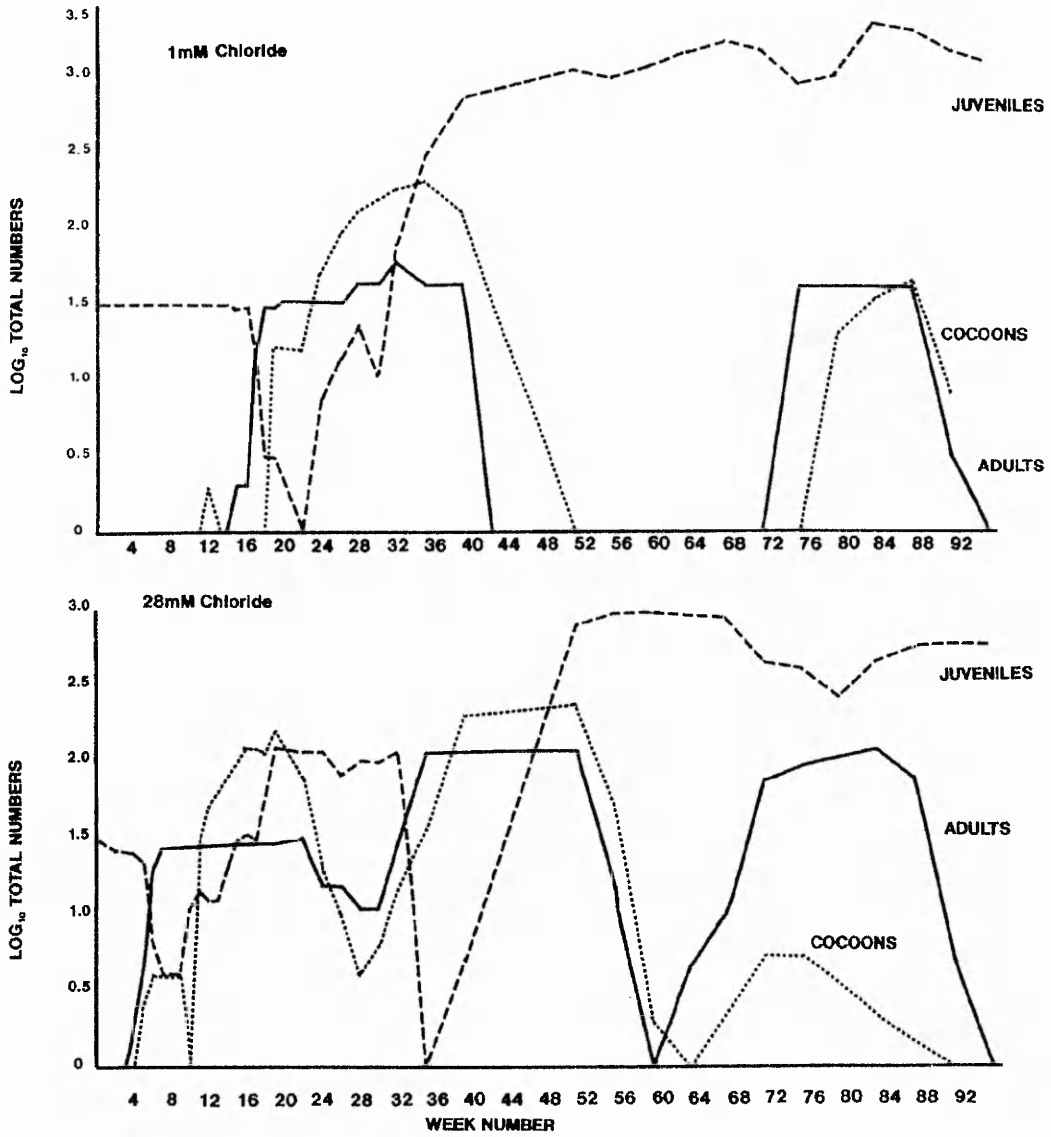
	T. tubifex			*L. rivalis*		
NaCl (mM)	mean	max	min	mean	max	min
1	22	26	14	26	35	20
28	31	25	40	24	40	19
56				28	38	21
113				30	45	22

iii) Number of Emerging Juveniles/Cocoon

	T. tubifex	*L. rivalis*
NaCl (mM)	mean	mean
1	3.88	4
28	2	6
56	0	5
113	0	3

FIGURE 5:3

Number of Mature (Adult) and Juvenile *Tubifex tubifex* in cultures at two salinities (1mM and 28mM NaCl), together with numbers of cocoons laid in the cultures, against week number


1mM Chloride
JUVENILES
COCOONS
ADULTS
LOG10 TOTAL NUMBERS
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92
28mM Chloride
JUVENILES
ADULTS
COCOONS
LOG10 TOTAL NUMBERS
3.0
2.5
2.0
1.5
1.0
0.5
0
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92
WEEK NUMBER

April, maximum in February). There were therefore 13 months between periods of maximum cocoon laying in this culture.

The cocoon-laying appeared to be more continuous in the 28mM culture, although there were peaks between days 79-225 (June to November, maximum July), 273 to 470 (December to June, maximum March) and 597 to 750 (November to April, maximum February). There were, therefore, eight months between the first two maxima and eleven months between the second and third.

The total population size in each of the cultures is shown in Figure 5:4. The numbers of worms in the two populations followed similar patterns of fluctuation. The numbers in the 1mM culture exceeded those of the 28mM by day 273, and then showed three peaks in population size, in May, September and January. Nearly all these individuals were juveniles. In the 28mM culture, there were maxima in August, June and February. These peaks were also mainly due to juveniles, and after the first, were lower in size than in the 1mM culture.

The numbers in the cultures can be calculated as a population size (m^{-2}) for comparison with field populations. The containers used in this experiment were 8cm x 8cm, giving a surface area of 64 cm^2.

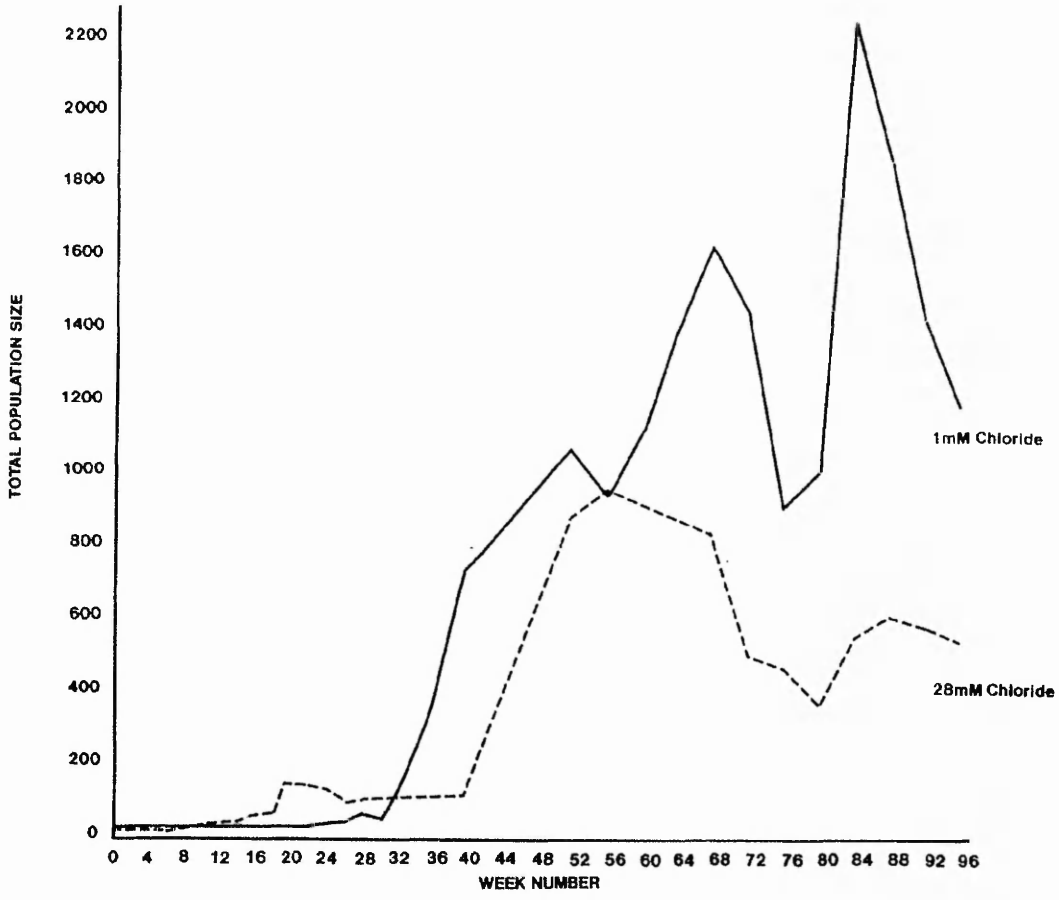
The maximum population size in the 1mM culture was at day 660, when 2222 juveniles and 42 adults were present, equivalent to a population density of 34.7 animals per cm^2, or 347,000 m^2

In the 28mM culture, the maximum population size was 960, found on day 439 (June). This was equivalent to 14.8 worms per cm^2, or 148,000m^{-2}

Nearly all the original juvenile worms matured in both cultures, but after this the rate of maturation was very

FIGURE 5:4

Total population size (matures plus juveniles) of *Tubifex tubifex* in cultures at two salinities (1mM and 28mM NaCl)


TOTAL POPULATION SIZE
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96
WEEK NUMBER
1mM Chloride
28mM Chloride

low, especially in the 1mM culture, where from the first generation of juveniles only 60 became mature, compared with 116 in the 28mM culture. It is, of course possible that some of these mature worms were survivors from the initial stock, as these worms may have regressed, then matured again to lay a second batch of cocoons. This would have reduced the numbers of worms maturing from the first generation to 27 and 89 respectively. In the second generation, there were 41 mature worms in the 1mM culture and 114 in the 28mM culture.

In the 1mM culture, there was little evidence that the juveniles which did not mature were dying, as although there was a decrease in numbers when some worms were mature on day 597, there were still approximately 900 worms in the culture, and juveniles from the next generation were added to these. It is possible that the very high population density in the culture was limiting food supplies and delaying growth and maturity.

In the 28mM culture, a large proportion of the 123 juveniles produced in the first generation matured, 116 from a maximum of 123 juveniles, a maturation rate of 93%. The time between peaks of juveniles and adults was 136 days (273-161), which compares with the maturation time of 31 days for the initiators. The initiating worms, may, however, have been emerged from the cocoon for some time before being selected. In the second generation, there was a peak of juveniles at day 439 (946 juveniles) and a peak of adults at day 660 (114 adults). This would give a maturation rate of 12% in the second generation. This reduction in maturation rate may be due to the increased population density in the culture, or it may be that the juveniles from the second generation have not matured, and the adults were those from the first

generation, which had regressed and matured for a second time.

The maximum and mean hatching times for *T. tubifex* in 1mM and 28mM NaCl solutions was estimated previously, and are shown in Table 5:2. The mean values were 26 days at 1mM and 25 days at 28mM, with a maximum value of 40 days. Using these figures to estimate cocoon hatching times in this experiment, the cocoon producing periods were divided into 25 day sections. The number of cocoons laid in each of these periods were added together to provide an estimate of the total number of cocoons produced in a period. The next maxima of juveniles present in the culture was then divided by this number to give an estimate of the numbers of juveniles survivng from each cocoon.

For example, 760 cocoons were produced between week 14 and week 42. The next maximum of juveniles was 1076, therefore the number of survivng juveniles per cocoon was 1.42. The number of cocoons produced in each period is shown in Table 5:4.

It should be appreciated, however, that these figures are estimates only, as cocoons hatching rapidly might have been missed between counting dates, and cocoons hatching more slowly may be counted twice.

Before the second generation of cocoons were laid, it is possible that some cocoons were missed in the cultures, as the number of juveniles continued to increase despite the fact that no cocoons could be found in the culture. This could be due simply to smaller juveniles becoming more easily visible as they grew, or to cocoons laid and hatched between counting dates, which in the second year were only monthly. The number of juveniles from this generation of cocoons could therefore be up to 1641,

TABLE 5:4

Number of cocoons per adult *Tubifex tubifex* Produced by Two Generations in Cultures at Two Salinities

NaCl (mM)	1		28	
Generation	1	2	1	2
Total No Cocoons	760	160	414	794
No cocoons/worm	12.7	2.6	13.8	6.9

TABLE 5:5

Numbers of Surviving Juveniles per cocoon laid by two Generations of *Tubifex tubifex* in cultures at two salinities

Salinity of Culture	1mM	28mM
Original worms	1.42-2.16	0.3
First generation	2.97	1.19

increasing the hatching success rate to 1641/760=2.16 juveniles per cocoon.

The total numbers of cocoons produced per worm can be estimated from the data in Figure 5:2, and are shown in Table 5:5.

For example, in the 1mM culture, 60 adults produced a total of 760 cocoons, *ie* 760/60=12.7 cocoons per individual. Table 5:5 shows the calculated survival rate per cocoon in the two cultures. The survival was highest in the 1mM culture in both generations, but was higher in the first generation of worms in both cultures.

Table 5:6 shows the k values obtained for *T. tubifex*. The maximum natality and actual natality were calculated using figures from Experiment 3.

The values of k_0 for both cultures were similar (0.2, 0.25 and 0.2), and quite low, indicating that the worms were producing cocoons with a high number of eggs when compared to the maximum. Mortality between the egg and juveniles stage was more variable, with a low value in the 1mM culture and in the second generation in the 28mM culture, and a higher value in the first 28mM generation. The k_2 value for the juvenile to adult mortality was highest in the 1mM culture, showing the failure of this generation of juveniles to mature, although it did not reflect actual mortality, as most of the juveniles were still alive. The values for the two generations in the 28mM culture were similar, perhaps indicating that a maximum number of adults had been reached.

Table 5:7 shows the summary statistics for the dry weights of worms from the two cultures. It can be seen that the mean weight of the worms from the 28mM culture was smaller than the mean weight from the 1mM culture, and this difference was found to be significant using a

TABLE 5:6

k-Values for *Tubifex tubifex* in cultures of increasing salinity

	NaCl (mM)			
	1	56	141	
Log_{10} max natality	3.66	3.65	3.67	
k_0	0.2	0.25	0.2	
Log_{10} no of eggs	3.46	3.4	3.47	
k_1	0.43	1.09	0.49	
Log_{10} no juvs	3.03	2.09	2.98	
k_2	1.42	0.03	0.92	
Log_{10} no adults	1.61	2.06	2.06	
Total *k*	2.05	1.37	1.6	

TABLE 5:7

Summary statistics of Dry Weights of
T. tubifex from 1mM and 28mM NaCl cultures

Weights in mg

NaCl (mM)	No.	Mean	Med	Max	Min
1	0.45	0.25	3.05	0.12	0.52
28	0.26	0.22	0.999	0.11	0.14

t-test. (t= 3.7, significane level 1%)

5:3:6 Reproductive Success of *Lumbricillus rivalis* in Increasing Salinity

The figures for the two replicate samples for each concentration were compared using one-way analysis of variance to compare all the replicated numbers, and by t-tests on the means of adults, juveniles and cocoons for each concentration. None of the comparisons showed a significant difference between replicates, and so the two replicates were added together and treated as one.

Table 5:8 shows the combined numbers of mature and juvenile *L. rivalis* and combined numbers of cocoons on all counting occasions.

In the 1mM and 56mM culture, *L. rivalis* had two periods of high breeding intensity, between May and September in the first year, and January and March in the second. In the 141mM culture the first breeding period was between May and September, as in the other cultures, but there was no further breeding after this time.

Figure 5:5 shows the total number of adults and juveniles in the cultures on each sampling occasion. The 200mM cultures produced no juveniles, although some cocoons were laid, and the adults were all dead by the 10th month of the experiment. The greatest density of animals was found in the 56mM culture in August. This culture had two population peaks, in August and January, while the 1mM culture had three, in July, December and February. The 141mM culture also showed three maxima, in September, November and February.

The maximum population size for each culture was converted to numbers per square metre using the surface

TABLE 5:8

Numbers of *Lumbricillus rivalis* in Laboratory Culture

NaCl (mM)		1			56			141		
		Stage in Life Cycle								
DAY No.	MONTH No.	M	J	C	M	J	C	M	J	C
1	5	20		20		20				
35	6	20	13	20	17	23	6	12	12	2
64	7	15	203	27	15	102	12	16	13	6
95	8	3	206	2	16	389	2	10	133	35
123	9	3	57	4	6	226	1	10	174	5
172	10	14	1	1	0	235	0	20	116	1
191	11	1	17	0	0	187	0	30	186	1
232	12	17	33	8	11	179	0	17	131	1
253	1	14	7	6	120	235	9	14	128	0
289	2	15	149	5	42	260	19	105	47	1
332	3	48	110	8	18	77	4	12	65	0
360	4	36	115	59	8	90	18	0	92	0

M= Mature animals

J= Immature animals

C= Cocoons

FIGURE 5:5

Total population size (matures plus juveniles) of *Lumbricillus rivalis* in cultures at four salinities (1, 56, 141 and 226mM NaCl)

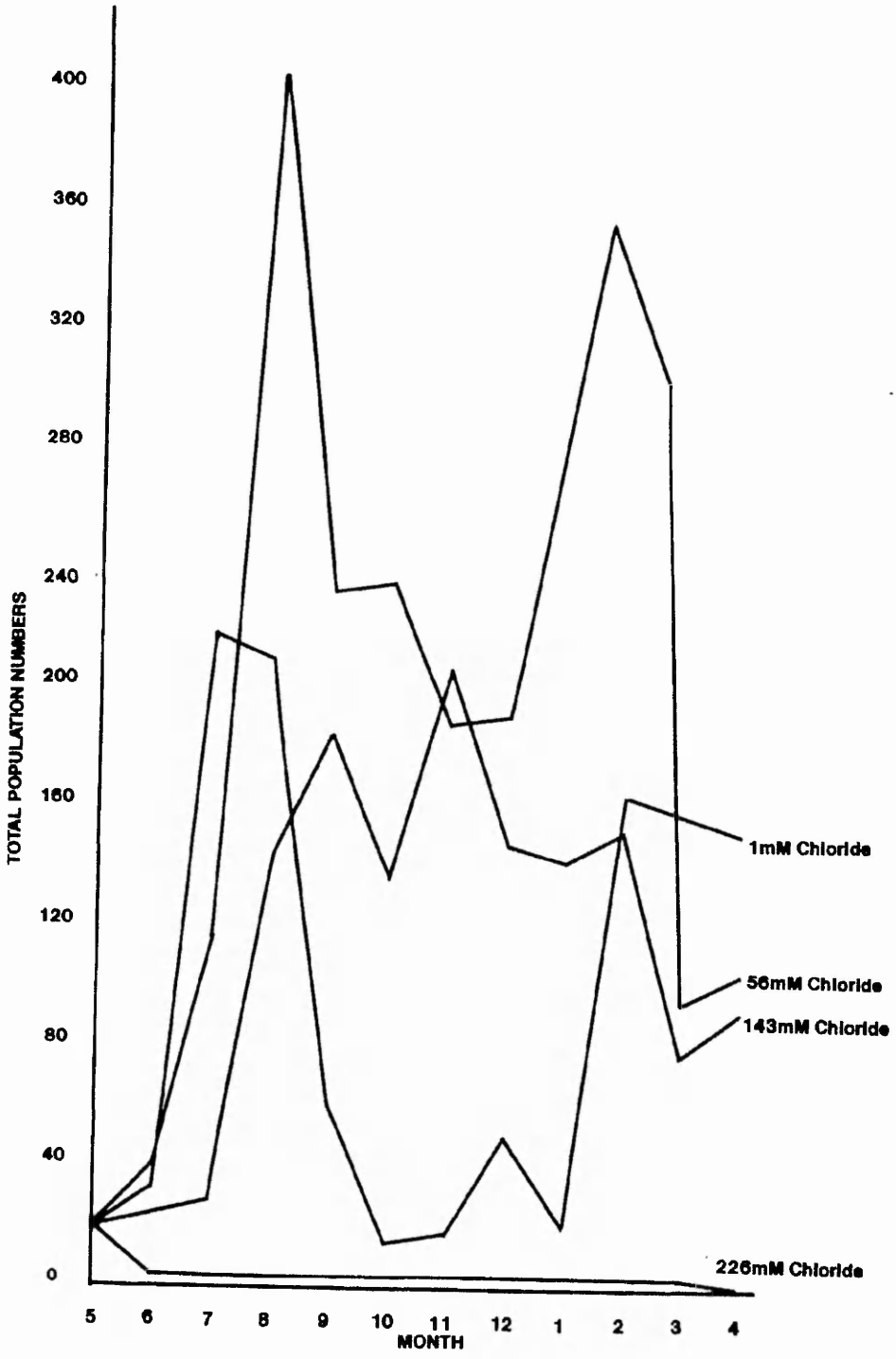
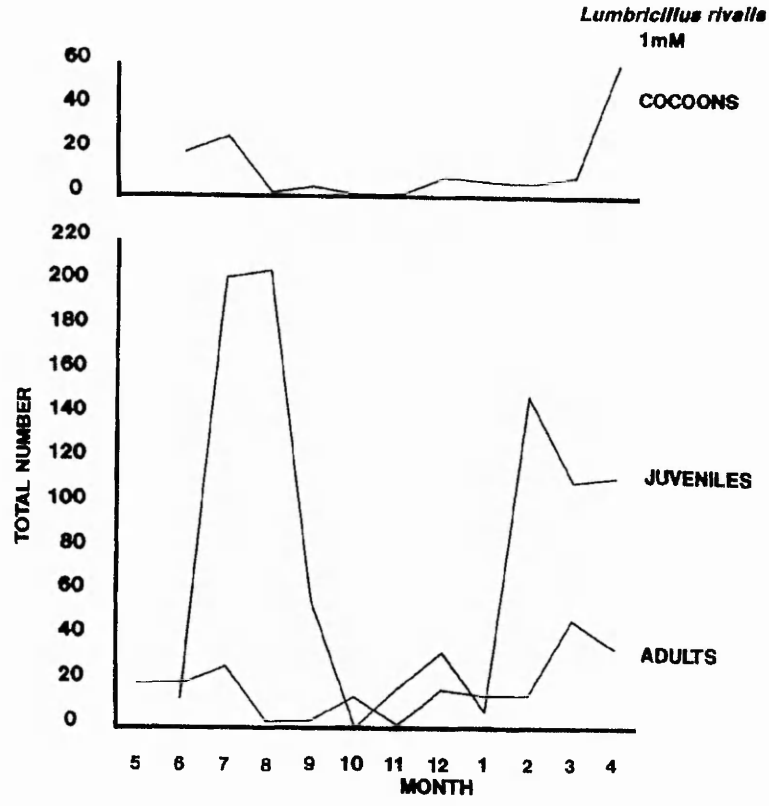

400
360
320
280
240
200
160
120
80
40
0
TOTAL POPULATION NUMBERS
5
6
7
8
9
10
11
12
1
2
3
4
MONTH
1mM Chloride
56mM Chloride
143mM Chloride
226mM Chloride

FIGURE 5:6

Numbers of adults, juveniles and cocoons of *Lumbricillus rivalis* in cultures at increasing salinity.

a) 1mM NaCl

b) 141mM NaCl


Lumbricillus rivalis
1mM
COCOONS
60
40
20
0
220
200
180
160
140
120
100
80
60
40
20
0
TOTAL NUMBER
JUVENILES
ADULTS
5 6 7 8 9 10 11 12 1 2 3 4
MONTH

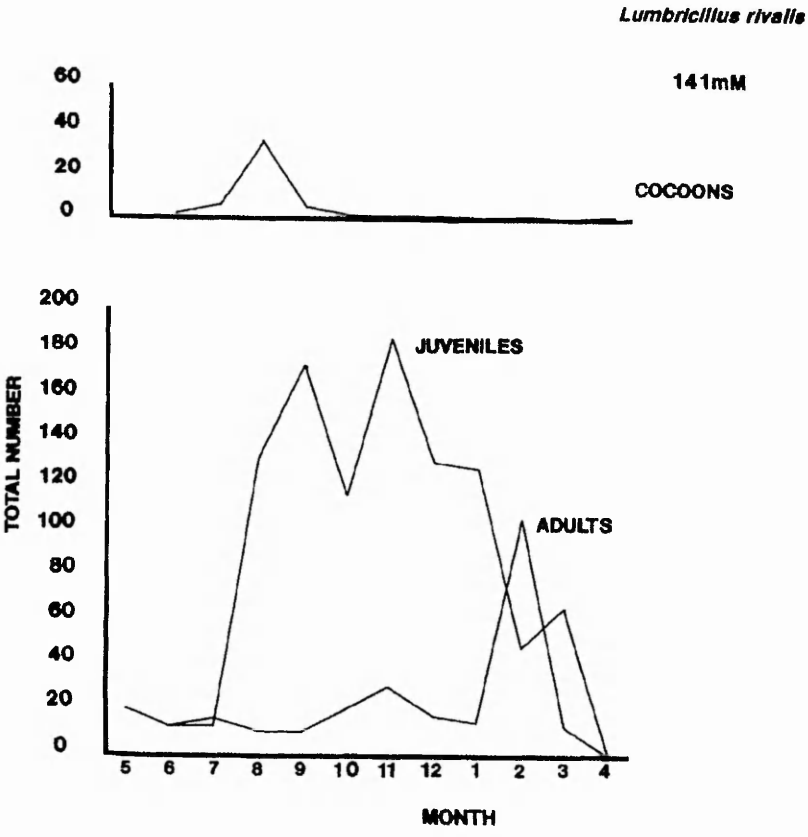
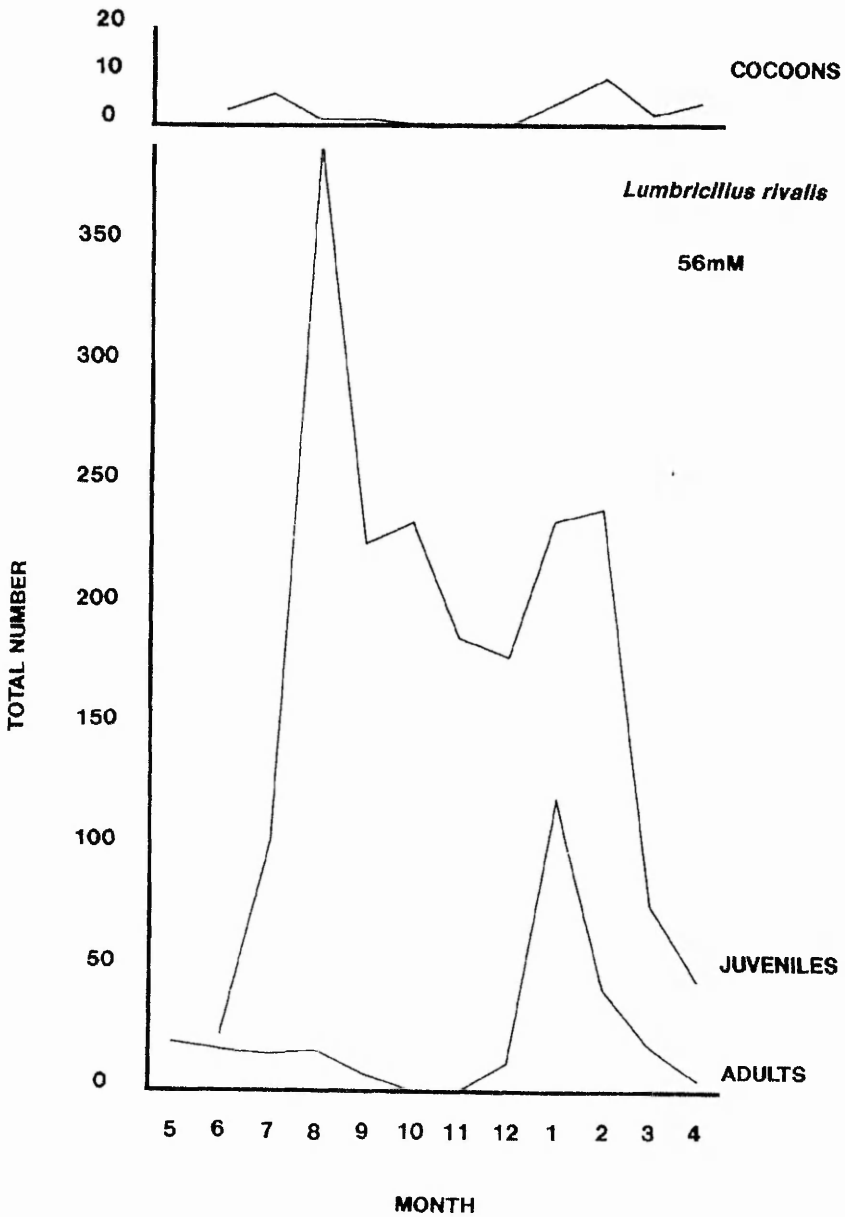

Lumbricillus rivalis
141mM
COCOONS
60
40
20
0
200
180
160
140
120
100
80
60
40
20
0
TOTAL NUMBER
JUVENILES
ADULTS
5 6 7 8 9 10 11 12 1 2 3 4
MONTH

FIGURE 5:6

Numbers of adults, juveniles and cocoons of *Lumbricillus rivalis* in cultures at increasing salinity.

c) 56mM NaCl


20
10
0
COCOONS
Lumbricillus rivalis
56mM
350
300
250
200
150
100
50
0
TOTAL NUMBER
JUVENILES
ADULTS
5 6 7 8 9 10 11 12 1 2 3 4
MONTH

area of each pot (=4cm^2). Since each population figure was a combination of the numbers in two pots, the numbers were divided by 8 to give numbers per square centimetre, then multiplied by 10,000 to give numbers per square metre, as shown below:-

1mM = 307,500 (maximum numbers per square metre)
56mM = 506,250
141mM= 270,000

The worms in the 1mM culture matured at between 60 and 77 days, while those in the 56mM culture matured over a longer period, between 60 and 158 days. Those in the 141mM culture matured between day 68 to day 88, although these did not produce cocoons.

The percentage of adults which matured increased with the salinity of the solution, with 23.3% at 1mM, 30.8% at 56mM and 56% at 141mM NaCl.

The mean numbers of juveniles which emerged were similar in the 1mM and 141mM cultures, with 4.5 and 4.04 respectively. In the 56mM culture, however, there was a mean of 21.6 juveniles emerging per cocoon.

The K values were calculated using data from Experiment 3, as for *T. tubifex*.

The K values for the difference between maximum and mean numbers of eggs per cocoon were similar (Table 5:9), indicating that the salt concentration did not affect the numbers of eggs produced. There was greater mortality between egg and emerging juveniles in the 1mM and 141mM cultures than in the 56mM culture. The mortality between juvenile and adult was lowest in the 141mM culture, and highest in the 1mM culture. The overall mortality was lowest in the 56mM culture.

TABLE 5:9

K-Values for Lumbricillus rivalis in cultures of increasing salinity

	NaCl (mM)		
	1	56	141
Log_{10} max natality	3.16	2.8	3.09
k_0	0.3	0.33	0.21
Log_{10} no of eggs	2.86	2.47	2.88
k_1	0.55	0.12	0.61
Log_{10} no juvs	2.31	2.59	2.27
k_2	0.63	0.51	0.25
Log_{10} no adults	1.68	2.08	2.02
Total k	1.48	0.96	1.07

5:4 Discussion

A summary of the results of laboratory experiments on the life-cycles of *T. tubifex* and *L. rivalis* is presented in Table 5:10. The maximum population densities of *T. tubifex* are at 1mM NaCl, and in *L. rivalis* at 56mM NaCl. This is despite the fact that both species show a greater reproductive effort, with earlier maturity, more cocoons produced per individual and often a higher percentage of juveniles maturing in a shorter time at concentrations other than this optimum. These strategies should produce an advantage in terms of numbers over those found in the optimum NaCl concentrations, but do not. This is due to the high juvenile mortality rate, which in *T. tubifex* at 28mM is two to three times greater than at 1mM, and in *L. rivalis* is five times greater in 1mM and 141mM than it is in 56mM. This suggests that, in order to maintain a population in other than optimal salinities, the worms must adopt a strategy of early reproduction, producing large numbers of juveniles, only a small percentage of which survive. In the optimal concentrations, the worms can delay reproduction and produce fewer young, as a larger proportion of those young will survive. The early maturity and larger numbers of young would indicate a reduction in size of both juveniles and adults, which was found in *T. tubifex*, where the worms weighed at the end of the experiment were significantly smaller in the 28mM culture than in the 1mM.

Lumbricillus rivalis had two breeding periods per year in the experimental cultures, between May and September,

TABLE 5:10

Summary of Results for *Tubifex tubifex* and *Lumbricillus rivalis* in Laboratory Culture

	T. tubifex		*L. rivalis*		
	NaCl (mM)		NaCl (mM)		
	1	28	1	56	141
Time to Mature (original worms)	79-178	27-58	-	-	-
Peak Reprod 1	9-3	6-11	5-9	5-9	5-9
(month number) 2	11-4	12-6	1-4	1-4	-
(January = 1) 3		11-4	-	-	-
Max Pop Density (Nm^2)	347,000	147,812	307,500	506,250	270,000
Time to Maturity (1st gen)	188	136	60-77	60-158	68-88
% Maturing (1st gen)	3.7	94	23.3	30.8	56
No J/cocoon					
(1st gen)	1.4-2.2	0.3	4.5	21.6	4.04
(2nd gen)	2.97	1.19			
No eggs/cocoon	3.82	3.65	15.6	18.7	15.4
No cocoons/adult					
(1st gen)	12.7	13.8	2.7	1.05	2
(2nd gen)	2.6	6.9	1.8	0.42	-
Cocoon Hatching Time (days)	14-26	25-40	20-35	21-38	22-45
Longevity (months)	-	-	<6	<6	<6
Fertility range NaCl (mM)	1-<56		1-<226		

and then between January and April. This suggests that there are two generations per year, with individuals born in late summer overwintering and breeding in spring, while those born in spring matured and reproduced in late summer. The adults appeared to die after breeding, as there was no increase in juveniles after September, and the juveniles present were not large, as they would be if adult *L. rivalis* were regressing to a juvenile state after breeding. Williams *et al.* (1969), in a study of *L. rivalis* in sewage filter beds, found maximum cocoon production in March/April each year, suggesting an annual life-cycle, while Learner (1972) found a generation time of 68-106 days at 8^0C in the laboratory, and suggested 1.4 generations per year at this temperature. Bird (1982) found an annual life-cycle in the enchytraeid *Propappus volki* inhabiting chalk streams. This would seem to suggest that *L. rivalis* under these laboratory conditions has a slightly shorter life-cycle than has been observed by other workers.

Tubifex tubifex, in contrast, had one breeding period per year in the 1mM culture, in winter/spring, while in the 28mM culture, breeding was almost continuous, but divided into two main periods, spring and winter. The original worms matured in 79-178 days in the 1mM culture and 27-58 days in the 28mM culture. The first generation of juveniles which were bred in the cultures, however, were slower to mature, requiring eight months in the 28mM culture and 13 months in the 1mM. These rates of maturity are speculative as there was no way to detect, with this method, if the worms which matured and produced the second generation were doing so for the first time, or if they were adults which had previously regressed. In view of the similarity in the numbers of adults in each

breeding period, it seems likely that the same adults were breeding twice in the cultures, and the length of time to maturity could be much longer. The length of the life-cycle of *T. tubifex* is known to be very variable, although most authors suggest 1 to 2 years (Brinkhurst and Jamieson, 1971). Adreani *et al.* (1984) suggested that the maturation rate in *T. tubifex* was density dependent, and found a correlation of r = 0.77 between initial density and maturation rate. They also suggested that, although number of eggs per cocoon varied with time in the breeding cycle, this was also density dependent. This could help to explain the length of time required for maturity in *T. tubifex* in the 1mM culture. The number of eggs per cocoon, however, does not seem to have been affected by increasing population density in this culture. It is possible that the original worms which began the cultures were still alive at the end of the experiment, as Timm (1984) found that some specimens of *T. tubifex* lived for up to eight years at seasonal temperatures in the laboratory. He also found that *T. tubifex* under experimental conditions was able to reproduce twice a year.

Since the time taken for cocoons to hatch was less than thirty days in both species, it seems likely that some cocoons were missed in the later stages of the *T. tubifex* experiment and in the *L. rivalis* experiments, as the cultures were only counted once per month. Accounting for these would increase the observed number of cocoons produced per worm and also reduce the juvenile survival rate. There is evidence of the missing cocoons in the low number of cocoons per worm produced by *T. tubifex* in the second generation in both 1mM and 28mM cultures. The number of cocoons per worm was reduced to between 2 and 7

per worm, from 12 to 14 in the first generation. This might also, however, have been due to a reduction in fertility caused by overcrowding in the cultures. The figure of 12-13 cocoons per worm is close to the number of fertile cocoons which Poddubnaya (1984) calculated that each *T. tubifex* worm can produce from one mating (=10-12). She suggested that further cocoons layed in a reproductive period were the result of parthenogenesis, and that worms were capable of laying up to 20 cocoons produced in this manner.

The present laboratory experiments were carried out at a temperature which was kept close to 10^0C (+ or - 1^0C) throughout the duration. Since temperature is shown by many authors to affect the breeding of both species of worm (eg Learner, 1972; Aston, 1973; Aston, 1982; Williams *et al.*, 1973), a temperature was chosen at which both species would breed and which could be constantly maintained. Aston (1973) demonstrated a steady rate of egg production in *T. tubifex* between 10 and 25^0C, while Learner (1972) demonstrated that *L. rivalis* could breed within the range 8-20^0C. A pH of above 7 was maintained in all experiments, as several authors (eg Whitley, 1967; Chapman *et al.*, 1982b) found during the course of toxicological work that Tubificidae were more tolerant of all toxicants if the pH was above 7. Chapman and Brinkhurst (1980) also found that sand as a substrate increased the salinity tolerance of *Limnodrilus hoffmeisteri*, a tubificid often found together with *T. tubifex*. These authors found an LC_{50} value of 282mM NaCl for *L. hoffmeisteri*, a value which is much higher than the concentrations at which *T. tubifex* is fertile, although *T. tubifex* can withstand salinities of 140mM for long periods.

In marine situations *Lumbricillus* spp. have been described as "stress-resistant" by Coates and Ellis (1980) when describing the effects of pulp-mill wastes on rivers in America. They found *L. lineatus* within 1.4km of a pulp-mill outfall into a river estuary, where it was the only oligochaete present in an area of *Ulothrix* spp. and *Oscillatoria* spp. "slime". Beyond this zone, *Enteromorpha* spp. were found supporting an oligochaete fauna of *Lumbricillus* spp., *Marionina* spp., and *Enchytraeus* spp. In Germany Wachs (1963) found *L. rivalis* together with *Enchytraeus albidus, Paranais litoralis* and *Nais elinguis* in a reach of the River Werra salinated by the potassium industry to 16% sea water. There are no records of *T. tubifex* from saline-polluted waters, although Brinkhurst (1971) records *Limnodrilus hoffmeisteri* from the Saginaw Bay, Lake Huron, close to the input of the salinated River Saginaw, where salinities were up to 10mM NaCl.

The distribution of *T. tubifex* and *L. rivalis* in Millwood Brook can be explained from the laboratory studies of reproductive biology, which demonstrate that *T. tubifex* was only fertile below a salinity of 56mM NaCl, while *L. rivalis* was fertile in a wide range of salinities, but has best reproductive success at a NaCl concentration of 56mM. Tubificidae were found at sites on Millwood Brook with a mean annual chloride below 22mM, wheras *L. rivalis* occured at all sites with chloride concentrations above 24mM, and also two where the chloride concentrations were lower. These sites, (M5 and D), were directly upstream from areas of high chloride and as *L. rivalis* can reproduce in chloride as low as 1mM, it seems able to extend into areas of low salinity close to areas of higher salinity where it has few competitors.

(*T. tubifex* was found at one site, site B, which had a mean annual chloride concentration of 34.85mM. This was, however, due to short periods of high cloride concentration, which *T. tubifex* is able to withstand, as demonstrated in the first survival experiment described in this chapter.)

The laboratory population densities of both *T. tubifex* and *L. rivalis* were ten times higher than those found in the field, where *T. tubifex* had a maximum density of 37,000 per m^2, and *L. rivalis* a maximum of 18,620 per m^2. However, Lazim and Learner (1986) found population densities of between 5420 and 613,000 per m^2 of *T. tubifex* in a moat-feeder stream in Wales, and Williams *et al.* (1969) found densities of *L. rivalis* of up to 1,520,000 per m^2 in sewage filters, it would seem that the numbers found in Millwood Brook were quite low.

All these observations, together with the experimental results presented in this chapter, emphasise the importance of oligochaete worms as indicators not only of pollution, but of the type of pollution, as large populations of Tubificidae suggest the presence of organic pollution, while populations of *Lumbricillus rivalis* coupled with the absence of Tubificidae indicate saline pollution. It also confirms the need to identify oligochaetes collected in field samples from polluted sites, as they can provide important information concerning the extent and type of pollution.

CHAPTER 6

Effects of Salinity on the Oxygen Uptake of *Gammarus pulex*, *Gammarus tigrinus*, *Gammarus zaddachi*, *Tubifex tubifex* and *Lumbricillus rivalis*

6:1 Introduction

Pollution of all types may affect the survival of freshwater animals either directly or indirectly by altering aspects of their physiology and behaviour at sub-lethal levels. This may reduce the competitive ability of a species at a polluted site when a better suited invading species is introduced.

The effects of increasing salinity on the reproductive strategy of *Tubifex tubifex* have been described in Chapter 5, where it was shown that increased salinity reduced the survival of juvenile worms, and might thus reduce the competitive ability of this species when compared to a more saline tolerant species such as *Lumbricillus rivalis*.

Increased salinity would be likely to increase the amount of osmo-regulation which freshwater animals need to undertake to maintain their salt balance, and since this regulation is to a large degree an active process (Rankin and Davenport, 1981), it is possible that one effect of increasing salinity on freshwater organisms could be an increase in respiration rate. There could also be a reduction in the energy available for activity if an increasing proportion of respiratory energy was to be used in osmo-regulation.

Several authors have measured the rates of oxygen uptake by marine and freshwater species in relation to pollutants, for example using Tubificidae and heavy metals (Brkovic-Popovic and Popovic, 1977a, Brinkhurst *et al.*, 1983). Studies have also been published on the effects of chlorinated organic chemicals such as PCP (O'Connor *et al.*, 1985) on the respiration of Gammaridae. The effects of salinity on the respiration of the marine amphipod *Corophium volutator* have also been measured by McClusky (1969), and *G. tigrinus* has been similarly investigated by Dorgelo (1973).

Although many of these authors did not find statistically significant results, they indicated that pollution at sub-lethal levels could affect the respiration rate of aquatic organisms. The effect of pollutants on respiration was not uniform, and depended on the nature of the substance itself, as some pollutants, such as lead in Tubificidae, were found to decrease the respiration rate, while others, such as sodium pentachlorophenate were found to increase it (Whitley and Sikora, 1970).

Saline pollution is assumed to produce osmotic stress in all freshwater aquatic animals which regulate their blood sodium and chloride levels, and which are permeable to some degree to the surrounding medium. If the animal is an osmoregulator, it must expend energy in order to maintain a constant internal environment, and gain or remove ions in response to the changing concentration of the external medium. This extra energy is presumed to be released from food via respiration (Milne and Ellis 1973). Gammarids such as *G. duebeni* have been shown to be osmoregulators (Lockwood and Inman, 1973), since their blood osmolarity is higher than that of the surrounding medium in fresh water, and lower in full sea water,

although the species can survive for long periods in both types of environment.

The respiration rates of five experimental species (*G. pulex*, *G. tigrinus*, *G. zaddachi*, *Tubifex tubifex* and *Lumbricillus rivalis*) were measured in increasing concentrations of NaCl to determine if any of these species were using respiratory energy to adapt to increasing salinity. The respiration of *G. pulex* and *G. tigrinus* in varying salinities were compared with that of *G. zaddachi*, a brackish water species which occurs at higher salinities than *G. tigrinus*, (Holland, 1976) and which, although able to tolerate freshwater for extended periods, is unable to breed in freshwater (Gledhill *et al.* 1976). It was therefore regarded as a true estuarine inhabitant with which to compare *G. tigrinus* taken from rivers.

The *G. zaddachi* used in the experiments were collected from Cresswell Quay (SN 050 067) near Creselly, in Dyfed, South Wales, where the animals were living in the mouth of a small stream, just above the shoreline.

6:2 Methods

The Hansatech equipment used in these experiments was designed to measure the oxygen production and consumption of isolated plant cells in aequeous solution, and it is small in scale and very sensitive to changes in oxygen concentration. It was therefore suitable for measurements with single animals when using *Gammarus* species, although ten oligochaetes were required to produce a decrease in oxygen large enough to measure accurately. Since the oxygen saturation of the water in Millwood

Brook was never recorded below 80% saturation, all experiments were carried out over short periods using solutions which were initially at 100% saturation. *Tubifex tubifex* is known to be tolerant of low oxygen concentrations (Brinkhurst *et al* 1983), although *G. pulex* has increased mortality below 50% oxygen saturation (Grant and Hawkes, 1982.) However, even in *T. tubifex* there is a sudden decline in the rate of respiration as the ambient oxygen level falls, once a certain critical point is reached. In order to avoid changes in respiration due to low external oxygen concentrations, short tests of one hour per animal were used in the experiments.

The "Hansatec" oxygen electrode and digital readout box were connected to a Gould chart recorder which produced a line trace showing changes in the oxygen saturation of the solution under test. The Hansatech electrode consisted of a 2mm diameter cathode and a silver anode immersed in, and linked by, a saturated solution of KCl. The electrodes were protected by a Teflon membrane which was permeable to oxygen and which trapped a thin layer of electrolyte over them. A "spacer" of "Rizla" cigarette paper was placed between the electrodes and membrane to ensure an even layer of electrolyte between anode and cathode. The electrode and membrane were enclosed in an experimental chamber of 4ml capacity, and were thus directly in contact with the solution in the chamber.

When this apparatus is switched on, a small voltage is applied across these electrodes and the platinum made negatively charged with respect to the silver. The current which flows is at first negligible as the platinum becomes polarised but as the potential is increased, oxygen is reduced at the platinum surface and

the current then flowing is stoichiometrically related to the oxygen consumed at the cathode. As the oxygen in the solution decreases, the amount of current flowing decreases proportionally, and the electrical current generated by the reduction of oxygen at the cathode was plotted by the chart recorder at the 1mV range.

The solution was continually stirred to ensure even mixing of the dissolved oxygen in the solution at all times. This mixing was achieved using a small magnetic follower on top of the membrane. The movement of solution caused problems for the animals, however, which when loose in the chamber were swirled around or hit by the follower when they settled. To avoid undue stress to the animals, small mesh cages were pushed into the chamber to allow the animals to settle and remain clear of the stirrer. The cages were made of inert nylon mesh, and sewn together with nylon thread. They were sterilised every night in methanol to prevent bacterial growth and rinsed thoroughly before use. The mesh size allowed easy circulation of liquid, and allowed the oligochaetes to pass through the mesh and entwine themselves in the threads to remain away from the stirrer. It was found that after a few minutes movement, the *Gammarus* settled in the cages and did not attempt to swim.

Salt solutions were made up using ANALAR sodium chloride dissolved in tap water and aerated using porous air-tubes and aquarium pumps. The aeration was continued overnight in tightly sealed containers (to prevent evaporation) at 10^{0}C in a constant temperature room, which also contained the electrode equipment at the same temperature. The oxygen concentration of all solutions was checked before use with a portable oxygen electrode, to ensure that they were all fully saturated.

The saturation level of the solutions was between 0.31 and 0.32mM O_2/l. The equipment was always calibrated using the current experimental solution. To do this, 4ml of the solution were pipetted into the chamber and allowed to equilibriate with the vessel closed by a plastic stopper. The magnetic follower in the chamber was switched on and adjusted to give a fast, steady rate of stirring.

A "base-line" value was then set using the controls on the digital readout box to represent electrical zero. The 100% saturation level was set using the output control and these two readings were adjusted to allow the full-scale deflection between 0 and 100% saturation to fit comfortably onto the chart recording paper. A trace was recorded from the electrode for several hours, until a straight horizontal trace was obtained on the chart recorder. This line was marked as the 100% saturation point. The zero oxygen reading was then obtained using sodium dithionate crystals to remove oxygen from the solution in the test chamber. A small quantity of sodium dithionate crystals were added to the solution in the electrode chamber. The crystals consume oxygen according to the equation:

$$Na_2S_2O_4 + O_2 + H_2O \rightarrow NaHSO_4 + NaHSO_3$$

Once the trace was recording a steady line close to zero, the chart was marked. The electrodes were then cleaned by washing ten times in distilled water to remove all traces of $Na_2S_2O_4$. Fresh solution was added to the chamber and allowed to equilibriate until the trace was steady. The mesh animal cage and test animal was then carefully pushed into the solution, and checked to ensure that no

air bubbles were trapped in the mesh. The chamber was sealed with a plastic stopper and the test continued for 1 hour 10 minutes to allow 10 minutes for the animals to settle and produce a 1-hour steady trace.

To calculate the amount of oxygen consumed by the test animal, the number of divisions on the chart recorder paper between 0 and 100% oxygen saturation was first counted. The oxygen content of air-saturated water was then calculated using the method of Truesdale and Downing (1954) which gives a value of 0.341 umol/ml at 10^0C and normal atmospheric pressure. There is a small reduction predicted in the oxygen content of air saturated water as salinity increases, but since this was small, and all solutions were checked for oxygen concentration before use, it was discounted from calculations.

Since there were 4mls of solution in the test chamber there were therefore 1.364 umoles of oxygen available in the chamber. This value was divided by the number of division on the chat paper between 100% and 0% saturation to give the change in oxygen saturation represented by one division. The amount of oxygen used by the test organism in one hour could then be calculated by subtracting the reading after one hour from the initial reading and multiplying this figure by the number of micromoles of oxygen represented by each division. The first ten minutes of each recording were discounted, to avoid recording oxygen used before the test organism had settled, and the last ten minutes were discarded in case of oxygen depletion at this time. The traces on the chart paper were found to be almost straight lines during the one-hour of the tests, indicating little change in the rate of oxygen uptake during the hour.

After removal from the test chamber, the animals were

immediately killed by immersing them in methanol. *Gammarus* were killed singly and oligochaetes in the groups of ten used for each test. The animals were then allowed to air dry for one week (or until a steady weight was obtained) and weighed on a Cahn mocrobalance. The oxygen consumption for each animal or group could then be calculated per mg dry weight.

All animals which were used in the tests were selected from stock cultures and appeared healthy. In *Tubifex tubifex* and *Lumbricillus rivalis* large mature worms were selected. The dry weight of the *T. tubifex* varied between 0.28 and 1.04mg per worm, and in *L. rivalis* between 0.2 and 0.61mg per worm. As oxygen uptake weights could not be related to specific individuals in these species, but were means of groups of ten, the oxygen uptake per mg dry weight of each group was calculated, and the mean values for the replicates in each salinity compared using one and two-way analysis of variance.

In the three *Gammarus* species, individuals of weights varying between 0.3 and 10mg dry weight were selected, as shown in Table 6:1. The dry weight of most individuals was in the range 0.3 - 8.0 mg, and although some *G. pulex* and *G. tigrinus* were larger than this, there were sufficient individuals of these species within the required weight range. The *G. zaddachi* in the stock culture were, however, much more uniform in size, and fewer individuals above 3 mg and below 0.5 mg dry weight were available for use. Specimens were selected to provide a comparable weight range to the other two species, but there was a greater concentration of replicates within the 1-2 mg weight range. This meant that, although the weight range used in the experiments for all three species is similar, (Table 6:1) the means

TABLE 6:1

Dry Weight Range (in mg) of *Gammarus* spp. used in Respiration Experiments

1 hour Acclimation n = 20

NaCl (mM)	*G. pulex* max	*G. pulex* min	*G. tigrinus* max	*G. tigrinus* min	*G. zaddachi* max	*G. zaddachi* min
1	5.046	0.325	10.48	0.328	3.569	0.743
28	6.994	0.426	9.925	0.566	7.656	0.536
56	8.902	0.356	6.643	0.388	4.853	0.754
85	9.660	0.337	7.258	0.459	3.565	0.640
113	12.73	0.238	8.908	0.402	8.830	0.734

3 day Acclimation

NaCl (mM)	*G. pulex* max	*G. pulex* min	*G. tigrinus* max	*G. tigrinus* min	*G. zaddachi* max	*G. zaddachi* min	
28	5.280	0.283	6.940	0.513	1.199	0.376	n = 10
56	6.435	0.241	2.240	0.803	2.100	0.327	n = 7

were often significantly different when tested using *t*-tests. Mean respiration rates could not, therefore, be compared using *t* tests or analysis of variance, as the distribution of weights was not equal in each case. The comparisons between respiration rates were therefore performed via regression lines relating respiration rates to dry weight, a method employed by several authors (eg McClusky, 1969; Rumpus and Kennedy, 1974; Wright and Wright, 1976; O'Connor *et al.*, 1985).

All test animals were acclimated to the experimental solutions for one hour or three days in large plastic beakers. All the animals were fed on trout-fry pellets during acclimation and thoroughly rinsed before use to remove any clinging food particles. Any *Gammarus* which moulted during acclimation were not used in the experiments. All the animals were apparently healthy after one hour acclimation, but after three days at 56mM NaCl, many *G. tigrinus* were dead or moribund.

6:3 Results

6:3:1 *Gammaridae*

Since the relationship between respiration rate and dry weight of *Gammarus* spp. was curvilinear, the data were $\log_{10}$ transformed and a $\log_{10}$ x $\log_{10}$ plot derived to produce linear results. The original data with the $\log_{10}$ line can be seen in Figure 6:1. (The data are for *Gammarus pulex* at the 1mM NaCl concentration).

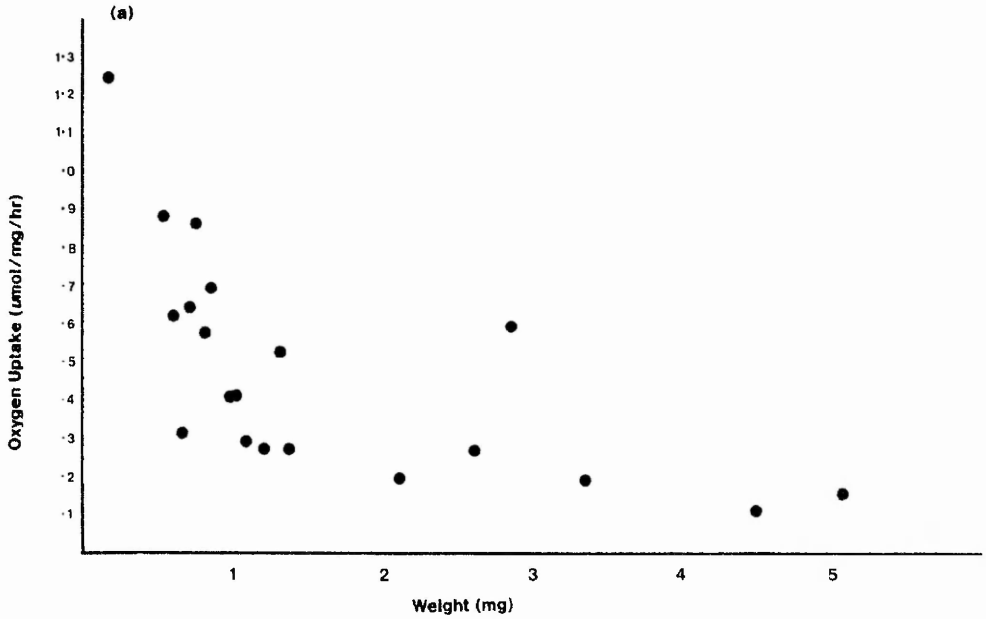
The regression equations for each calculated line are shown in Table 6:2. All the lines for the one-hour acclimated animals (with 20 replicate measurements) were

FIGURE 6:1

Oxygen Consumption ($\mu molmg^{-1}h^{-1}$) of *Gammarus pulex* in 1mM NaCl solution against dry weight (mg)

a) Untransformed data

b) Log_{10} transformed data together with the regression line calculated from the data


(a)
Oxygen Uptake (μmol/mg/hr)
1·3
1·2
1·1
·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
1
2
3
4
5
Weight (mg)

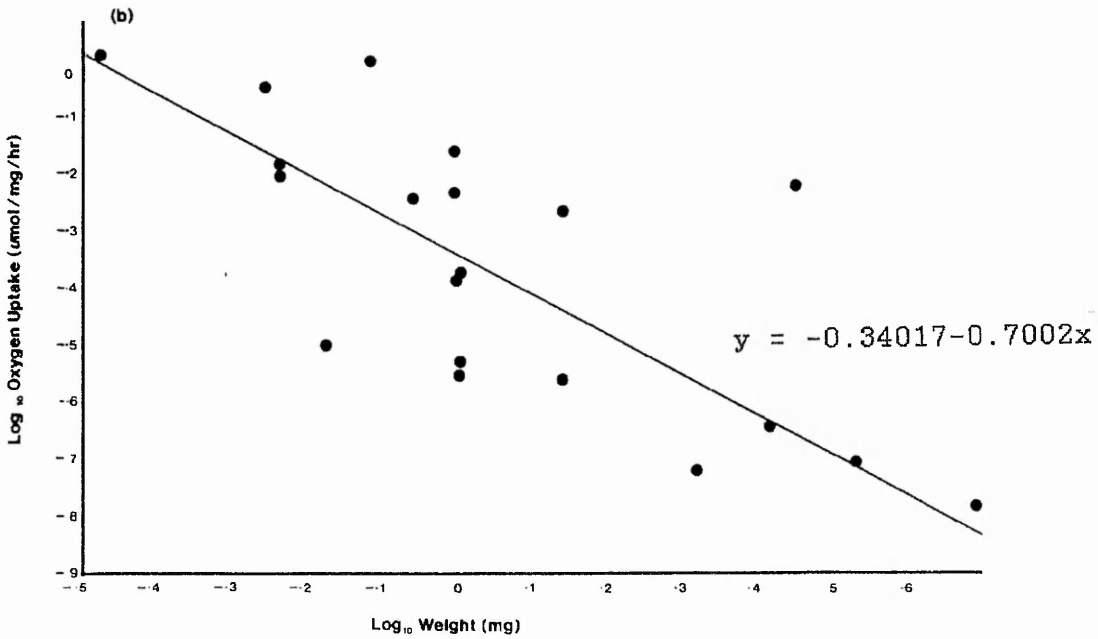

(b)
Log ₁₀ Oxygen Uptake (μmol/mg/hr)
0
−1
−2
−3
−4
−5
−6
−7
−8
−9
−5
−4
−3
−2
−1
0
·1
·2
·3
·4
5
·6
Log₁₀ Weight (mg)
y = -0.34017-0.7002x

TABLE 6:2

Regression Equations Relating Body Weight (x) to Oxygen Uptake (y) using $\log_{10}$ Transformed data for Three Species of *Gammarus* acclimated for 1 hour or 3 days to increasing NaCl concentrations The Variance Ratio, F, and the significance Level of the Relationship are given

1) *Gammarus pulex*

n = 20

NaCl (in mM)	Equation	F	Sig Level	
1	y=-0.34017-0.7002x	36.53	1%	
28	y=-0.31380-0.9389x	90.11	1%	
56	y=-0.33199-0.9341x	181.86	1%	
85	y=-0.29281-1.0045x	485.31	1%	
113	y=-0.23012-1.0857x	489.57	1%	
3 day-acclimation				
28	y=-0.3181-0.6648x	22.83	1%	n = 10
56	y=-0.0126-0.1928x	9.35	5%	N = 7

TABLE 6:2 (COntinued)

Regression Equations Relating Body Weight (x) to Oxygen Uptake (y) using $\log_{10}$ Transformed data for Three Species of *Gammarus* acclimated for 1 hour or 3 days to increasing NaCl concentrations
The Variance Ratio, F, and the significance Level of the Relationship are given

2) *G. tigrinus*

n = 20

NaCl (in mM)	Equation	F	Sig Level	
1	y=-0.42984-0.9259x	179.97	1%	
28	y=-0.38546-0.7676x	139.38	1%	
56	y=-0.36388-0.9331x	377.64	1%	
85	y=-0.37526-0.9042x	199.94	1%	
113	y=-0.34134-0.9517x	184.52	1%	
3 day acclimation				
28	y=-0.45063-0.7542x	17.10	5%	n = 10
56	y=-0.37519-0.9561x	4.93	NS	n = 7

TABLE 6:2 (Continued)

Regression Equations Relating Body Weight (x) to Oxygen Uptake (y) using $\log_{10}$ Transformed data for Three Species of *Gammarus* acclimated for 1 hour or 3 days to increasing NaCl concentrations
The Variance Ratio, F, and the significance Level of the Relationship are given

3) *Gammarus zaddachi*

n = 20

NaCl (in mM)	Equation	F	Sig Level	
1	y=-0.32133-0.8671x	69.85	1%	
28	y=-0.35122-0.7622x	100.71	1%	
56	y=-0.36246-0.6738x	45.22	1%	
85	y=-0.33976-0.8179x	128.79	1%	
113	y=-0.38899-0.8113x	45.28	1%	
3-day acclimation				
28	y=-0.33816-0.2740x	0.52	NS	n = 10
56	y=-0.24446-0.9551x	41.24	1%	n = 7

significant at the 1% level, but two lines for the 3-day acclimated animals (with 10 and 7 replicates) were not significant.

There was a strong negative relationship between respiration rate and weight in all species at all NaCl concentrations, that is, the smaller individuals had a much higher oxygen consumption per mg body weight than larger individuals. The calculated regression lines are shown in Figure 6:2. It is apparent from these lines that there was not a consistent relationship between respiration rate in each species and increasing salinity when the differences were compared over a wide weight range, as in Figure 6:3. To test for any significance in the regression coefficients, the method described by Parker (1979) was used, which compares the variances around the points on each line with the regression coefficients, and calculates F-values with 1 and N-2 degrees of freedom (N=the total numbers of points on both lines). The regression coefficients within a species were first compared with the coefficient for the 1mM line of that species, then the differences between species at each concentration were compared (Tables 6:3 and 6:4).

In *G. pulex*, after 1 hour's acclimation, the 85mM and 113mM regression lines were significantly different from the 1mM regression line, but this was the only significant difference between regression lines in any species as NaCl concentration increased. After three days's acclimation, there was no change in the respiration rate at 28mM NaCl, but in *G. pulex* there was a significant change at 56mM NaCl. The lack of significance when testing regression lines was also found by Rumpus and Kennedy (1974) studying the effects of parasitism on *G. pulex*, despite differences in mean

FIGURE 6:2

Regression lines relating Log_{10} Oxygen Uptake of *Gammarus pulex*, *Gammarus tigrinus* and *Gammarus zaddachi* to Log_{10} dry weight

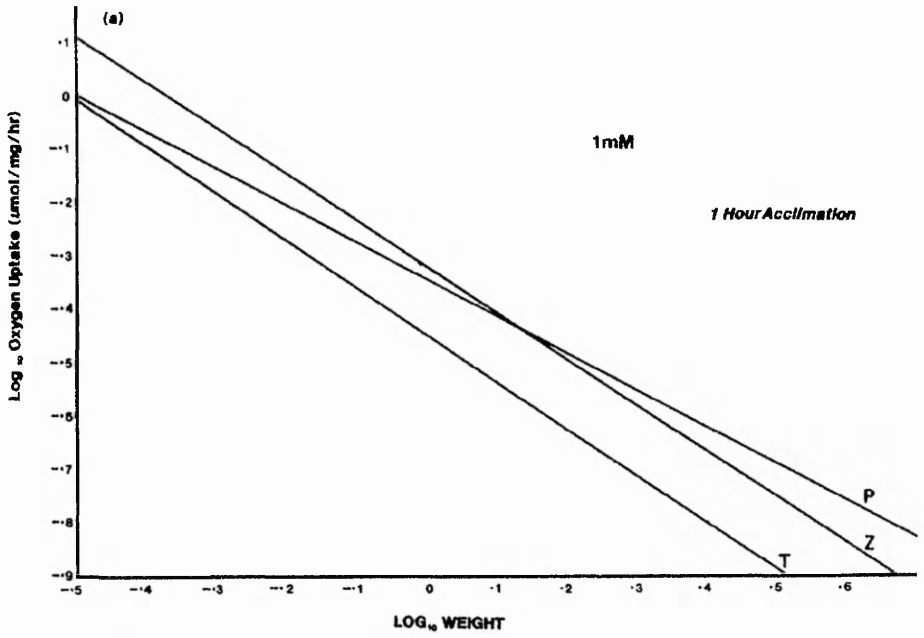
a) After 1 hour acclimation to 1mM NaCl

b) After 1 hour acclimation to 28mM NaCl

P = *Gammarus pulex*

T = *Gammarus tigrinus*

Z = *Gammarus zaddachi*


(a)
1mM
1 Hour Acclimation
P
Z
T
Log ₁₀ Oxygen Uptake (μmol/mg/hr)
LOG₁₀ WEIGHT

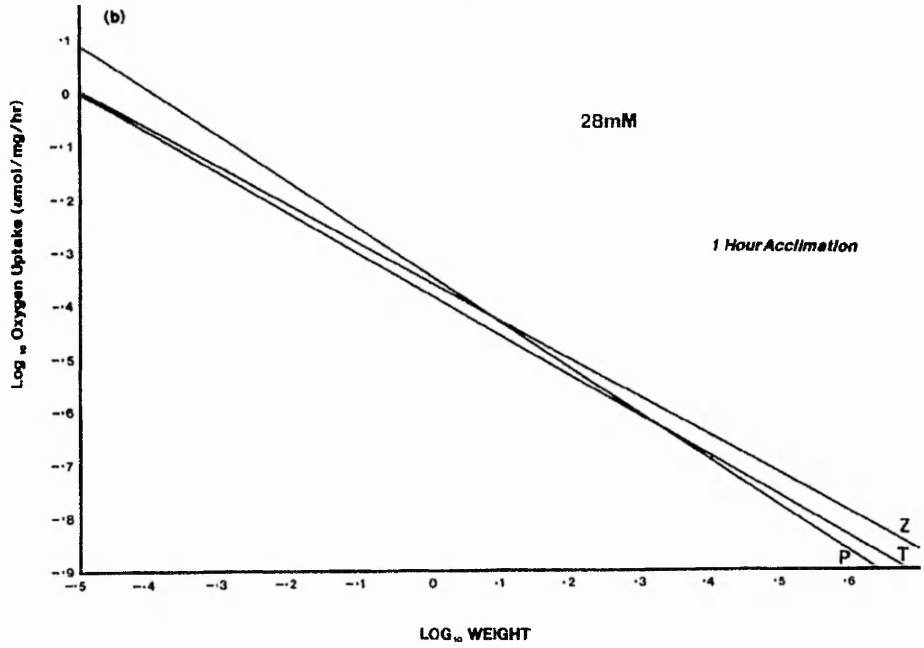

(b)
28mM
1 Hour Acclimation
Z
T
P
Log ₁₀ Oxygen Uptake (μmol/mg/hr)
LOG₁₀ WEIGHT

FIGURE 6:2

Regression lines relating Log_{10} Oxygen Uptake of *Gammarus pulex*, *Gammarus tigrinus* and *Gammarus zaddachi* to Log_{10} dry weight

c) After 1 hour acclimation to 56mM NaCl

d) After 1 hour acclimation to 85mM NaCl

P = *Gammarus pulex*

T = *Gammarus tigrinus*

Z = *Gammarus zaddachi*

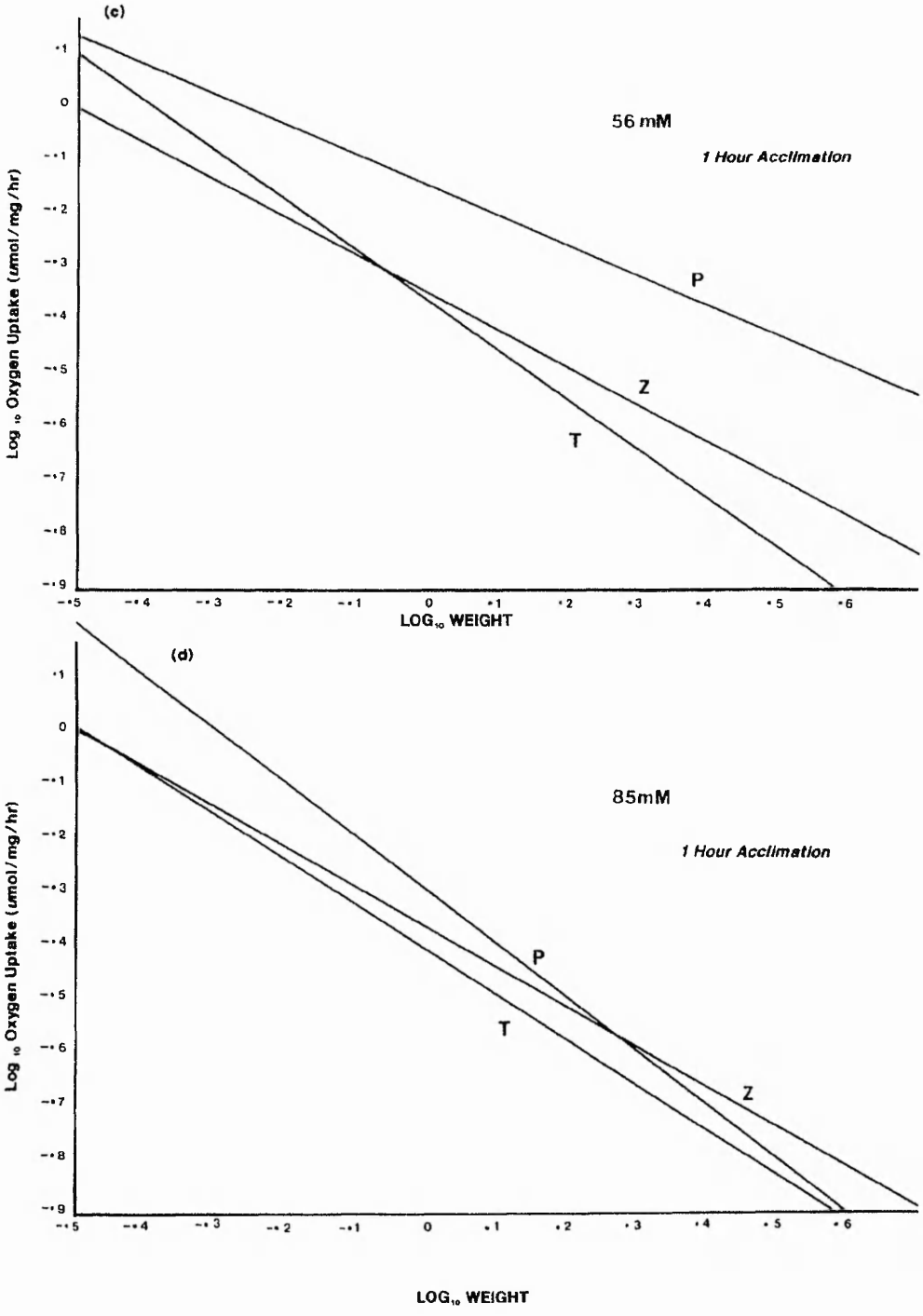

(c)
56 mM
1 Hour Acclimation
P
Z
T
Log ₁₀ Oxygen Uptake (umol/mg/hr)
LOG₁₀ WEIGHT
(d)
85mM
1 Hour Acclimation
P
T
Z
Log ₁₀ Oxygen Uptake (umol/mg/hr)
LOG₁₀ WEIGHT

FIGURE 6:2

Regression lines relating Log_{10} Oxygen Uptake of *Gammarus pulex*, *Gammarus tigrinus* and *Gammarus zaddachi* to Log_{10} dry weight

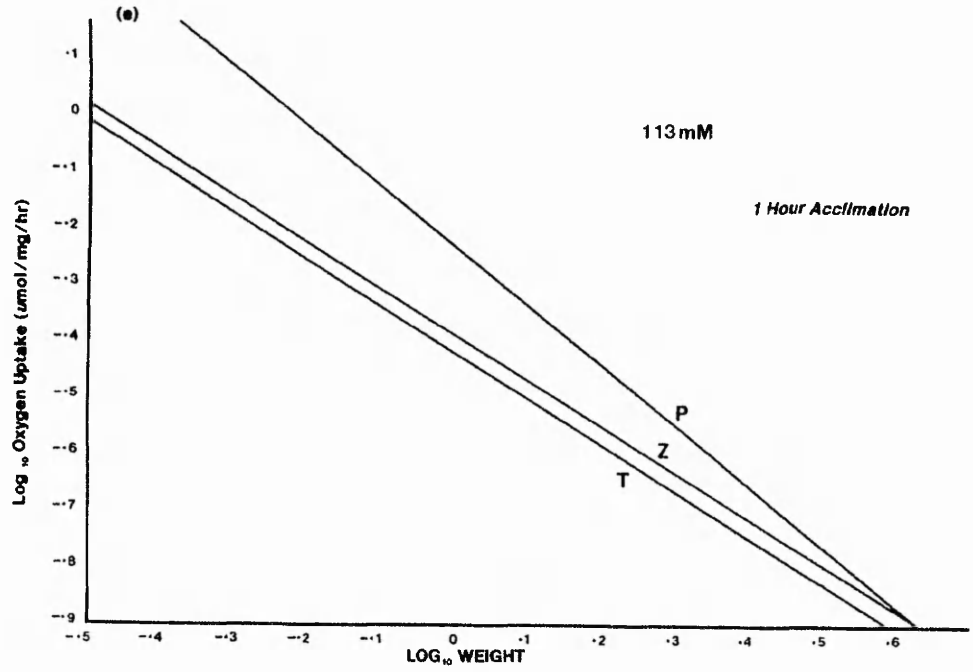
e) After 1 hour acclimation to 113mM NaCl

f) *Gammarus pulex*after 3 day acclimation to 1, 28 and 56mM NaCl

P = *Gammarus pulex*

T = *Gammarus tigrinus*

Z = *Gammarus zaddachi*


(e)
113mM
1 Hour Acclimation
P
Z
T
Log ₁₀ Oxygen Uptake (umol/mg/hr)
LOG₁₀ WEIGHT

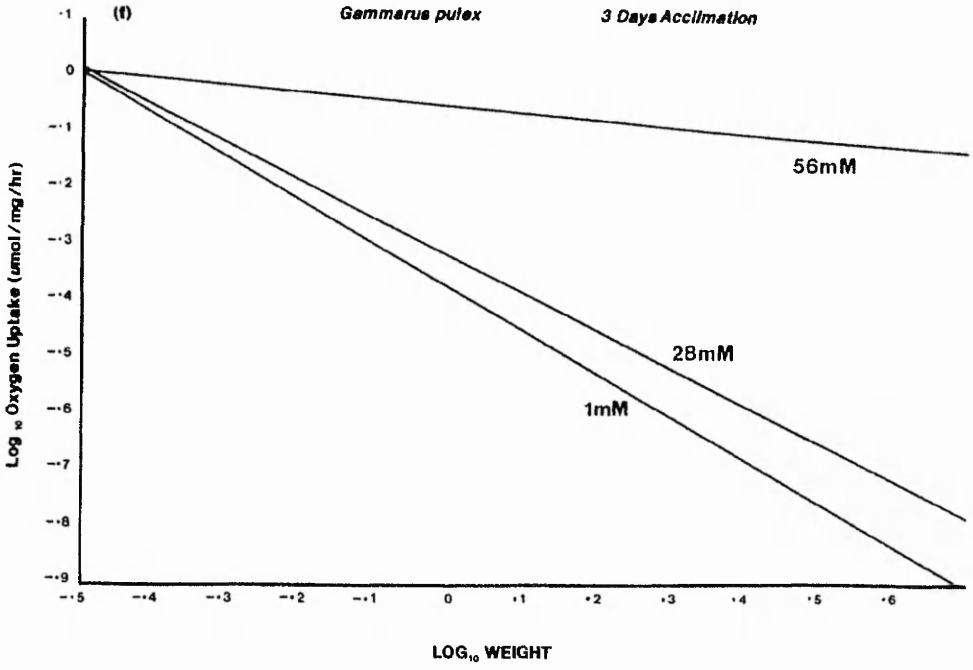

(f)
Gammarus pulex
3 Days Acclimation
56mM
28mM
1mM
Log ₁₀ Oxygen Uptake (umol/mg/hr)
LOG₁₀ WEIGHT

FIGURE 6:2

Regression lines relating Log_{10} Oxygen Uptake of *Gammarus pulex*, *Gammarus tigrinus* and *Gammarus zaddachi* to Log_{10} dry weight

g) *Gammarus tigrinus*after 3 day acclimation to 1, 28 and 56mM NaCl

h) *Gammarus zaddachi* after 3 day acclimation to 1, 28 and 56mM NaCl

P = *Gammarus pulex*

T = *Gammarus tigrinus*

Z = *Gammarus zaddachi*

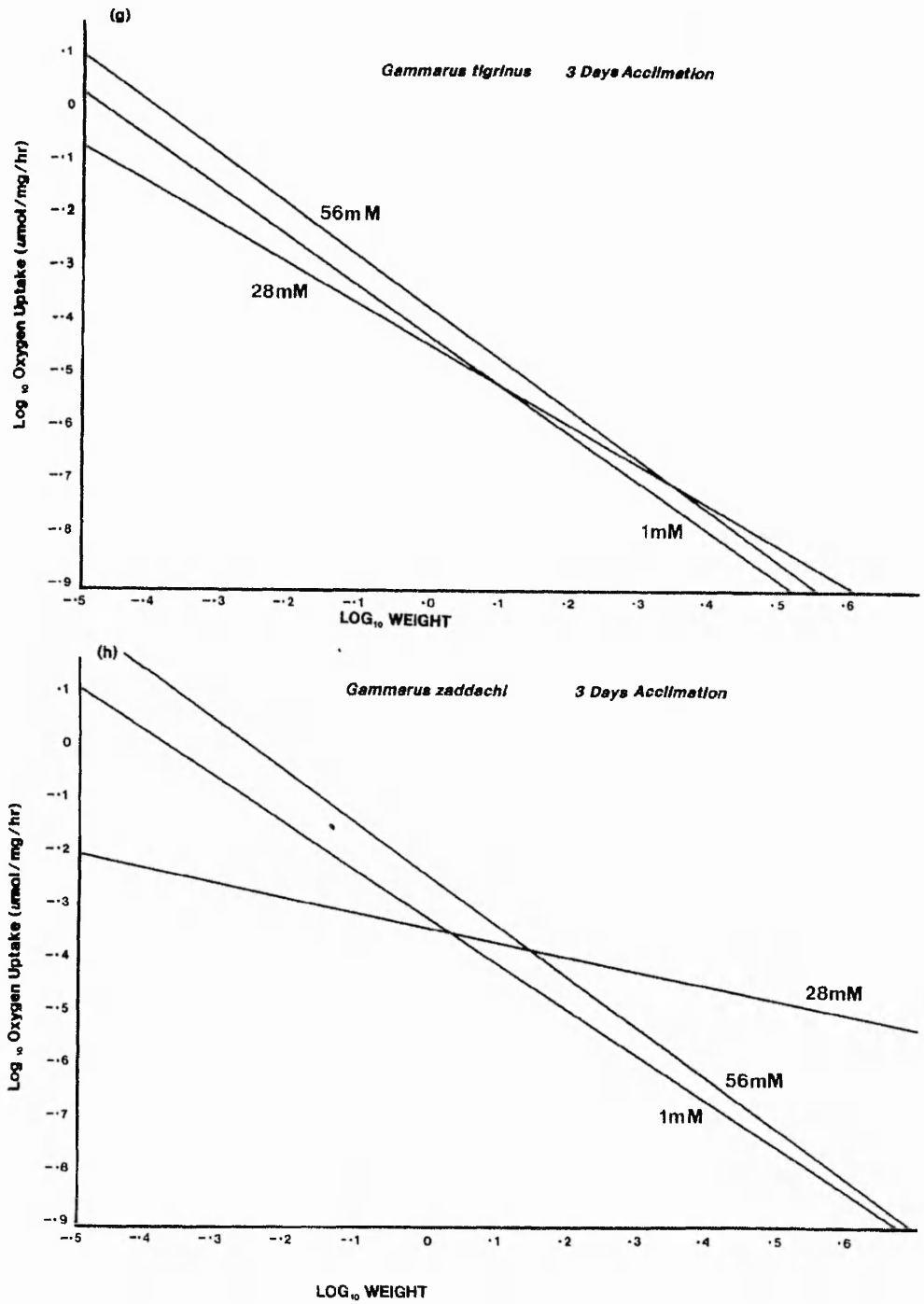

(g)
Gammarus tigrinus 3 Days Acclimation
56m M
28mM
1mM
Log ₁₀ Oxygen Uptake (µmol/mg/hr)
LOG₁₀ WEIGHT
(h)
Gammarus zaddachi 3 Days Acclimation
28mM
56mM
1mM
Log ₁₀ Oxygen Uptake (µmol/mg/hr)
LOG₁₀ WEIGHT

TABLE 6:3

Respiration rates ($\mu molmg^{-1}h^{-1}$)
of three species of *Gammarus* at 6 weights
calculated from the Regression Equations
shown in Table 6:2

One hour Acclimation

NaCl (mM)	1			28			56		
Wt	P	T	Z	P	T	Z	P	T	Z
0.1	2.3	3.1	3.5	4.2	2.4	2.6	4.0	3.7	2.1
0.3	1.1	1.1	1.4	1.5	1.0	1.1	1.43	1.3	0.98
0.5	0.74	0.71	0.87	0.93	0.70	0.75	0.89	0.82	0.69
1.0	0.46	0.37	0.48	0.48	0.41	0.45	0.47	0.43	0.43
2.0	0.28	0.19	0.26	0.25	0.24	0.26	0.24	0.23	0.27
5.0	0.15	0.15	0.12	0.11	0.12	0.13	0.10	0.10	0.15

NaCl (mM)	85			113		
Wt	P	T	Z	P	T	Z
0.1	5.2	3.4	3.0	7.2	2.0	2.6
0.3	1.7	1.3	1.2	2.2	1.43	1.08
0.5	2	0.79	0.80	1.3	0.60	0.72
1.0	0.51	0.42	0.46	0.59	0.46	0.41
2.0	0.25	0.23	0.26	0.28	0.21	0.23
5.0	0.10	0.10	0.12	0.10	0.11	0.11

P = *Gammarus pulex*: T = *Gammarus tigrinus*: Z = *Gammarus zaddachi*

TABLE 6:3 (Continued)

Respiration rates ($\mu molmg^{-1}h^{-1}$)
of three species of *Gammarus* at 6 weights
calculated from the Regression Equations
shown in Table 6:2

Three-day Acclimation

NaCl (mM)	28			56		
Wt	P	T	Z	P	T	Z
0.1	2.22	2.01	0.86	1.02	3.81	5.14
0.3	1.07	0.88	0.64	1.22	1.33	1.8
0.5	0.76	0.60	0.55	1.11	0.82	1.1
1.0	0.48	0.35	0.46	0.62	0.42	0.57
2.0	0.30	0.21	0.38	0.85	0.22	0.29
5.0	0.16	0.11	0.29	0.71	0.09	0.12

P = *Gammarus pulex*: T = *Gammarus tigrinus*: Z = *Gammarus zaddachi*

TABLE 6:4

Comparison of regression coefficients calculated for the oxygen uptake ($\mu molmg^{-1}h^{-1}$) of three species of *Gammarus* in 1mM NaCl and four higher NaCl solutions

Species	*G. pulex*			*G. tigrinus*			*G. zaddachi*		
1) 1 hour acclimation									
NaCl (mM)	F	DF	Sig	F	DF	Sig	F	DF	Sig
28	2,5	1,36	NS	2.6	1,49	NS	O.6	1,40	NS
56	3.2	1,42	NS	0.0	1,49	NS	1.4	1,40	NS
85	7.0	1,35	5%	0.1	1,48	NS	0.2	1,38	NS
113	11.2	1,44	1%	0.7	1,50	NS	0.1	1,38	NS
2) 3-Day Acclimation									
NaCl (mM)	F	DF	Sig	F	DF	Sig	F	DF	Sig
28	2.8	1,27	NS	0.0	1,32	NS	3.1	1,28	NS
56	36.4	1,36	1%	0.0	1,29	NS	2.3	1,29	NS

respiration rates when specific weights were compared.

To compare the weight-specific oxygen uptake rates more directly, therefore, the respiration rates for each species at six different weights in all the NaCl concentrations were calculated from the regression lines (Table 6:5). The calculated rates for 0.1mg animals (slightly above the size at which individuals are released from the brood pouch - see Chapter 3) were very high, up to 70 times the rate per mg of a 5mg individual of *G. pulex* in 113mM NaCl. The rates at higher weights became progressively more similar, and in 5mg animals were little different in any species or NaCl concentration.

When the rates for three weights (0.3, 1.0 and 5.0mg) were plotted against NaCl concentration, as shown in Figure 6:3, the differences between the species can be seen to be largest at 0.3mg. At this weight, *G. pulex* had the highest rate above 28mm NaCl, and the lowest at 1mM. *G. tigrinus* at 1mM had a rate mid-way between that of *G. pulex* and *G. zaddachi*, but the lowest rate at 28mM NaCl. Above this concentration, the oxygen uptake rate in *G. tigrinus* increased until it was above that for *G. zaddachi*. The oxygen uptake rate in *G. zaddachi* was highest in 1mM NaCl, then decreased in 58mM, after which the rate fluctuated.

At 1.0mg, the relationship between the species was similar, except that at 1mM NaCl, *G. tigrinus* had a slightly higher rate that *G. pulex*. At 5.0mg, the pattern for *G. pulex* and *G. tigrinus* was almost identical, with *G. zaddachi* having a lower rate at 1mM NaCl, and a higher rate at 58mM NaCl. The rate at 120mM NaCl was almost identical in all three species.

The rates in 28 and 56mM NaCl after 3 days acclimations

TABLE 6:5

Comparison of Regression coefficients between three *Gammarus* species at Five NaCl concentrations The Variance Ratio, F, Degrees of Freedom (DF) and Significance Level of F are shown

Comparison	P x T			P x Z			T x Z		
NaCl (mM)	F	DF	Sig	F	DF	Sig	F	DF	Sig
0	3.0	1,43	NS	0.7	1,38	NS	0.1	1,45	NS
28	2.2	1,4	NS	1.8	1,38	NS	0.0	1,44	NS
56	0.0	1,48	NS	4.0	1,44	5%	6.3	1,44	5%
85	1.6	1,40	NS	3.2	1,35	NS	0.5	1,41	NS
113	2.4	1,51	NS	4.4	1,44	5%	1.0	1,43	N

P = *G. pulex*

T = *G. tigrinus*

Z = *G. zaddachi*

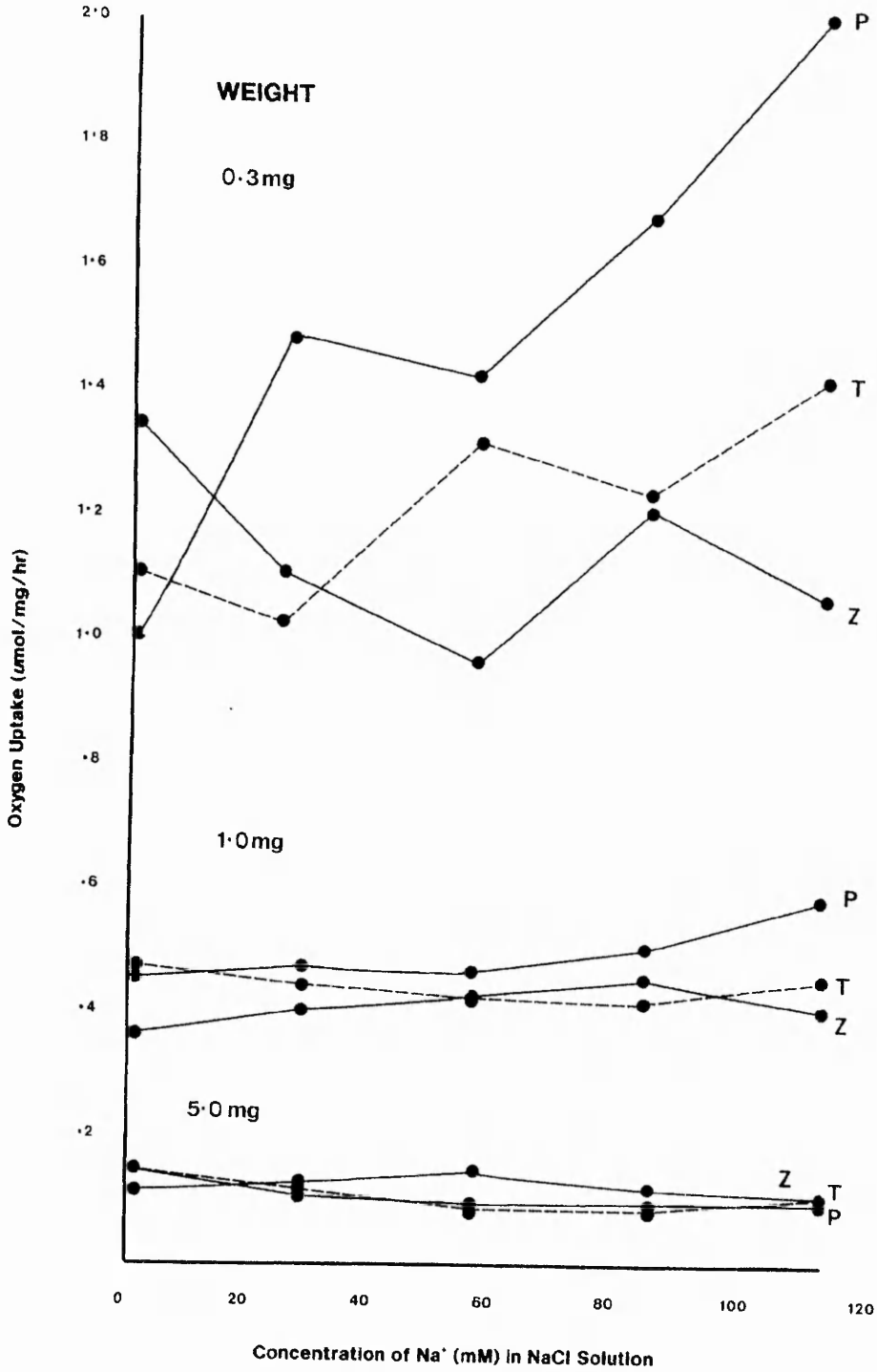
FIGURE 6:3

Oxygen uptake of *Gammarus pulex*, *Gammarus tigrinus* and *Gammarus zaddachi* at three different weights calculated from regression lines

P = *Gammarus pulex*

T = *Gammarus tigrinus*

Z = *Gammarus zaddachi*


WEIGHT
0·3mg
1·0mg
5·0 mg
P
T
Z
Oxygen Uptake (umol/mg/hr)
2·0
1·8
1·6
1·4
1·2
1·0
·8
·6
·4
·2
0
20
40
60
80
100
120
Concentration of Na⁺ (mM) in NaCl Solution

are shown in Table 6:3. There was little change in the 28mM solution in any species. In 56mM solutions, however, *G. pulex* had a reduction in respiration rate in smaller individuals, but an increase in larger ones. There was little difference in the rates of *G. tigrinus*, but *G. zaddachi* showed an increase at low weight, and a decrease at higher weights.

6:3:2 *Tubifex tubifex* and *Lumbricillus rivalis*

The oxygen consumption of *T. tubifex* in increasing NaCl concentrations is shown in Table 6:6 . and Figure 6:4a shows the mean oxygen consumption in each NaCl concentration together with the 95% confidence limits for the mean. The oxygen consumption per mg can be seen to rise to a maximum in 56mM NaCl, then decrease, although the rate at 141mM NaCl was still greater than that at 1mM.

The eleven replicate values for oxygen consumption in each of the NaCl concentrations were tested in a two-way analysis of variance to determine if there was any significant difference between the respiration rate in the increasing NaCl concentrations and also within each NaCl concentration. The result is shown in Table 6:7. There was a significant difference in respiration rate both between the NaCl concentrations, and within the replicates at each treatment. This difference within the replicates was probably due in part variations in the size of worms used in tests, and since the between treatment differences were highly significant, the differences between respiration rates at each NaCl level were analysed using one-way analysis of variance. This tested whether there were significant differences between

FIGURE 6:4

Oxygen consumption ($umolmg^{-1}h^{-1}$) of *Tubifex tubifex* and *Lumbricillus rivalis* in increasing salinity

Mean and 95% confidence limits are shown

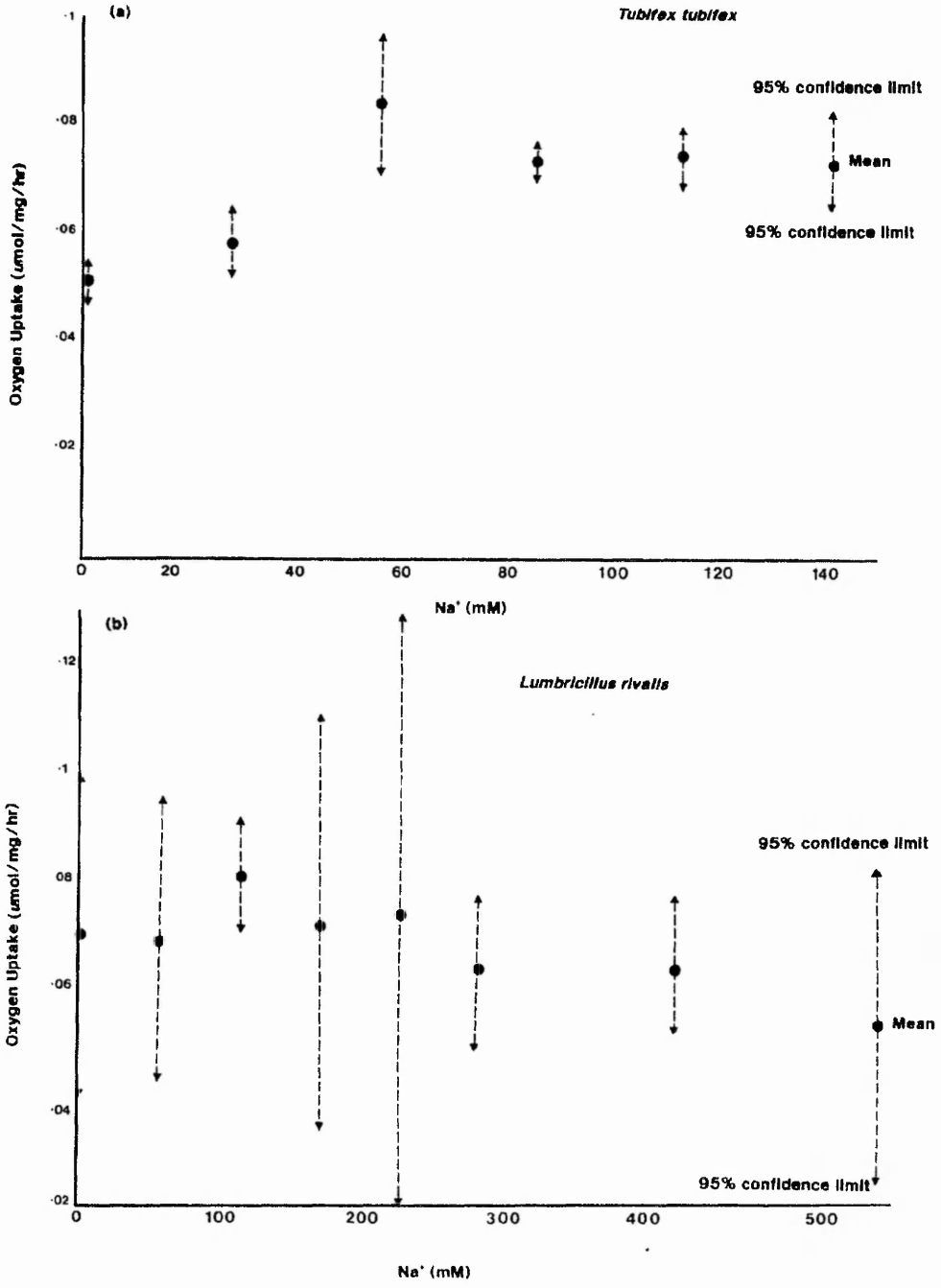

(a)
Tubifex tubifex
95% confidence limit
Mean
95% confidence limit
Oxygen Uptake (umol/mg/hr)
·1
·08
·06
·04
·02
0
20
40
60
80
100
120
140
Na⁺ (mM)
(b)
Lumbriculus rivalis
95% confidence limit
Mean
95% confidence limit
Oxygen Uptake (umol/mg/hr)
·12
·1
08
·06
·04
·02
0
100
200
300
400
500
Na⁺ (mM)

TABLE 6:6

Mean, Minimum and Maximum Oxygen consumption

($\mu molmg^{-1}h^{-1}$)

of *Tubifex tubifex in increasing salinity*

NaCl (mM)	Mean	Max	Min	95% CL (upper)	95% CL (lower)	SD
1	0.0513	0.0598	0.0416	0.0558	0.0468	0.0660
28	0.0585	0.0699	0.0428	0.0655	0.0521	0.0867
56	0.0840	0.1096	0.0582	0.0974	0.0685	0.0170
85	0.0738	0.0816	0.0586	0.0785	0.0685	0.0681
113	0.0759	0.0897	0.0639	0.0805	0.0689	0.0089
141	0.0733	0.0846	0.0620	0.0835	0.0659	0.0084

95% CL = 95% confidence limits for the mean

Means are from 11 replicates using 10 worms in each.

TABLE 6:7

Two-Way Analysis of Variance of Oxygen Consumption of *Tubifex tubifex* in 6 NaCl Concentrations showing Degrees of freedom, Sum of Squares, Mean square and Variance Ratio (F), together with the significance level of F

Source	DF	SS	MS	F	Sig
Between Concs	5	0.008	0.002	79.4	1%
Within Concs	10	0.005	0.0005	25.2	1%
Error	50	0.001	0.00002		
Total	65	0.014			

TABLE 6:8

F Values and significance levels
(1 and 21 degrees of freedom)
from One-Way Analysis of Variance between Respiration rates at Different Salinities in *Tubifex tubifex*

NaCl (mM)	28	56	85	113	141
1	63.1	34.3	61.5	53.5	15.2
Significant at	1%	1%	1%	1%	1%
28		19.0	21.3	21.4	16.7
Significant at		1%	1%	1%	1%
56			3.1	1.8	3.2
Significant at			NS	NS	NS
85				0.4	0.0
Significant at				NS	NS
113					0.5
Significant at					NS

each concentration despite the significant within concentration difference. The results are shown in Table 6:8.

The analyses showed that the respiration rates in the NaCl concentrations above 56mM were not significantly different from each other, but were significantly higher than the rates in both the 1mM and 28mM NaCl concentrations, and that respiration rate in 28mM NaCl was higher than in 1mM NaCl.

The respiration rate in all NaCl concentrations is shown in Table 6:9, and the means and 95% confidence limits are plotted against NaCl concentration in Figure 6:4b. The results for each concentration were analysed in a two-way analysis of variance, as used for *T. tubifex*, and the results of this analysis are shown in Table 6:10. It can be seen that in *L. rivalis* there was no significant difference in respiration rate between the NaCl concentrations, and neither was there any significant difference between the replicates in each concentration. The range of respiration rates found between replicates was greatest in the 564mM concentration, when one group of worms had a very low respiration rate.

The mean respiration rate in each NaCl concentration was similar to the corresponding rate in *T. tubifex*, but slightly higher, probably due to the smaller size of *L. rivalis* used in the experiments.

6:4 Discussion

The oxygen uptake rates derived using the method in this study are compared with those found by previous authors (using different methods) in Table 6:11. All the figures

TABLE 6:9

Oxygen consumption ($\mu molmg^{-1}h^{-1}$)

of Lumbricillus rivalis in increasing salinities

NaCl (mM)	Mean	Max	Min	95% CL (upper)	95% CL (lower)	SD
1	0.070	0.091	0.049	0.100	0.039	0.019
56	0.069	0.092	0.053	0.096	0.043	0.016
113	0.081	0.089	0.074	0.092	0.071	0.064
170	0.072	0.096	0.051	0.111	0.034	0.024
226	0.074	0.099	0.048	0.129	0.019	0.022
282	0.064	0.072	0.055	0.078	0.049	0.009
423	0.064	0.072	0.055	0.078	0.049	0.009
564	0.054	0.086	0.011	0.103	0.004	0.003

95% CL = 95% Confidence Limits

SD = Standard deviation of the Mean

Figures are calculated from 4 replicates of 10 worms each

TABLE 6:10

Two-Way Analysis of Variance of Respiration Rates of *Lumbricillus rivalis* at increasing NaCl Concentrations Showing Degrees of Freedom, Sum of Squares, Mean Square Variance Ratio (F) and Significance Level of F

Source	DF	SS	MS	F	Sig
Between Concs	7	0.0007	0.0001	0.3	NS
Within Concs	3	0.0018	0.004	1.0	NS
Error	21	0.0076	0.0004		
Total	31	0.009			

in the Table have been converted from their original units to uMmg dry weight^{-1}h^{-1}, and are from experiments conducted either in fresh water or in very dilute sea water solutions (less than 10mM chloride). The figures obtained are similar to those from the present work in *G. pulex*, *G. zaddachi* and *T. tubifex*, although the rates found were slightly higher in this study than in previous work. The respiration rates calculated by previous authors for *G. tigrinus* were much more variable, which is unsurprising given the poor response of this species to handling. In this study, however, the oxygen uptake rates by this species were similar to those of *G. pulex* and *G. zaddachi*.

In *T. tubifex*, respiration per mg was lower than in any of the *Gammarus* species. The rate did, however, increase significantly as the salinity of the external solution was raised, reaching a maximum rate at 56mM NaCl in the external solution. Above this level, the respiration rate fell slightly, although still remaining higher than in 1mM. From previous experiments, *T. tubifex* was known to be unable to reproduce successfully in NaCl concentrations above 56mM, and in 28mM solutions to produce fewer young and smaller adults than in 1mM (Chapter 4). It would seem, therefore, that this species was using increasing amounts of respiratory energy to regulate internal conditions as external salinity rose. Above a salinity of approximately 56mM NaCl, however, it was unable to compensate further, and death followed. The high respiration rate at 56mM NaCl, a concentration at which this species cannot reproduce successfully, suggested that at this point, so much energy was being channeled into osmoregulation that the individuals had no further resources for reproduction. Also, if, as in

TABLE 6:11

Oxygen Uptake Rates of *Gammarus* spp. and Tubificidae from Published Literature

Figures are Converted to $\mu molmg^{-1}h^{-1}$ where appropriate

Species	Conditions wt	temp	Rate $\mu molmg^{-1}h^{-1}$	Authors
T. tubifex		20°C	0.03	Brkovic-Popovic 1972
T. tubifex		Room	0.03	Whitley & Sikora 1970
T. tubifex		10°C	0.02	Brinkhurst *et al* 1983
T. tubifex		10°C	0.05	Present study
L. hoffmeisteri		pH4	0.01	Chapman *et al* 1982
G. tigrinus	10mg	15°C	0.56	Dorgelo 1973
G. tigrinus	1mg	20°C	0.11	O'Connor *et al* 1985
G. tigrinus	5mg	10°C	0.10	This study
G. pulex	1mg	10°C	0.40	Wright & Wright 1974
G. pulex	1mg	10°C	0.36	Rumpus & Kennedy 1974
G. pulex	1mg	10°C	0.46	This study
G. zaddachi	2mg	15°C	0.31	Bulnheim 1972
G. zaddachi	2mg	15°C	0.26	This study

Gammarus spp., smaller individuals have higher specific respiration rates, the juvenile Tubificidae at 56mM NaCl would have an extremely high respiration rate, and juveniles did not, in fact, survive at this NaCl concentration. In Millwood Brook, Tubificidae were not found at any sites with a mean annual NaCl concentration of above 28mM, and it would seem that this approximates to the upper range at which Tubificidae were able to successfully reproduce.

Tubificidae generally feed with their anterior segments buried in sediments, and most gaseous exchange is performed across the body wall of the posterior segments, which have a convoluted surface to increase the surface area for gaseous exchange (Kaster and Wolfe, 1982). The worms also perform rhythmic respiratory movements with their posterior segments to facilitate oxygen uptake, which would be expected to increase as respiration increases, and further reduce the energy available for growth and reproduction in increasing salinities.

Lumbricillus rivalis, in contrast, showed no clear change in respiration rate at any of the salt concentrations used. The rates at 282 and 423mM were lower than the rates in less saline solutions, but not significantly so, and the decrease in respiration rate at 564mM was due to approaching death of some worms at these concentrations. This species did not seem to be using extra respiratory energy for internal ion and water regulation as the salinity of the external solution increased, and it is possible that this species adapts to a wide range of salinities by tolerating very variable internal ionic conditions. In Millwood Brook, this species was found at sites of nearly all salinities, and was most frequently found at saline sites from which Tubificidae had been

eliminated.

The results from the experiments using *Gammarus* species are more difficult to interpret due to the decreasing effect of salinity on respiration rate as the size of the individual increased. The *Gammarus* were tested while they were still adapting to new salinities, as Lockwood *et al.* (1973) found that *G. duebeni* required three hours to adapt to changes in salinity and it is assumed that the three species used here would require at least this time. However, after three days acclimation, there was no further change in respiration rates in either *G. zaddachi* or *G. tigrinus* although the respiration rate in *G. pulex* in 56mM NaCl declined slightly.

In small animals, where the effect of salinity on respiration rate was greatest, the rate of oxygen uptake in *G. pulex* steadily increased as salinity rose. In *G. tigrinus* and *G. zaddachi*, however, the rates, which were higher than in *G. pulex* in 1mM NaCl, decreased to a minimum at 28mM in *G. tigrinus* and 56mM in *G. zaddachi*. The NaCl concentrations which produced the minimum rates in *G. tigrinus* and *G. zaddachi* were in the range of LC_{50} values for these species, which also showed a declining numbers of swimming movements in increasing salinity. Since chloride ions have an effect on the exciteability of nerve cells and high cellular levels are known to be associated with paralysis, (Komnick *et al.*, 1972), the increase in chloride concentration within the animals could both restrict their movements and respiration rate, as *Gammarus* need to move their pleopods to create water currents over their gills. This was further illustrated when, after three days acclimation, few *G. tigrinus* survived and those which did had a low respiration rate and very little capability for movement.

It seems that the smaller animals have to change their respiration rate very much more than larger ones when subjected to changing salinity, perhaps due to the increased surface area to volume ratio which allows increased fluxes of water and ions across the body wall. *G. pulex* appeared the most able to increase its respiration rate in these conditions, and this species had the highest LC_{50} in NaCl solutions. It would appear that the freshwater species *G. pulex* (in particular small individuals) and *T. tubifex* were using increasing amounts of respiratory energy to osmoregulate as the external chloride concentration of the medium increased in NaCl solutions. This increase did not occur in the brackish water species *G. zaddachi*, *G. tigrinus* and *L. rivalis*, which had a low tolerance for sodium chloride in solution. It would be interesting to investigate the respiration of the brackish water species using sea-water dilutions in which they were more tolerant of increasing sodium and chloride, to see if under these conditions there were changes in respiration rate, perhaps including a reduction when in sea-water dilutions which are isotonic to their blood.

It has been suggested by Milne and Ellis (1973) that the coxal gills of *Gammarus* are the sites of most ion and water exchange, in addition to gaseous exchange. The gills consist of single layers of cells with lacunae containing haemolymph between them. When Milne and Ellis tested the chloride ion uptake of all the tissues of *Gammarus oceanicus* (a sea-shore species), they found that only the gill lamellae were permeable to this ion. They also found that when *G. oceanicus* was acclimated to 20% sea-water, it had larger lacunae and more mitochondria in the cells lining the lacunae than when it was in

freshwater, and concluded that this species required more respiratory energy to achieve ionic balance in dilute sea-water.

Taylor (1985), working with the amphipods *Corophium volutator* and *C. curvispinum*, found that the rate of haemolymph flow through the gills of the euryhaline *C. volutator* was markedly reduced following transfer to a hypersmotic saline medium, but not following transfer to hypoosmotic salinities. This reduction in flow was dependent on the magnitude of the ionic gradient across the gill surface, not to the osmotic gradient, and was postulated to be under neural control. This restriction in haemolymph flow reduced the uptake of ions across the gill surface while the animal was adapting to a hyperosmotic environment, and was reversible once the animal was transferred back to its acclimation salinity. In the freshwater *C. curvispinum*, there was evidence of a similar restriction in flow, although this species does not tolerate hyperosmotic salinity transfers. Harris (pers. comm), found that in *G. pulex*, the restriction in gill perfusion occurred after transfer to 100% sea-water, and that the change was irreversible, the affected gills becoming necrotic. The animal was, however, able to maintain oxygen uptake for some time after this change, and eventually died through loss of osmoregulatory control. In *G. duebeni*, however, this change was reversible, although the process was slow and could take up to 20 hours. A blackening and swelling of the gills was often seen in both *G. pulex* and *G. tigrinus* during LC_{50} experiments (Chapter 8), when the animal was approaching death, and it is possible that this change was due to the reduction of haemolymph flow in the gills, which might have caused the tissues to die.

It would be interesting to study the gill structure in *G. pulex* and *G. tigrinus* after transfer to hyperosmotic media, both NaCl solution and sea water, to see if this change in haemolymph flow was triggered in both species. It is possible that in *G. tigrinus*, the restriction in haemolymph flow and thus in ion influx, does not occur in NaCl solutions, resulting in a lower tolerance of this species for Na^+ and Cl^- in NaCl solution than in sea water. If this was true, increasing levels of potassium ions in sea-water would be one of the triggering factors for this response in *G. tigrinus*, but not in *G. pulex*.

CHAPTER 7

Effects of Salinity on the Swimming Activity of *Gammarus pulex* *Gammarus tigrinus* and *Gammarus zaddachi*

7:1 Introduction

Salinity was shown in Chapter 6 to affect the respiration rate of *Gammaridae*, especially in small specimens. In *G. pulex*, a fresh water species, respiration increased in higher salinity, while in the brackish water *G. tigrinus* and *G. zaddachi*, the rate initially declined in increasing salinity, then rose again. The increased amount of energy available to the animal as respiration rate increases could be used in osmoregulation, or if the energy requirement for ion regulation did not increase, for greater mobility. The frequency of swimming movements in an unnatural situation can be easily measured using a light beam and swimming chamber, and this method was used to test the amount of energy available to each species in increasing salinities.

The ability to maintain position and move upstream, either by swimming or crawling, is obviously of importance to all stream dwellers which are components of drift, and these abilities are even more important for estuarine inhabitants, which are subjected to strong tidal currents in addition to river flow, and which would be quickly washed out to sea and away from suitable habitats if they did not resist the currents. Any decrease in the energy available for mobility could affect the competitive ability of a species.

Many freshwater species have adaptations designed to maintain position in a strong current, such as adhesive suckers (eg leeches), hooks (eg *Hydropsyche spp.*, *Simulium spp.*) , or extra weight as in the cased caddis. *Gammarus* species have none of these adaptations, and maintain position by crawling under stones and clinging to vegetation. *Gammarus pulex* has, in fact, been described by Hynes (1970) as a "clumsy creature which seems ill-adapted to stream life", and which has little swimming ability. *Gammarus tigrinus* has, however, been described as an active predator by Savage (1981) and Wachs (1963), and has many more long saetae on its swimming legs than *G. pulex* in both the sexes, but partcularly in males (Lincoln, 1979), which might prove to be an advantage in swimming.

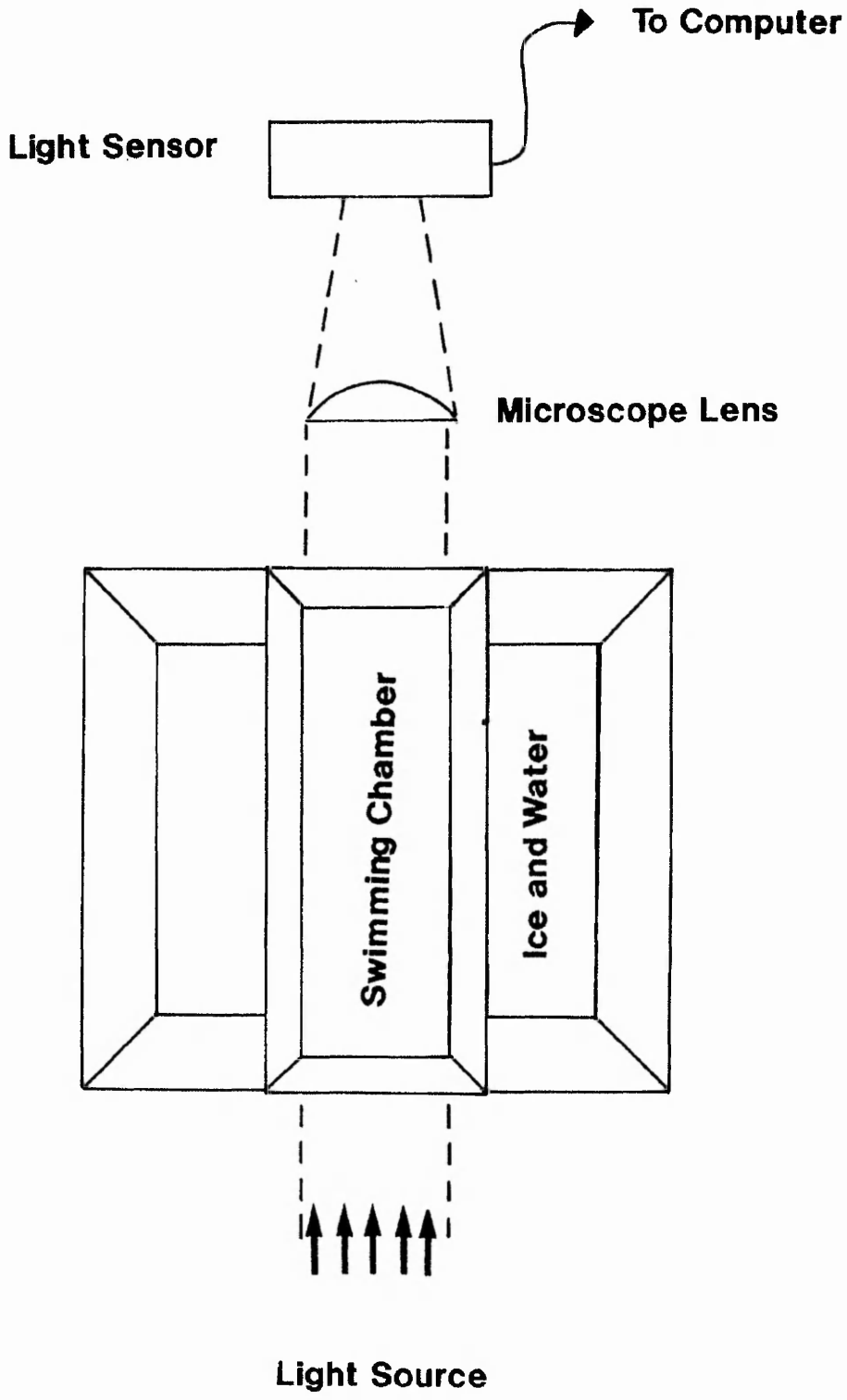
The comparative swimming abilities of *G. pulex* (a river inhabitant), and *G. tigrinus* (an estuarine species) were therefore studied in a series of experiments designed to show whether *G. tigrinus* was a more active species than *G. pulex*, and whether increasing salinity had a detectable effect on the level of swimming activity in both species. The swimming ability was also compared with that of *G. zaddachi*.

7:2 Methods

The equipment used in the experiments is shown in Figure 7:1 and consisted of light sensors connected to a BBC computer which ran a commercial program designed to record reductions in intensity in a light beam on a datadisc and display a graph showing these reductions as straight vertical lines. The size of the vertical lines was proportional to the reduction in light intensity. The

FIGURE 7:1

Diagram showing the equipment used in swimming experiments using three species of *Gammarus* in increasing salinity


To Computer
Light Sensor
Microscope Lens
Swimming Chamber
Ice and Water
Light Source

sensors and software used were designed by and purchased from Phillip Harris Ltd.

The experimental swimming chambers were designed to show the frequency of swimming movements in *G. pulex*, *G. tigrinus* and *G. zaddachi*. Each chamber consisted of an oblong plastic tube 107mm long x 16mm wide x 20mm deep, which was completely filled with the appropriate solution, and which was inserted into a plastic box filled with crushed ice and water to maintain the temperature in the swimming chamber at 10^0C. The temperature was checked before and after each experiment. A light beam from a fibre-optic source (to avoid heating) was shone along the length of the swimming chamber and was focused onto the light sensor using a microscope eye-piece lens. The whole apparatus was covered with a cardboard box during the recording to prevent disturbance of the *Gammarus* and to reduce fluctuations in external light which would affect the recording.

Before each experiment, the computer software was calibrated using full light and zero light (obtained by holding a piece of card across the light beam) and set to record for 1 hour. Two large male *Gammarus*, which had been acclimated to the experimental solution and temperature for one hour, were then placed in the swimming chamber and the recording begun.

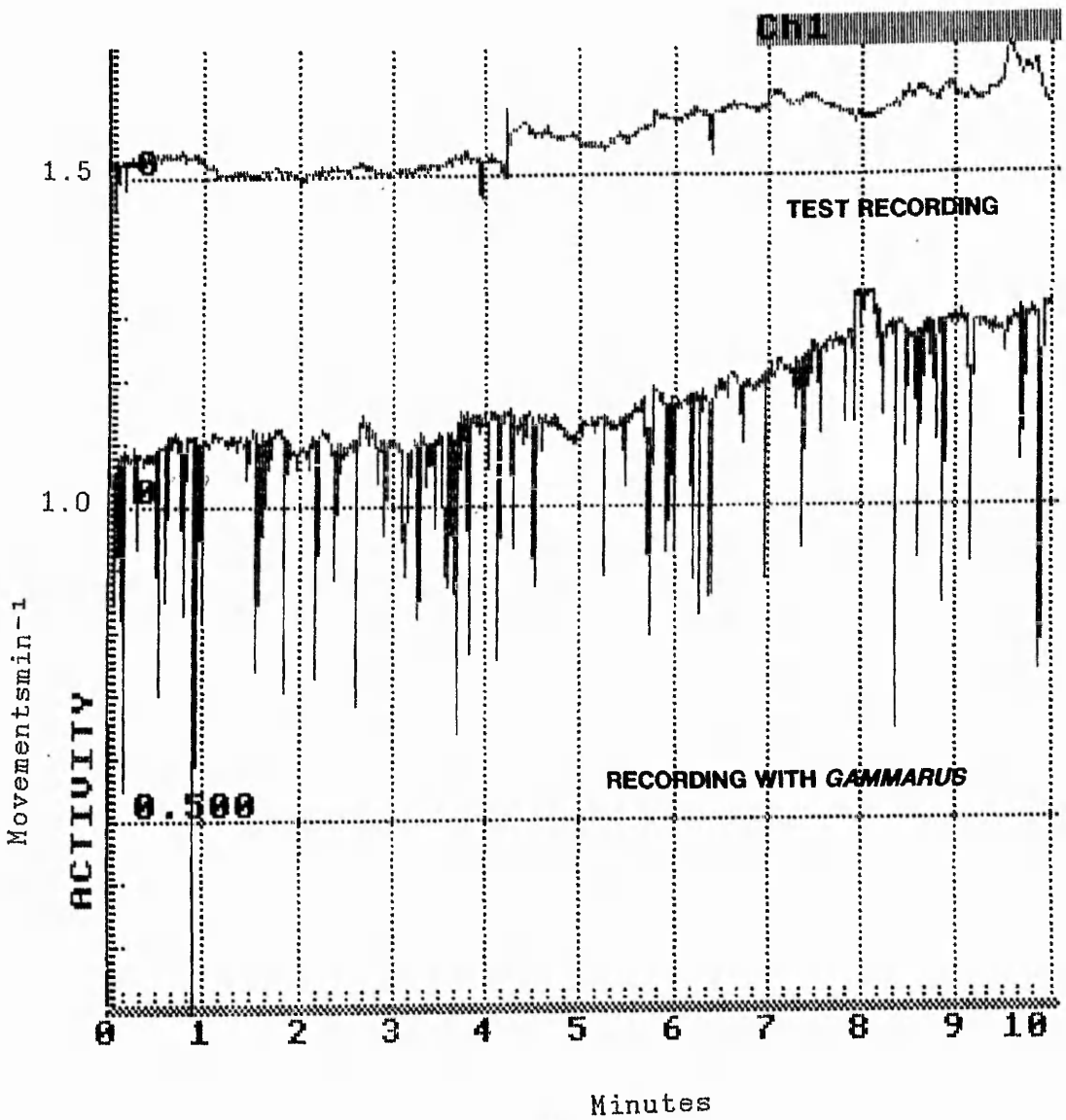
The experiment was repeated using tap water and four NaCl solutions, 28, 56, 84 and 112mM, with 16 replicates for each concentration and species. The *Gammarus* used had been acclimated to the experimental solutions at 10^0C for 24 hours in the first experiments and 96 hours in the second.

The graphs from each replicate were then plotted directly from the software, and the number of movements across the

light beam calculated. From tests with no *Gammarus* in the swimming chamber, the amount of "noise" present in the system was calculated. (This "noise" appeared on the final graphs as small vertical lines.) To eliminate this background level of fluctuation from calculations, horizontal lines were drawn at the top and bottom of the vertical lines in each section of the graph. (See Figure 7:2). The distance between the horizontal lines was measured, divided by two, and a horizontal line drawn at the mid-point. The length of lines in the lower section from each ten-minute recording period was measured, and was found always to be less than 20mm. The graphs for experiments with *Gammarus* were analysed in the same manner, with all vertical lines exceeding 20mm in length in the lower sector recorded as a movement of the test individuals. The software was calibrated so that reductions in light appeared as downward vertical lines, and all such lines were therefore due either to "noise" or movements by the test animals. The "test" graphs (without *Gamarus* present) were drawn every day, to check the apparatus, and the software re-calibrated at 0 and full light after each recording.

FIGURE 7:2

Graph showing the numbers of movements of two *Gammarus pulex* in an experimental test chamber, and a "test" line produced with no *Gammarus* in the experimental chamber, to demonstrate the amount of "noise" in the system


Ch1
1.5
1.0
TEST RECORDING
RECORDING WITH GAMMARUS
ACTIVITY
0.500
Movementsmin-1
0 1 2 3 4 5 6 7 8 9 10
Minutes

7:3 Results

Table 7:1 shows the mean number of movements per animal per minute for the sixteen replicates at concentration after one hour's acclimation, and these results are shown graphically in Figure 7:3. *Gammarus pulex* was the least active species in all the NaCl concentrations, while *G. tigrinus* was the most active species in the 1mM solution, although less active than *G. zaddachi* in the 28, 56, 84 and 112mM concentrations. *G. zaddachi* had the least variation in activity between NaCl concentrations, although it had least activity in the in the 84mM concentration. *G. pulex* had a marked drop in activity in the 28mM concentration, followed by a rise in higher concentrations, while *G. tigrinus* had its lowest activity in the 56mM concentration.

After 24 hours acclimation to the sodium chloride solutions, the amount of activity of each species was reduced in all concentrations, although the numbers of movements followed the same pattern as in the 1 hour acclimation experiments, with least movement in the 28mM concentration in *G. pulex* and in the 56mM for *G. tigrinus* (Table 7:1). The numbers of movements in the higher concentrations were, however, very small, and many of the animals, particularly *G. tigrinus*, were unable to swim well. The 96 hour acclimation time did not cause any significant change in the number of movements, although the numbers of *G. tigrinus* surviving for this time above 28mM NaCl was very small, and the results were therefore based on few replicates.

The difference between numbers of movements in each species and NaCl concentration was tested using two-way

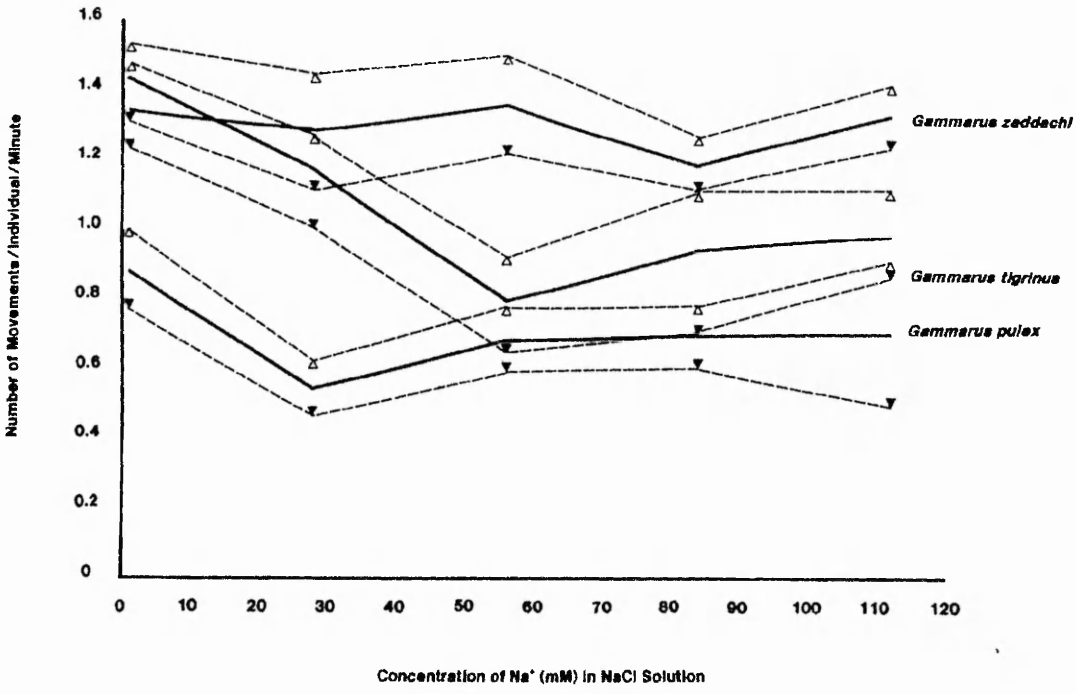
TABLE 7:1

Number of movements per individual per minute
Together with 95% confidence limits in three
species of *Gammarus* at four chloride concentrations
After 1 hour Acclimation

		NaCl Concentration (mM)				
		1	28	56	84	112
Species						
G. pulex	mean	0.87	0.54	0.68	0.69	0.69
	95% upper	0.98	0.62	0.8	0.8	0.9
	lower	0.77	0.47	0.6	0.6	0.5
G. tigrinus	mean	1.43	1.17	0.79	0.94	0.98
	95% upper	1.51	1.2	0.9	1.1	1.1
	lower	1.3	1.1	0.7	0.8	0.8
G. zaddachi	mean	1.34	1.29	1.36	1.19	1.32
	95% upper	1.51	1.4	1.5	1.3	1.4
	lower	1.2	1.1	1.2	1.1	1.2

FIGURE 7:3

Mean number of movements per minute of *Gammarus pulex*, *Gammarus tigrinus* and *Gammarus zaddachi* in experimental conditions in increasing salinity


1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70
80
90
100
110
120
Number of Movements / Individual / Minute
Concentration of Na⁺ (mM) in NaCl Solution
Gammarus zaddachi
Gammarus tigrinus
Gammarus pulex

analysis of variance, which could show significant changes in activity both within the replicates at each concentration and between each concentration and species. Table 7:3 shows the significance level of the calculated F values for each comparison. There were significant differences between the number of movements in increasing concentrations in all three species, but there were also significant differences within the replicates for each NaCl level. To reduce the effect of the within level differences, the data from experiments using NaCl concentrations of 28, 56, 84 and 112mM were re-analysed, after transformation. The transformation was calculated by first sorting the replicates in each concentration from the lowest to the highest, and then subtracting the sorted values for the 1mM experiments from the replicate values for each concentration. The resulting values were the difference in activity between the 1mM and higher NaCl concentrations in each species. The F values following two-way analysis of variance of the transformed data are shown in Table 7:3. The significant differences between the concentrations remained when transformed data was analysed, while only in *G. tigrinus* were there significant differences within NaCl levels.

Figure 7:4 shows the mean values of the transformed data. *G. zaddachi* had the least difference in activity between the NaCl concentrations, as the mean values for this species are close to zero (a zero value would result from identical values to the 1mM concentration). *G. pulex* was the next least affected by increasing salinity, and *G. tigrinus* was affected most by the increasing salinity, as the mean values for this species are the furthest from zero in all concentrations except 28mM, where *G. pulex* had the lowest value.

TABLE 7:2

Number of Movements per minute of *Gammarus pulex* and *Gammarus tigrinus* in increasing NaCl concentrations

a) After 24 hours Acclimation

NaCl (mM)	Mean Number of Moves per minute Species	
	G. pulex	*G. tigrinus*
1	0.07	0.65
28	0.17	0.31
56	0.21	0.07
84	0.22	0.09
112	0.12	0.07

b) After 96 hours Acclimation

NaCl (mM)	Mean Number of Moves per minute Species	
	G. pulex	*G. tigrinus*
1	0.39	0.48
28	0.12	0.44
56	0.09	0.09
84	0.21	0.04
112	0.1	0.03

FIGURE 7:4

Mean number of movements per minute of *Gammarus pulex*, *Gammarus tigrinus* and *Gammarus zaddachi* in experimental conditions in increasing salinity

Data are transformed by subtraction from control values.

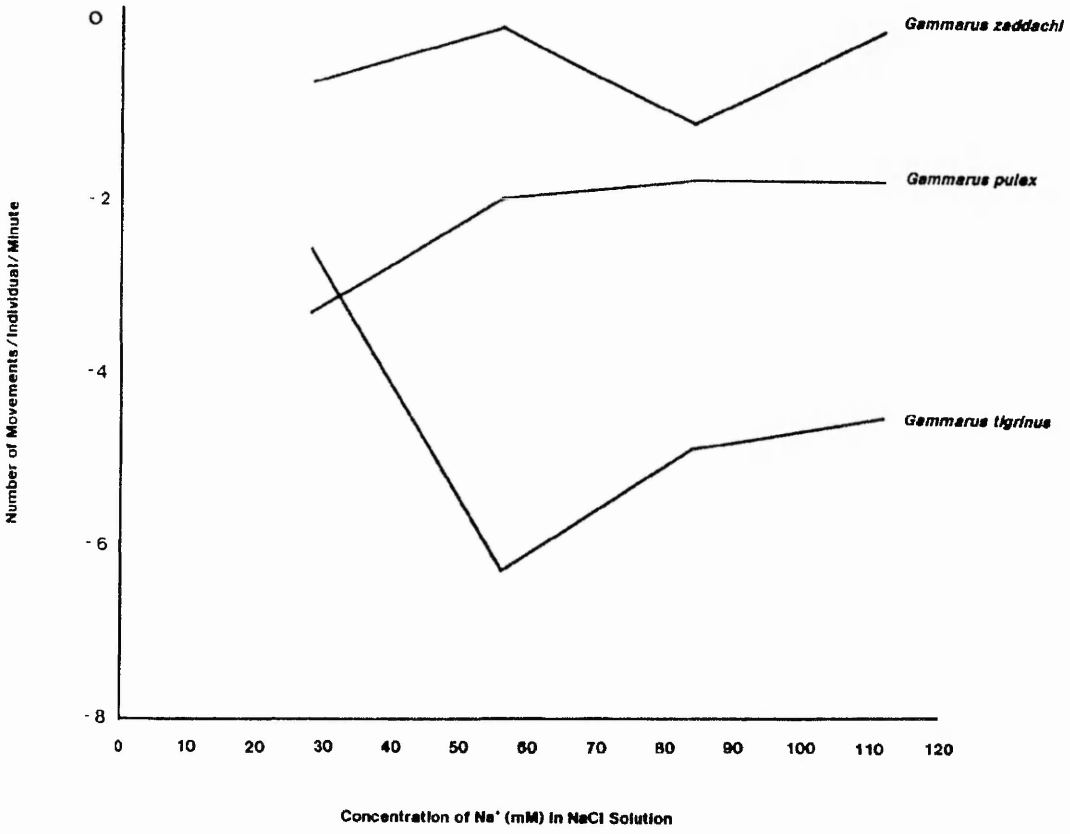

Gammarus zaddachi
Gammarus pulex
Gammarus tigrinus
0
-2
-4
-6
-8
0
10
20
30
40
50
60
70
80
90
100
110
120
Number of Movements / Individual / Minute
Concentration of Na⁺ (mM) in NaCl Solution

TABLE 7:3

Comparison of Number of Movements made by *Gammarus pulex*, *Gammarus tigrinus* and *Gammarus zaddachi* in swimming experiments using Five NaCl concentrations. Significance Level of F values from Two-Way Analysis of Variance for untransformed data and data transformed by subtraction from Control Values

a) Untransformed Data

	Species		
	G. pulex	*G. tigrinus*	*G. zaddachi*
Between concentration	1%	1%	1%
Within concentrations	1%	1%	1%

b) Transformed Data

	Species		
	G. pulex	*G. tigrinus*	*G. zaddachi*
Between concentration	1%	1%	1%
Within concentrations	NS	1%	NS

c) Between species comparisons (untransformed data)

	Species Combination		
	P x T	T x Z	P x Z
Between Concentrations	1%	1%	1%
Within Concentration	1%	1%	1%

P = *Gammarus pulex*

T = *Gammarus tigrinus*

Z = *Gammarus zaddachi*

TABLE 7:4

Comparison of Number of Swimming Movements made by *Gammarus pulex*, *Gammarus tigrinus* and *Gammarus zaddachi* using five NaCl Concentrations

Significance Level of t between means of numbers of movements at different NaCl concentrations

a) *Gammarus pulex*

		NaCl concentration (mM)				
		1	28	56	84	112
NaCl	1	-				
(mM)	28	1%				
	56	1%	5%			
	84	1%	1%	NS		
	112	NS	NS	NS	NS	

b) *Gammarus tigrinus*

		NaCl concentration (mM)				
		1	28	56	84	112
NaCl	1	-				
(mM)	28	1%				
	56	1%	1%			
	84	1%	1%	NS		
	112	1%	5%	5%	1%	

c) *Gammarus zaddachi*

		NaCl concentration (mM)				
		1	28	56	84	112
NaCl	1	-				
(mM)	28	NS				
	56	NS	NS			
	84	5%	NS	5%		
	112	NS	NS	NS	5%	

When the values for each species were compared, there were significant differences between each species combination using both transformed and untransformed data.

To test the differences between the means of each concentration for each species, t-tests were used. The results of these are summarised in Table 7:2, with the significance levles. In *G. pulex*, activity in all the NaCl levels except 112mM was significantly lower than the 1mM value, and in 56mM there was greater activity than in 28mM. In *G. tigrinus* the only concentrations in which activity was not significantly different from the 1mM level were the 56 and 84mM. In *G. zaddachi*, there were differences only between the 56 and 84mM activity levels and between the 84 and 112mM concentration activity levels.

7:4 Discussion

The numbers of swimming movements made by the three species demonstrate clearly that *G. tigrinus* and *G. zaddachi* swim more actively than *G. pulex* when in unusual conditions, as in the swimming chamber. It is interesting that *G. tigrinus* has a number of movements mid-way between the number for *G. pulex* (the river species), and *G. zaddachi* (the estuarine species). It also has a drop in numbers of movements at a NaCl concentration between the 28mM of *G. pulex* and 84mM of *G. zaddachi*.

It is also interesting to note that both the more active species, *G. tigrinus* and *G. zaddachi* have a striped colouration (this is quite faint in *G. zaddachi* and confined to the boundaries between tergites, but very striking in *G. tigrinus*, which has a broad dark stripe

vertically down each tergite, hence its name). Since *G. pulex* is known to form a large part of the diet of many fish, for example, brown trout (*Salmo trutta* L) (Elliott, 1975), and invertebrates eg damselfly nymphs and Dytiscid larvae (personal observation), it is possible that the more active *G. tigrinus* and *G. zaddachi* have cryptic colouration in order to reduce predation, while the less active *G. pulex* avoids predators by remaining hidden for longer periods.

CHAPTER 8

Relative Toxicities of NaCl Pumped Mine Drainage Water and Sea Water to Fresh Water Species

8:1 Introduction

A toxicant can be defined as "an agent which can produce an adverse effect in a biological situation" (Rand and Petrocelli, 1985). The adverse effect may be described as any response is outside the normal range for healthy organisms. Within the terms of this definition, the solutes comprising the saline mine drainage water pumped from Creswell Colliery into Millwood Brook are clearly toxicants, in that they produce adverse effects on organisms exposed to them. However, all the major ions in the mine drainage water appear to be innocuous; indeed most are necessary for the healthy life of aquatic organisms. No chemical, however, is completely safe and the safety of a substance is determined by the relationship between the concentration to which an organism is exposed and the length of time the exposure continues. Below a minimum level (the "threshold"), a substance may produce no measureable response, but above this threshold may show sudden or gradually increasing deleterious effects.

Standard 96-hour acute toxicity tests (Sprague, 1969; 1973; 1976) using the major components of mine pumping water (in particular sodium, chloride, potassium and calcium) were therefore used to evaluate the level at which these components caused harmful effects. These experiments are tests only of acute exposure to high

concentrations, conducted over hours or days, and are often criticized as having limited application in the field, when compared with chronic exposure tests. However, since some of the effects of chronic exposure to low concentrations of sodium chloride on the life history and physiology of *Gammarus*, *Tubifex tubifex* and *Lumbricillus rivalis* were described in Chapters 4,5 and 6, it was felt that additional information about the ranges of salinity which species could withstand would be useful in exploring the mechanisms by which the animals tolerate salinity. In addition, a sudden exposure to high levels of salinity is paralleled immediately below the point where mine pumping water is released into Millwood Brook, and the length of time which a species is able to withstand this "shock" could determine whether or not they were able to survive when washed downstream through the area close to the mine input.

A range of freshwater species from Millwood Brook were used in initial trials, but further experiments were confined to the five species used in the preceding studies (*G. pulex*, *G. tigrinus*, *G. zaddachi*, *T. tubifex* and *L. rivalis*). The ability of these five species to tolerate NaCl solutions, mine pumping water dilutions, and solutions of commercial (Griffin and George) sea water were tested, in addition to some of the other components of the mine drainage water. Sea water was used in toxicity experiments as it is chemically similar to mine drainage water and can be made up to a standard concentration for each experiment.

8:2 Methods

Acute toxicity tests using the standard 96 hour time were

used to calculate LC_{50} values which could be compared with published results. The tests were carried out using the following standardised method.

Organisms used for toxicity testing were collected from the field and maintained in aerated 10l tanks in a 10^0C constant temperature room for twenty-four hours before use.

All solutions were made up using tap water and Analar chemicals where required. Mine drainage water was cooled to 10^0C before use and always used within one week of collection. The artificial sea water solutions were made up and tested as described in Chapter 3. All solutions were tested for conductivity and pH before use. The pH of solutions used was always between 7.0 and 8.0.

The prepared solutions were divided into containers appropriate to the size of the species under test. The containers were Petri dishes (used for oligochaetes), 200ml plastic cups (used for *Gammarus*, Chironomidae and Planariidae), and clear plastic sandwich boxes (used for Baetidae, Hydropsychidae and *Potamopyrgus jenkinsi*).

Organisms for testing were taken from the holding containers or stock cultures. All animals used were selected for uniformity of size (*ie* they were large adults unless juveniles were specifically under test), and were apparently healthy and undamaged. Ovigerous females and mated animals were excluded. Where there were adequate numbers of a species, 10 individuals were introduced into each container, with two replicates of each concentration. If 20 individuals of a particular species were not available for each concentration, those which could be obtained were divided equally between the containers.

The containers were covered, placed in the constant

temperature room, and left undisturbed for 96 hours, when the animals were counted and the number of dead recorded. Death was defined in all species (except *Potamopyrgus jenkinsi*) as the point at which an animal could not move away when prodded with a needle, even if there were feeble movements still present, for example, slow movements of the pleopods in *Gammarus*. It was found in preliminary experiments that *Gammarus* in that condition did not recover when placed in carbon-filtered tap water and fed.

Death of *Potamopyrgus jenkinsi* could not be decided by this method, as, when placed in unfavourable conditions, these small gastropods can close their operculae and remain apparently inert for several days before moving again when placed in fresh water. For this species, therefore, all animals were removed from the test solution at the end of the experiment, and placed in sandwich boxes on a layer of fine sand so that when the snails moved, trails were left in the sand. The snails were left in the boxes for a further 96 hours, and any which did not move by the end of this period were scored as dead.

The number of dead in the pairs of replicates were compared, and if these were not significantly different (this was tested using one-way analysis of variance), the two replicates were combined. If the two replicates were significantly different, the experiment was repeated.

The concentration of the test solution, number of animals dead and number tested were entered into the GLIM (Baker and Nelder, 1978; Bratby, 1985) computer package which was used to calculate LC_{50} and 95% confidence limits for each using a log/probit method (Finney, 1971).

Four sets of experiments were conducted using the above

method.

The first set of experiments were LC_{50} tests on a range of freshwater invertebrate species using NaCl solutions. These tests were performed on a range of species collected from sites M3, M12 and M19 on Millwood Brook, and *G. zaddachi* from the site described in Chapter 6.

The second set of experiments compared the toxicity of mine drainage water to *Gammarus spp.In these experiments, Gammarus pulex* and *G. tigrinus* were exposed to the mine drainage water dilutions shown in Table 8:1, the containers examined and the numbers of dead recorded after 30mins, then every hour for 6 hours, then at 24, 48 and 96 hours.

The third set of experiments was a comparison of the toxicity of NaCl solutions, mine drainage water and sea water dilutions. The LC_{50} ranges of five species, *Gammarus pulex*, *G. tigrinus*, *G. zaddachi*, *Tubifex tubifex* and *Lumbricillus rivalis* were compared in increasing concentrations of three types of saline solution; mine drainage water, NaCl solution and Griffin and George artificial sea water.

The fourth set of experiments determined the LC_{50} ranges of separate components of artificial sea water. This was prepared using the components recommended by Spotte (1970), (*ie* NaCl 26g/l, $MgSO_4$ 6.4g/l, $MgCl_2$ 2.3g/l, KCl 0.74g/l, $NaHCO_3$ 0.09g/l, $CaCl_2$ 2.03g/l). This gave an ionic compostion of Cl^- 519mM, Na^+ 444mM, Ca^{2+} 9.25mM, SO_4^{2+} 26mM, Mg^{2+} 50mM and K^+ 10mM.

An LC_{50} range for this solution was established for *G. pulex* and *G. tigrinus*, then each component of the sea water (except NaCl) was used individually in LC_{50} tests, together with 0, 44, 133, 354 and 444mM Na^+ (in NaCl).

LC_{50} tests were then conducted with each component added

TABLE 8:1

Conductivity and Chloride Concentrations of Mine Drainage Water Dilutions

Mine Drainage Water %	Conductivity (mS/cm)	Chloride (mM)
0	0.9	4
5	1.7	40.3
10	3.6	82.7
20	10	160.3
30	14	220
40	14.7	340
50	20.3	455
60	22.9	570
80	27.3	740
100	39.7	855

to the last, so that the solutions became increasingly complex. These LC_{50} experiments were conducted concurrently, using two replicates for each mixture.

8:3 Results

Table 8:2 shows the calculated LC_{50} values for NaCl solutions in a range of species collected from Millwood Brook, together with the 95% confidence limits for these values.

The lowest LC_{50} range (15-28mM NaCl) among the tested species was that of *Baetis vernus*, although in the field this species was present at sites M17, M18 and M19, where mean chloride concentrations were higher. This apparent discrepancy was not due to poor survival of this mayfly species in still water conditions, as there were no mortalities in the filtered tap-water controls, demonstrating that *Baetis vernus* could survive the experimental conditions for the required length of time. This was not the case in two other species of Ephemeroptera which were tested, *Baetis rhodani* and *Ephemerella ignita*, in which there were large numbers of deaths in the controls, and the results from these species could not therefore be used to calculate LC_{50} values.

Four species (*Lumbricillus rivalis*, *Hydropsyche angustipennis*, *Conchapelopia* spp. and *Prodiamesa olivacea*) had LC_{50} ranges above 200mM Cl^-, with *Conchapelopia* spp. and *H. angustipennis* tolerating NaCl in close to sea water strength.

Five species had LC_{50} values in the rance 110-180mM Na^+, (30-40% sea water), *Polycelis tenuis*, *Potamopyrgus jenkinsi*, *Nais elinguis* and adult *G. pulex*. The ranges

TABLE 8:2

LC_{50} (mM) Values for NaCl solutions in a range of freshwater invertebrate species at 10°C

Taxon	Number of animals Used	LC_{50} (mM)	95%CL
Planariidae			
Polycelis tenuis	15	157	+/- 10.7
Gastropoda			
Potamopyrgus jenkinsi	20	170	+/- 17.6
Oligochaeta			
Nais elinguis	10	112	+/- 15.5
Lumbricillus rivalis	10	228	+/- 8.8
Tubifex tubifex	10	63	+/- 6.1
Crustacea			
Asellus aquaticus	30	143	+/- 20.6
Gammarus pulex (<1mg dry weight)	20	43	+/- 6.1
G. pulex (adults)	70	124	+/- 4.9
Gammarus tigrinus (<1mg dry weight)	20	52	+/- 6.8
G. tigrinus (adults)	70	21	+/- 5.5
G. zaddachi	20	41	+/- 9.7

All tests were of 96 hour duration

TABLE 8:2 (Continued)

LC_{50} (mM) Values for NaCl solutions in a range of freshwater invertebrate species at 10°C.

Taxon	Number of animals Used	LC_{50} (mM)	95%CL
Ephemeroptera			
Baetis vernus	20	22	+/- 6.7
Trichoptera			
Hydropsyche angustipennis	25	313	+/- 63.5
Chironomidae			
Conchapelopia spp.	18	394	+/- 194.2
Prodiamesa olivacea	10	226	+/- 10.7
Cricotopus spp.	10	72	+/- 28.3
Chironomus spp.	10	87	+/- 24.8
Simuliidae			
Simulium argyreatum	18	52	+/- 10.0

All tests were of 96 hour duration

for the other species were low, although all except *G. tigrinus* and *G. zaddachi* were higher than the range of salinity in which the species was found in the field.

The LC_{50} values for *G. tigrinus* and *G. zaddachi* were surprisingly low, only 20-50mM Na^+, which is below the chloride concentration at which they were found in the field.

Table 8:3 shows the cumulative number of dead *Gammarus* in dilutions of mine drainage water with an initial conductivity of 39.7mS/cm.

Gammarus pulex were killed in increasing numbers in all mine drainage water concentrations, and above 10% mine drainage water, 50% of the *Gammarus* were killed after 96 hours. In 100% mine drainage water, the first deaths occurred within half an hour of exposure, and all the *G. pulex* were dead after one hour. Above 60% mine drainage water, all animals except one were dead within six hours of exposure, and this individual was dead within 24 hours.

In *G. tigrinus*, however, there were no deaths for five hours in any concentration, and only above 50% mine drainage water were there numbers of deaths which were significantly different from the controls after that time.

The LC_{50} values for the five species used to compare the toxicity of NaCl, mine drainage water and sea water are shown in Tables 8:4, 8:5 and Figure 8:1. It can readily be seen from these that the tolerance of *G. pulex* for salinity was almost identical in NaCl, mine drainage water and sea water, and this this was also true for *Tubifex tubifex*. In *G. tigrinus* and *G. zaddachi*, however, tolerance to salinity increased almost ten-fold in mine drainage water and sea water, with the highest tolerance

TABLE 8:3

Cumulative Mortality of *Gammarus* spp. in Increasing Percentages of Mine Drainage Water

1) *Gammarus pulex*

Time in Hours

%	0.5	1	2	3	4	5	6	24	48	72	96
0								0	0	1	1
5								1	2	3	3
10								2	2	5	6
20								2	5	7	7
30						1	1	4	6	6	10
40						1	1	6	6	6	9
50						3	4	10	10	10	10
60			4	5	7	9	9	10	10	10	10
80		5	9	10	10	10	10	10	10	10	10
100	5	10	10	10	10	10	10	10	10	10	10

TABLE 8:3 (Continued)

Cumulative Mortality of *Gammarus* spp. in Increasing Percentages of Mine Drainage Water

2) *Gammarus tigrinus*

Time in Hours

%	0.5	1	2	3	4	5	6	24	48	72	96
0											2
5										4	4
10								1	1	3	3
20									1	1	2
30										1	2
40										1	2
50									5	5	10
60						1	1	6	6	6	9
80								9	9	10	10
100						1	1	1	9	9	10

Figures given are actual numbers of dead animals out of 10

being in sea water. *Lumbricillus rivalis* also showed this pattern of increasing tolerance in mine drainage water and sea water, although the increase was less marked.

In tests using the chlorides of calcium, magnesium and potassium alone, *G. pulex* again showed the highest tolerance to the solutions, although *G. zaddachi* had a similar tolerance to calcium and potassium chlorides.

Table 8:6 shows the percent mortality of the *Gammarus* in separate components of sea water. In 44mM sodium and chloride, the addition of extra ions, particularly potassium, increased the mortality of *G. pulex*, while in *G. tigrinus* at this salinity, sodium bicarbonate and calcium produced a change in mortality, sodium bicarbonate greatly reducing mortality, and calcium increasing it. At 133mM sodium and chloride and above, all the *G. pulex* died except for those with added calcium in the 133mM solution. In *G. tigrinus*, however, once sodium and chloride concentrations were above 44mM, the addition of potassium to the salt solution produced a reduction in mortality which was maintained up to 443mM sodium and chloride, although all animals died in other ion combinations.

When the sea water components were added together one-by-one, mortality of *G. pulex* was the same and all individuals died in these tests. In *G. tigrinus*, however, all animals died until potassium was added to the mixture, which enabled 50% of the animals to survive. It was not until all the elements of the artificial sea water were added together, however, that all the *G. tigrinus* survived for 96 hours.

TABLE 8:4 (a)

LC_{50} (mM) Values for *Gammarus pulex* in NaCl, $CaCl_2$, $MgCl_2$ and KCl solutions, Mine Drainage Water and Artificial Sea Water

Solution	Ion	Number used in tests	LC_{50} (mM)	95%CL (+/-)
NaCl	Cl^-	70	123.8	4.9
Mine	%	20	38.6	11.5
	Na^+		125.8	37
	Cl^-		232.3	69
	Ca^{2+}		11.8	3.5
SW	%	20	32.1	5.3
	Na^+		121.8	20
	Cl^-		149.2	25
	Ca^{2+}		3.21	0.5
CaCl2	Ca^{2+}	30	21.6	3.12
$MgCl_2$	Mg^{2+}	30	22.9	3.55
KCl	K^+	20	3.25	1.3

All tests were of 96 hour duration

TABLE 8:4 (b)

LC_{50} Values (mM) for *Gammarus tigrinus* in NaCl, $CaCl_2$, $MgCl_2$ and KCl solutions, Mine Drainage Water and Artificial Sea Water

Solution	Ion	Number used in tests	LC_{50} (mM)	95%CL (+/-)
Mine	%	20	76.1	15
	Na^+		248.3	49
	Cl^-		458.5	90.3
	Ca^{2+}		23.25	4.6
SW	%	20	97	16.2
	Na^+		368.5	61
	Cl^-		451.2	74
	Ca^{2+}		9.7	1.6
CaCl2	Ca^{2+}	30	2.3	0.7
$MgCl_2$	Mg^{2+}	30	6.18	0.9
KCl	K^+	20	3.75	0.7

All tests were of 96 hour duration

TABLE 8:4 (c)

LC_{50} (mM) Values for *Gammarus zaddachi* in NaCl, $CaCl_2$, $MgCl_2$ and KCl solutions, Mine Drainage Water and Artificial Sea Water

Solution	Ion	Number used in tests	LC_{50} (mM)	95%CL (+/-)
NaCl	Cl^-	20	41	9.7
Mine	%	20	104.5	10.4
	Na^+		340.7	34
	Cl^-		629.2	63
	Ca^{2+}		31.9	3.18
SW	%	20	155	5.93
	Na^+		588.5	118
	Cl^-		720.6	144
	Ca^{2+}		15.5	3.1
CaCl2	Ca^{2+}	20	20.3	12
$MgCl_2$	Mg^{2+}	30	10.68	1.95
KCl	K^+	20	2.89	0.5

All tests were of 96 hour duration

TABLE 8:5

LC_{50} Values (mM) for Two Oligochaete Species in NaCl, Mine Drainage Water and Sea Water

a) *Tubifex tubifex*

Solution	Ion	Number used in tests	LC_{50} (mM)	95%CL (+/-)
NaCl	Cl^-	30	128.1	9.3
Mine	%	20	29	4.4
	Na^+		102	4.5
	Cl^-		133	5.8
	Ca^{2+}		9	1.3
Sea water	%	20	23	3.0
	Na^+		105	11
	Cl^-		128	14
	Ca^{2+}		7	0.9

b) *Lumbricillus rivalis*

Solution	Ion	Number used in tests	LC_{50}	95%CL (+/-)
NaCl	Cl^-	20	228	8.8
Mine	%	20	85	7.4
	Na^+		304	26.6
	Cl^-		394	34.4
	Ca^{2+}		26	2.3
Sea water	%	20	105	11.5
	Na^+		400	43.7
	Cl^-		488	53.5
	Ca^{2+}		11	1.2

All tests were of 96 hour duration

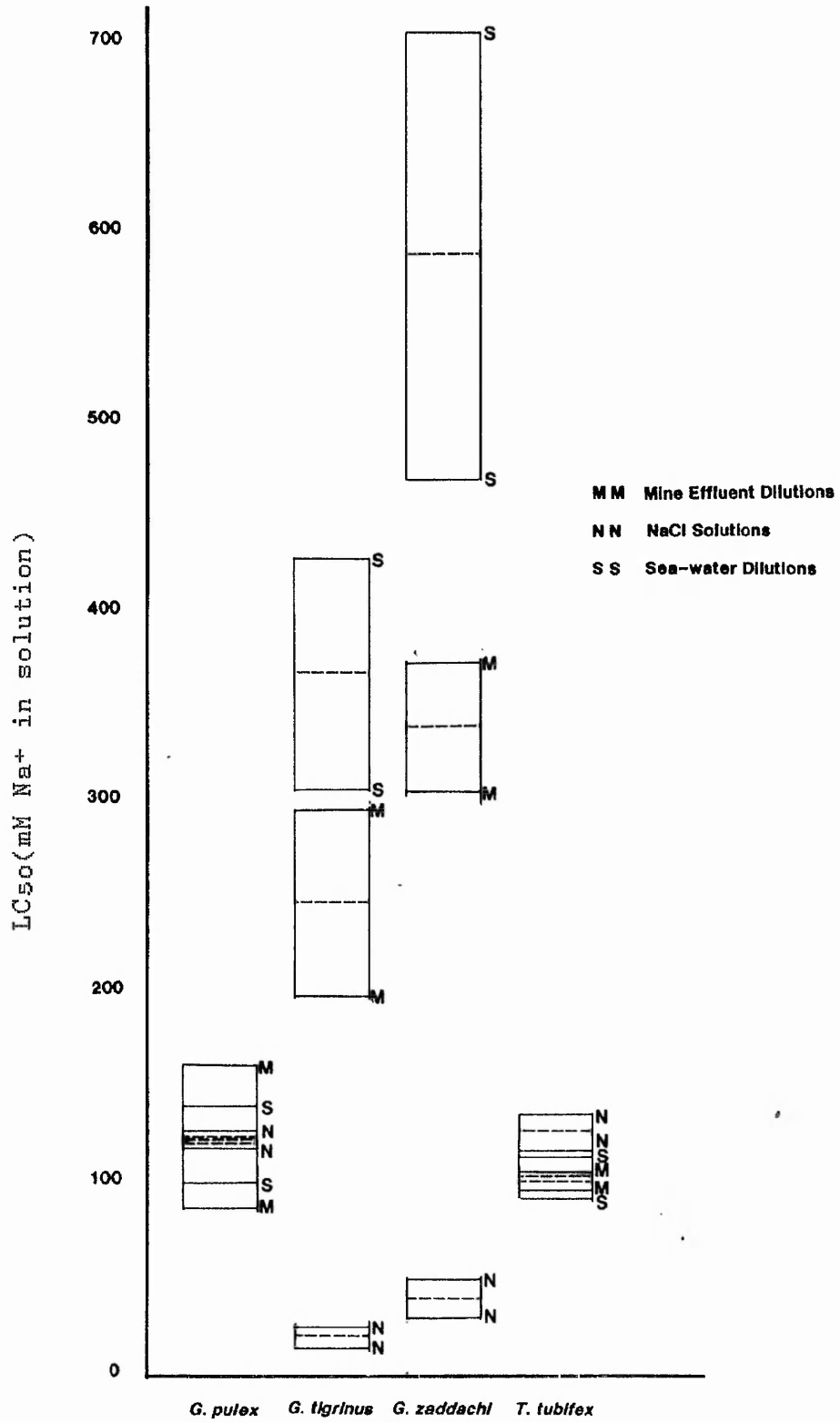
FIGURE 8:1

LC_{50} ranges for NaCl, Mine drainage water and sea water for three species

Mean LC_{50} values are shown as dotted lines, and 95% confidence limits for the mean as solid lines.

M = Mine drainage water
N = NaCl solution
S = Sea water solutions

All tests were of 96 hour duration


LCso(mM Na+ in solution)
700
600
500
400
300
200
100
0
S
S
S
S
M
M
S
M
M
S
N
N
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M
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M
S
G. pulex
G. tigrinus
G. zaddachi
T. tubifex
M M Mine Effluent Dilutions
N N NaCl Solutions
S S Sea-water Dilutions

Discussion

Ultimately, all freshwater animals are descended from marine ancestors, but some groups, such as the Crustacea, have colonised fresh waters directly via brackish waters, (Newell, 1976) and others, such as some Insecta, have colonised fresh waters from a terrestrial existence (Wigglesworth, 1953). Only organisms which can osmoregulate (control the ionic composition of body fluids) can colonise fresh water, as the internal fluids of all animals are more concentrated than typical fresh waters, resulting in a continual loss of ions from the body and a gain in water across the body surface.

Organisms can adapt to fresh water in a variety of ways, for example, by reducing the concentration of their blood to lower the osmotic gradient between their body fluids and the external medium, by reducing the permeability of their integument to water and ions, by employing excretory organs to eliminate water and by an active mechanism of taking up salts against the concentration gradient. (Rankin and Davenport, 1981). Most freshwater animals osmoregulate by a combination of all four methods. *Gammarus duebeni*, for example, is known to produce large quantities of dilute urine when in fresh water (Lockwood, 1964), and all fresh and brackish water Crustacea have a reduced permeability across their body wall when compared to marine forms (Lockwood, 1962). The low permeability of the body wall, however, reduces the diffusion of oxygen, and many freshwater organisms have areas of thinner integument ("gills") which are also the sites of active ion exchange (Rankin and Davenport, 1981). Many insects, such as water bugs and beetles, with

impermeable integuments, breathe atmospheric air and thus avoid the need for permeable gills.

The ionic strength of the blood is lower in most freshwater species when compared to marine or brackish species, for example, *G. tigrinus* has a blood sodium concentration of 300-400mM Na^+, compared with 120mM in *G. pulex*. (Sutcliffe, 1971a and b).

It is noticeable from these experiments that the LC_{50} range for adult *G. pulex* almost exactly corresponded with the blood sodium concentration of this species. The most striking point to emerge, however, was the difference in tolerance of NaCl solutions between fresh water and brackish water species. The freshwater species such as *T. tubifex* and *G. pulex* appeared to have an equal tolerance of Na^+ or Cl^- ions regardless of the other ions in solution, while the brackish-water *G. tigrinus* and *G. zaddachi* appeared to need other ions in solution in order to tolerate excess sodium and chloride. The experiments in section 4 demonstrated that *G. tigrinus* could only tolerate NaCl at near seawater concentrations when there was potassium present, and only had its maximum survival when all the major components of sea water were present in the correct proportions.

Potassium is a very important ion in the regulation of cell volume, and is also involved in nerve excitability (Wood, 1974). The concentration of potassium in the blood of most freshwater animals is about 4mM (Sutcliffe, 1971a), but this rises to approximately 10mM in brackish water animals (Sutcliffe, 1971b). The internal cell concentration of this ion is, however, very different in both freshwater and brackish water animals, being about 140mM (Rankin and Davenport, 1981). Sodium concentration follows a reverse pattern with higher concentrations in

the blood (120-500mM) than in the cell (15mM). Sodium and potassium ions are exchanged via an active ion exchange "pump" situated in cell membranes, which is used to maintain a constant intracellular concentration of the ions (Rankin and Davenport, 1981). It would appear that the brackish water *G. tigrinus* and *G. zaddachi* are unable to obtain sufficient potassium for survival when the sodium content of the external medium rises, unless the potassium concentration also increases. The sodium uptake of the three *Gammarus* species was further investigated and the results are presented in Chapter 9.

The results of the first experiment, where the time taken by mine drainage water to kill *G. pulex* was measured, demonstrated that few, if any, of these animals would survive if washed from Crags Pond into Millwood Brook where the mine drainage water enters. The time taken for *G. pulex* to become incapacitated is only a matter of minutes, and this observation was repeated using *T. tubifex*, *Chironomus* spp. and *Asellus aquaticus*. These animals could not survive being washed through the very saline waters close to the mine drainage water, and so could not recolonise downstream sites through downstream drift except in times of very heavy spate. *G. tigrinus*, however, could survive in mine drainage water for five hours, and it is possible that this species could cross the very saline area close to the mine drainage water input to Crags Pond, if it migrates further upstream from its present range. It would then be able to spread upstream into the area inhabited at present only by *G. pulex*.

It is also clear that the mine drainage water is more toxic than sea water, when the tolerance of sodium ions is considered. This is probably due to the presence of

iron salts and phenolic compounds which are in the drainage water in much higher quantities than in normal sea water, and are in themselves, toxic to *G. pulex* (Dodd, 1985).

CHAPTER 9

Uptake of the Radioisotope ^{22}Na by Three *Gammarus* species in NaCl Solutions and Sea Water Dilutions

9:1 Introduction

The results of the LC_{50} tests described in Chapter 8 show a marked difference in the tolerances of the brackish water species *Gammarus tigrinus* and *G. zaddachi* between NaCl solutions and in sea water dilutions. In similar tests, however, the freshwater species *G. pulex* and *Tubifex tubifex* showed no change in their tolerance of NaCl solutions, mine drainage water or sea water dilutions, and contrary to expectations, were more tolerant of NaCl solutions than either of the brackish water gammarids, although they were less tolerant of sea water and mine drainage water. In the oligochaetes, this relationship was repeated, with the freshwater *Tubifex tubifex* tolerant of the same concentrations of Na^+ and Cl^- in NaCl solutions and sea water dilutions, whilst *Lumbricillus rivalis* was more tolerant of these ions in sea water. *L. rivalis* was, however, tolerant of very high levels of these ions in all solutions.

These observations raise the question of whether brackish water species have a different ionic regulating system to freshwater species, or whether there is in sea water a combination of ions which modifies ionic exchange.

The blood ionic composition of Gammaridae has been

investigated by many authors, for example Lockwood, 1962; 1964; 1970; Dorgelo, 1977 and Sutcliffe, 1971a and b and 1978, and the blood sodium concentrations which they found in sea water dilutions are shown in Table 9:1.

It can be seen that all the species alter their blood sodium ion concentration in response to the external sodium concentration. *Gammarus zaddachi*, in particular, has been shown to change its internal blood ion concentration from approximately isosmotic with the external medium in sea water, to much higher than the external concentration in near fresh water. There are fewer studies on *G. tigrinus*, but the euryhaline species *G. duebeni* has been extensively studied and also has the ability to change the ionic composition of its blood (Lockwood, 1964). This regulatory capacity is considered an "active" process (Lockwood, 1962) in that the animal selectively controls its uptake of ions and can alter the composition of its urine to regulate its internal osmotic pressure. The "passive" uptake of ions, when the animal has no control of influx and efflux is considered to be the major method of ion uptake only in those sea water species which do not penetrate freshwater (Rankin and Davenport, 1981).

It seems likely that the blood sodium concentration in the *G. tigrinus* and *G. zaddachi* used in these experiments was close to the lowest that they could tolerate, since the stock cultures of these species were collected from very low salnity waters and maintained in filtered tap water, with a sodium ion content of approximately 1mM.

All Gammarids require a small intake of sodium and chloride ions for activity (Komnick *et al.*, 1972), and in low chloride freshwater must either actively absorb these ions or conserve them by reabsorption from their urine.

TABLE 9:1

Published Values for Blood Sodium Concentrations (in mM Na_+) in Sea-Water Dilutions

	% Sea-Water						
	0	2	30	50	70	80	100
Species							
G. pulex (Sutcliffe 1971a)	106	179	179	250			
G. tigrinus (Dorgello 1977)	268					326	
G. zaddachi (Sutcliffe 1971b)	180		228				518
G. duebeni (Lockwood 1970)			179	250	328		498

They must simultaneously respond to osmotic influxes of water as their internal osmotic pressure is higher than that of the external medium. In sea water, the freshwater *G. pulex* has the problem of preventing the influx of ions and loss of water, as it cannot increase its blood osmotic pressure to equal that of sea water (Sutcliffe, 1971a), while *G. tigrinus* and *G. zaddachi* can raise their blood ion concentration until they are much nearer to being in balance with the surrounding medium.

Both *G. tigrinus* and *G. zaddachi* are able to survive for long periods in NaCl solutions of up to 28mM made up in filtered tap-water containing 1.6mM Ca^{2+}. This is equivalent to an osmotic pressure of 63mOsm (Table 9:2). (1 Osmol is defined as the osmatic pressure of a 1 molar solution of an ideal solute: Rankin and Davenport, 1981.) Above this concentration, however, both species are rapidly killed, while *G. pulex* survives up to 120mM Cl^-.

To attempt an explanation of this observation, the sodium influx and efflux of each species was investigated in NaCl solutions and sea water dilutions using the radio-isotope $^{22}Na^+$. The aim of these experiments was to discover whether the greater mortality of *G. tigrinus* and *G. zaddachi* in NaCl solutions was due to differential uptake of ions in the two types of solution. The radio-isotope of sodium ($^{22}Na^+$) was chosen for these experiments as the radioactivity absorbed by the animal can be measured directly in a gamma-counter in a live specimen, and the same animal can then be used to measure efflux. In the case of other important ions such as chloride, the animal must be liquidised before uptake can be measured using the beta emitter, $^{36}Cl^-$.

9:2 Methods

For each species and in each solution the following procedures were adopted: Either 10ml of unlabelled sodium chloride solution (made up using ANALAR sodium chloride dissolved in tap water) or 10ml of artificial sea water (Griffin and George sea-salt) made up using tap water to the appropriate concentration were measured into a 25ml conical flask maintained at 13^0C in a water bath. Ten *Gammarus* were then introduced into each flask. These animals came from stock cultures maintained in the laboratory and were mature adults of size range 1.5 -10mg dry weight. No ovigerous females were used in the experiments.

A calculated amount of $^{22}Na^+$ of 100-1000*u*Ci/mg sodium activity (obtained from Amersham International plc) was added to each flask. The amount of radioactive sodium added to obtain sufficient counts was negligable compared to the amount of non-radioactive sodium in the solution and could be ignored in calculations, as was any which might be absorbed onto the surface of the experimental vessels, since this was constant for each species. The amount added depended on the concentration of nonradioactive sodium in the solution. 1*u*Ci was added to the 1.45mM NaCl solution, 3*u*Ci to a 30mM Na^+ solution, 6*u*Ci to a 60mM Na^+ solution and 12 *u*Ci to a 120mM Na^+ solution.

The animals were left in the flasks for two hours, without any shaking.

After two hours, the *Gammarus* were removed and rinsed for approximately one minute in unlabelled solution at the same experimental concentration, to remove surface radioactivity. The final rinsing water was retained for

counting, and the animal rewashed if there was significantly more than background radioactivity in this solution, as this would have indicted that the animal had not been properly cleaned of surface radioactivity.

The *Gammarus* were then placed individually in plastic vials each containing 1ml of non-radioactive sodium solution and their radioactivity counted for one minute in a Hewllett-Packard 800 gamma counter which automatically subtracted the background counts from the total count for each animal. The 10 animals which originally came from the same flask, together with the solution in which they had been counted, were next poured into a clean 25ml conical flask and a 0.5ml sample immediately withdrawn and counted. The time that this sample was taken was recorded and the flask was returned to the 13^0C incubator for a further two hours.

Samples of 0.5ml were taken from the flask at 10, 20, 30, 40, 50, 60 and 120 minutes from the first sample and counted for one minute. After 2 hours, the *Gammarus* were rinsed in distilled water, then killed in methanol and dried to constant weight before weighing on a Cahn microbalance. After weighing, the *Gammarus* were again rinsed with distilled water to remove any remaining surface sodium salts. The 10 animals from each test were then placed in a plastic container and 0.5ml of concentrated hydrochloric acid was pipetted onto them. The acid dissolved all the soft tissues of the animal and left only small pieces of integument intact. The acid solutions were left overnight to ensure that all the soft tissues from the *Gammarus* were dissolved, then diluted with distilled water to 10ml. A 'blank' containing 0.5ml of concentrated hydrochloric acid and 9.5ml of distilled water was prepared.

The sodium concentration of the solutions was measured on a flame photometer using the 'blank' as the zero point. (There was no measurable sodium in this solution). The figures for sodium influx and efflux were then calculated as follows.

Calculation of Sodium Influx rates Using a Specimen Calculation *(G. pulex* in 1.45mM NaCl Solution)

$cmin^{-1}$ = Counts per minute

544,482 $cmin^{-1}$ = 1*u*Ci

1*u*Ci was added to 1.45mM Na^+

10ml 1.45mM Na^+ contained 14.5*u*mol Na^+

544,482 $cmin^{-1}$ = 14.5*u*mol Na^+

Hence Specific activity = 544,488/14.5

= 37,551 $cmin^{-1}umol^{-1}$

Mean weight of *Gammarus* = 5.15mg

Mean $cmin^{-1}$ after 2 hours = 2946

Hence 2946/37551 *u*mol were taken up in 2 h

= 0.07845 *u*mol in 2h in *Gammarus weighing 5.15mg*

= 0.00762umolmg^{-1}h^{-1}

Calculation of Sodium Efflux rates Using a Specimen Calculation *(G. pulex* in 1.45mM NaCl Solution)

Total amount of Na^+ in 10 animals = 12.261 *u*mol

Total $cmin^{-1}$ in 10 animals = 29459

(after 2 hours)

Total ^{22}Na lost in 1 h = 2448 $cmin^{-1}$

Hence specific activity = 29459/12.261

= 2402 $cmin^{-1}umol^{-1}$

Hence efflux from 10 animals (51.493mg) = 2448/2402

= 1.019 *u*mol

Hence efflux per unit weight = 1.019/51.493

= 0.0198 *u*molmg^{-1}h^{-1}

9:3 Results

The mean weights for the *Gammarus* of each species used in the experiments are shown in Table 9:3. The *G. pulex* and *G. tigrinus* were of similar size in all experiments, but the *G. zaddachi* were smaller than these two species, as in the oxygen uptake experiments (Chapter 6).

The methods used here provide estimates the influx and efflux rates of sodium ions, as the *Gammarus* are gaining and losing ions simultaneously. The calculated uptake is therefore a measure of the portion of sodium ions which the individual retains after a defined time period, and the efflux rates are a measure of the proportion of that sodium which the animal loses in a defined time, while still taking up non-radioactive sodium ions.

Table 9:4 and 9:5 show the calculated flux rates from the experiments. In 1mM NaCl solutions, in which all three species were able to live for long periods, all species had a small negative flux (net loss), as might be expected in experimental animals under stress. *Gammarus pulex* had the lowest influx and net flux in this solution (half the influx of *G. zaddachi* and three times that of *G. tigrinus*). The net flux was nearly four times higher in *G. zaddachi* and *G. tigrinus* than in *G. pulex* in this concentration.

At 11.4mM NaCl, the influx increased in all three species and was again highest in *G. tigrinus*, which, although it had the highest efflux, had a net sodium loss three times that of *G. pulex* and *G. zaddachi*.

Gammarus pulex appeared to be able to maintain a small net flux in all the NaCl solutions tested,

The LC_{50} range for this species was

TABLE 9:2

Osmotic Pressure and Na^+ concentration (mM) of NaCl solutions, Sea Water and Mine Drainage Water Dilutions

NaCl		Mine Drainage Water			Sea Water		
Na^+	mOsm	%	Na^+	mOsm	%	Na^+	mOsm
1	10						
28.2	63	10	21	85	10	38	99
56	118	30	63	261	30	90	277
85	170	80	168	682	80	342	724
113	218	100	210	870	100	380	900

TABLE 9:3

Mean Weight (mg) of *Gammarus* used in Sodium Uptake Experiments

	NaCl Solutions (mM)				Sea Water Solutions (%)		
(mM)	P	T	Z	(%)	P	T	Z
1	4.01	2.01	0.96				
10	4.14	3.39	1.15	10	4.10	2.01	0.96
30	4.01	2.73	1.24	30	3.6	1.73	1.44
60	3.13	3.12	1.02	70	3.06	2.21	0.95
120	4.37	2.97	1.24	120	3.22	1.80	0.99

P = *Gammarus pulex*

T = *Gammarus tigrinus*

Z = *Gammarus zaddachi*

above 100mM Na^+, indicating that for the short period of the experiment, this species was able to tolerate the solutions and increase efflux as influx rose.

In *G. zaddachi*, the influx and efflux increased together, leaving the animals with a small positive or negative flux, until in the final concentration (121.4mM), very large fluxes were measured, perhaps indicating that the animals were losing control of sodium regulation.

In *G. tigrinus*, large fluxes were measured in all the solutions. There was no clear pattern of net loss in this species, with net losses in 1, 11 and 61mM and small net gains in 31 and 121mM. The influx increased rapidly above 61mM NaCl, then fell in the highest concentration.

In sea water dilutions, the net loss of Na^+ in *G. pulex* in 38mM Na^+ was greater than in comparable NaCl solutions, but in *G. tigrinus* and *G. zaddachi* the loss was much less than in the comparable NaCl solution. The influx was identical in *G. pulex*, but higher in *G. tigrinus* and *G. zaddachi*, as was the efflux.

The influx into *G. pulex* remained lower than in *G. tigrinus* and *G. zaddachi* in sea water up to 226mM, when *G. pulex* showed a large increase in sodium influx and net flux, which increased in 456mM. This concentration was above the LC_{50} range of *G. pulex*, and the animals appeared to be losing control of sodium balance at this point.

In *G. zaddachi* the influx and net flux remained relatively low until the 456mM solution, when influx more than doubled, as did net gain. In *G. tigrinus*, there was a net loss of Na^+ in 38 and 114mM Na^+, but an increasing net gain above this.

The influx in NaCl solutions and sea water are shown in Figures 9:1 and 9:2.

TABLE 9:4

Sodium flux rates ($\mu molmg^{-1}h^{-1}$)
of three species of *Gammarus*
measured using radio-active sodium
Measured in NaCl solutions

Na^+ (mM)	Species	J_{in}	J_{out}	Net Flux
1	P	0.008	0.02	-0.012
	T	0.023	0.069	-0.046
	Z	0.017	0.06	-0.043
11.4	P	0.013	0.045	-0.032
	T	0.084	0.175	-0.091
	Z	0.06	0.096	-0.036
31.4	P	0.043	0.023	+0.02
	T	0.128	0.091	+0.037
	Z	0.162	0.051	0.111
61.4	P	0.035	0.036	-0.001
	T	0.148	0.146	+0.002
	Z	0.143	0.064	+0.079
121.4	P	0.043	0.038	+0.003
	T	0.099	0.149	-0.005
	Z	0.146	0.314	-0.168

P = *Gammarus pulex*, T = *Gammarus tigrinus*, Z = *Gammarus zaddachi*

TABLE 9:5

Sodium flux rates ($\mu molmg^{-1}h^{-1}$)
of three species of *Gammarus*
measured using radio-active sodium
Measured in Sea Water solutions

Na^+ (mM)	Species	J_{in}	J_{out}	Net Flux
38	P	0.008	0.119	-0.111
	T	0.064	0.075	-0.011
	Z	0.055	0.095	-0.04
114	P	0.044	0.068	-0.024
	T	0.078	0.109	-0.031
	Z	0.098	0.079	+0.019
266	P	0.283	0.1	+0.183
	T	0.116	0.098	+0.018
	Z	0.111	0.045	+0.066
456	P	0.355	0.052	+0.303
	T	0.241	0.194	+0.047
	Z	0.271	0.068	+0.203

P = *Gammarus pulex*, T = *Gammarus tigrinus*, Z = *Gammarus zaddachi*

FIGURE 9:1

Sodium Uptake ($\mu molmg^{-1}h^{-1}$) by three *Gammarus*species from NaCl solutions measured using radioactive sodium

LC_{50} ranges (in mM Na^{+}) are indicated for each species by arrows

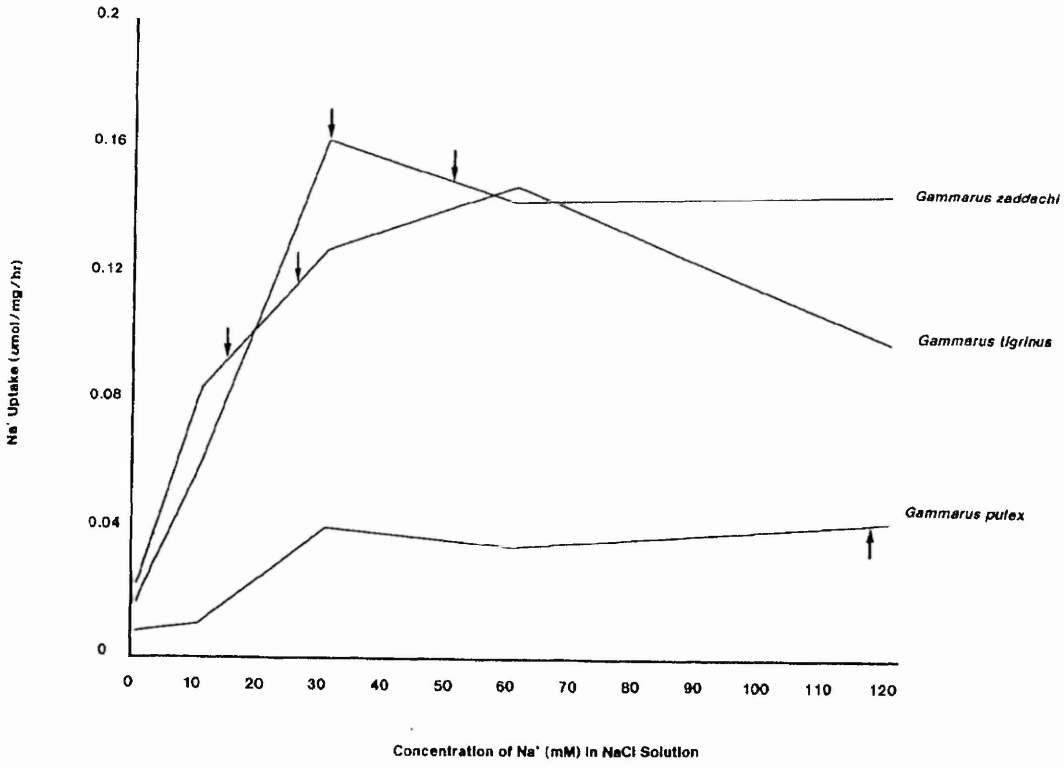
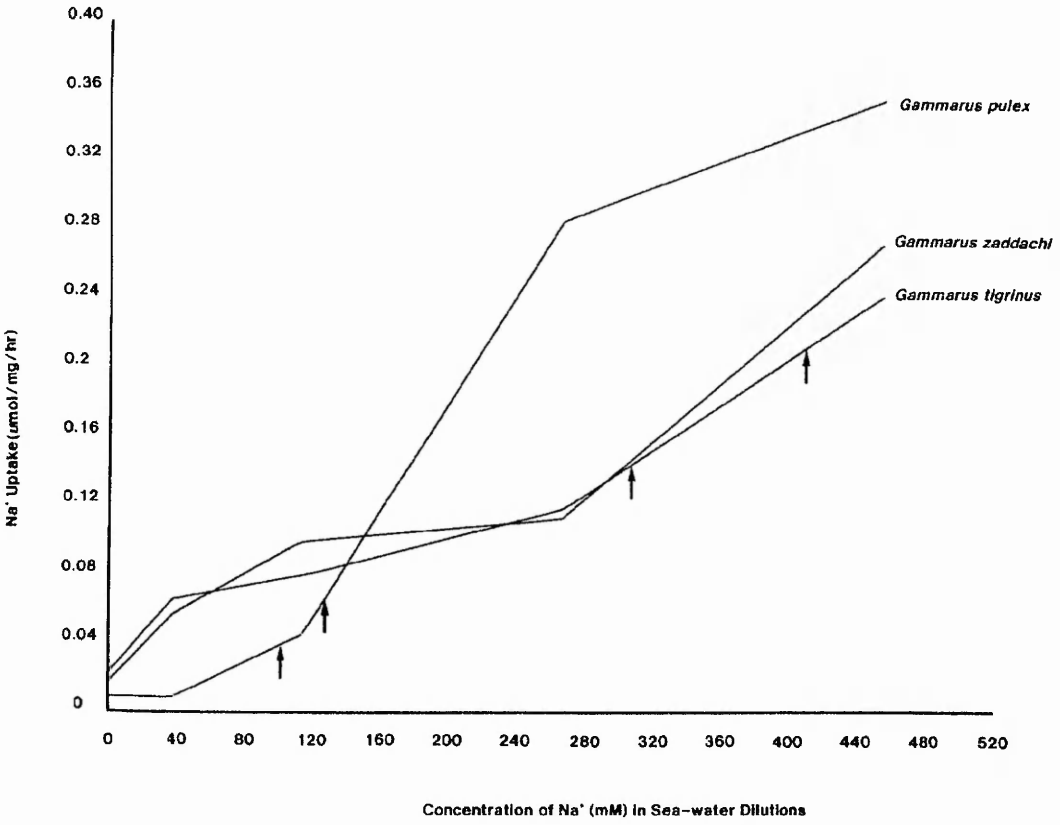

0.2
0.16
0.12
0.08
0.04
0
Na⁺ Uptake (μmol/mg/hr)
0
10
20
30
40
50
60
70
80
90
100
110
120
Concentration of Na⁺ (mM) in NaCl Solution
Gammarus zaddachi
Gammarus tigrinus
Gammarus putex

FIGURE 9:2

Sodium Uptake ($\mu molmg^{-1}h^{-1}$) by three *Gammarus* species from sea water solutions measured using radioactive sodium

LC_{50} ranges (in mM Na^{+}) are indicated for each species by arrows


0.40
0.36
0.32
0.28
0.24
0.2
0.16
0.12
0.08
0.04
0
Na⁺ Uptake (μmol/mg/hr)
0
40
80
120
160
200
240
280
320
360
400
440
480
520
Concentration of Na⁺ (mM) in Sea-water Dilutions
Gammarus pulex
Gammarus zaddachi
Gammarus tigrinus

9:4 Discussion

In the interpretation of the experiments described above, following the fluxes of sodium ions in *Gammarus* spp., several assumptions are made in methods and calculations. As in all radioactive tracer experiments, the uptake of ^{22}Na is assumed to be equivalent to that of ^{23}Na. Given this assumption, the net uptake rates of Na^+ ions can be simply and accurately calculated. However, measurements of the efflux rates are subject to several inaccuracies. Sodium efflux is usually measured in either distilled water or sugar solutions of equivalent osmotic pressure to the salt solutions used during experiments (eg Sutcliffe, 1967; Lockwood, 1970) to prevent uptake of sodium during the measurements. This uptake reduces the proportion of ^{22}Na to ^{23}Na in the *Gammarus* under test in the present experiments, although in calculation it was assumed that the proportion of the two ion species remained constant. In addition, the amount of sodium in the individuals was measured after four hours in the experimental conditions, which in higher sodium concentrations could result in considerable sodium uptake. However, it was decided not to use different external solutions to measure influx and efflux rates, as the effect of changing solutions on efflux rates was unknown. Despite these potential inaccuracies, however, the rates of influx are comparable with those found by previous authors (Table 9:6).

When the 96-hour LC_{50} values for sodium toxicity in the three species are compared with the calculated net uptake rates, there appears to be a relationship between the two

TABLE 9:6

Published Sodium Flux Rates in *Gammarus* spp.

Data are recalculated from original units to $\mu molmg^{-1}h^{-1}$ where appropriate

a) Influx

Species	Na^+ (mM)	SW/FW	Influx $\mu molmg^{-1}h^{-1}$	Authors
G. duebeni	9	SW	0.02	Shaw and Sutcliffe, 1961
G. duebeni	3	SW	0.015	Shaw and Sutcliffe, 1961
G. pulex	1	FW	0.009	Shaw and Sutcliffe, 1961
G. pulex	1	FW	0.008	Present Study
G. lacustris	1	FW	0.01	Sutcliffe and Shaw, 1967
G. pulex	0.8	FW	0.004	Sutcliffe, 1967
G. pulex	0.06	FW	0.001	Sutcliffe, 1967

b) Efflux (To distilled water)

Species	Na^+ (mM)	SW/FW	Influx $\mu molmg^{-1}h^{-1}$	Authors
G. duebeni	0.08	FW	0.003	Sutcliffe, 1967
G. duebeni	177	SW	0.006	Shaw and Sutcliffe, 1961
G. pulex	1	FW	0.009	Shaw and Sutcliffe, 1961
G. lacustris	100	SW	0.007	Sutcliffe and Shaw, 1971
G. lacustris	225	SW	0.02	Sutcliffe and Shaw, 1971

SW = Sea water, FW = Fresh water

which is similar in all three species, *ie*, the LC_{50} range for sodium occurs either during or just above concentrations at which influx increases sharply.

The difference in Na^+ uptake rates in NaCl solutions and sea water dilutions confirms the results of the toxicology tests which showed that Na^+ in NaCl solutions was more "toxic" than in sea water or mine drainage water dilutions in the brackish water species *G. tigrinus* and *G. zaddachi*, but of the same toxicity to the freshwater *G. pulex*.

Gammarus pulex appeared to take in Na^+ at a rate which increased in proportion to the external Na^+ concentration regardless of the other ions present in the external medium, up to approximately 120mM, which co-incides with the blood sodium levels found in this species by Shaw and Sutcliffe (1961). It could be argued that *G. pulex* simply takes in Na^+ ions passively until the the internal concentration of these ions exceeds the tolerance of the species, and that the lower rates of uptake when compared to *G. tigrinus* and *G. zaddachi* are due to lower permeability of the body wall in *G. pulex*. A reduction in permeability of the body wall when compared to marine members of the same genera is a common adaptation to freshwater in crustacea (Lockwood, 1962, Taylor and Harris, 1986). Since, however, *G. pulex* must take up sodium ions from an external medium more dilute than its own body fluids, passive uptake does not seem to be a likely method of sodium gain. Also Lockwood (1962) considered that the permeability of freshwater species such as *G. pulex* and brackish water species such as *G. tigrinus* and *G. zaddachi* was the same, and that the active uptake mechanisms in *G. pulex* had a greater affinity for sodium ions in low external concentrations

than in the brackish water species. Shaw and Sutcliffe (1961) found that the K_m (Michaelis Constant) for Na^+ ions in *G. pulex* was 0.06mM external sodium, which suggests that the sodium uptake routes for *G. pulex* would be able to operate at maximum efficiency in all the solutions used in the present experiments. The response of *G. pulex* to the high sodium concentrations could be explained if the species continued to absorb sodium at its maximum rate in all solutions, and to gain additional sodium through drinking and diffusion across the body wall. It is then able to excrete most of the sodium in excess of its requirements until the external medium reaches a sodium concentration approximately equal to that in its blood, and is thereafter unable to excrete all the excess sodium which enters and the animal rapidly dies. *G. pulex* produces a dilute urine whatever the external concentration of the medium (Sutcliffe, 1971a) and could be unable to obtain enough water against an osmotic gradient to excrete additional sodium ions once the external sodium has risen above its internal concentration.

G. zaddachi is tolerant of a wider range of internal sodium concentrations when compared to *G. pulex* (Sutcliffe, 1971b), as is *G. tigrinus* (Dorgelo, 1977). Dorgelo found that *G. tigrinus* had a blood sodium concentration above that of the external medium in sea water dilutions from 1-100%, although the lowest blood sodium concentration found in this species was 280mM, higher than the 190mM found in *G. zaddachi* and *G. pulex*. The osmolarities of NaCl solutions, mine drainage water and sea water dilutions are shown in Table 9:2. These demonstrate that the LC_{50} range for *G. pulex* occurred at similar osmotic pressures in NaCl solutions and sea

water, while in *G. tigrinus* and *G. zaddachi*, mortality occured at higher osmotic pressure in sea water and mine drainage water than in NaCl solution. This, together with the sodium uptake results, suggests that in the brackish water species, sodium uptake is limited in sea water by competition with another ion or ions which are present in sea water but not in sufficient quantity in fresh water. Many monovalent cations occur in sea water in small amounts, but the next most frequent ion after sodium is potassium. There are also large amounts of divalent cations such as calcium and magnesium. The toxicity experiments described in Chapter 8, however, indicate that the presence of potassium ions in sea water is the most important factor in reducing mortality in *G. tigrinus*.

Although potassium is found at lower concentration in the blood and other extracellular fluids (approximately 4mM), it is present in high concentration within cells (approximately 160mM), and is important in many cellular functions (Wilson, 1979). Potassium is taken into cells in exchange for the Na^+, brought into cells in exchange for H^+ and NH_4^+, by-products of respiration and the de-ammination of proteins. Potassium is therefore necessary to remove this sodium, and the uptake is an active process. Cell membranes are more permeable to K^+ than to Na^+, and if there were no active uptake of this ion, the internal cellular concentration of K^+ would rapidly be depleted. Potassium is also involved in the limitation of swelling of cells when an animal is moved to a hypo-osmotic medium (Vernberg and Vernberg, 1985).

Since K^+ is such an important ion in Crustacea, in fresh water it must be taken up from a solution in which it is at a very low concentration, implying a low value for K_m,

or obtained from food. For both *G. pulex* and *G. tigrinus*, there is enough K^+ in freshwater for animals to survive for long periods. However, when Na^+ concentration in the external solution rises, *G. pulex* is still able to obtain sufficient K^+ for its requirements, as adding extra potassium to more concentrated NaCl solutions does not improve the survival of this species (Chapter 8).

Gammarus tigrinus, on the other hand, is unable to tolerate increased Na^+ without the addition of extra K^+, suggesting that increased Na^+ is either inhibiting the uptake of K^+ in this species, or that increased amounts of K^+ are required to compensate for the increased uptake of Na^+.

Further experiments are therefore required to discover the nature of the regulation of sodium uptake in sea water in brackish water gammarids. The uptake of chloride should also be investigated, as this is an important ion in regulating nerve excitability. Beadle and Cragg (1940) working with *G. duebeni* and *G. pulex* found that *G. pulex* was less able to maintain a low tissue chloride concentration when the blood chloride level was raised, and that a high tissue chloride induced immobility. This reduction in mobility has also been seen in the present study, when, in increasing chloride concentrations, the number of swimming movements declined in all three species as chloride concentration increased (Chapter 6). Chloride is, however, present in sea water and mine drainage water in greater quantities than sodium, together with other anions such as bicarbonate and sulphate, unlike in NaCl solution, where the sodium and chloride are present in equal amounts and the amounts of other ions is small. Komnick *et al.* (1972), when working on chloride uptake in Baetidae in hypotonic solutions,

found that there were "chloride cells" in the epithelium of the tracheal gills which were used for chloride transport in dilute external concentrations and were also involved in osmoregulation. If there are similar cells on the gills of crustaceans, which most authors agree to be the main site of most osmoregulatory activity (Lockwood, 1962), the individual would be able to osmoregulate only when these cells were intact. It is also interesting to note that only *G. pulex* significantly increased its respiration rate in NaCl solutions up to 113mM chloride, and this is the only species tested which could survive in this chloride concentration. This suggests that *G. pulex* is using the extra respiratory energy for osmoregulation in NaCl solutions, while *G. tigrinus* and *G. zaddachi* suffer damage to the gill structure at lower concentrations and could not continue to osmoregulate.

Taylor and Harris (1986a, b; 1988), in experiments with the amphipod *Corophium curvispinum*, an Eastern European brackish water species which is a recent coloniser of fresh water in the English Midlands, found that passive Na^+ loss in this species was much higher than in other freshwater species, and also that it was more permeable to Cl^- than other freshwater organisms. They also found considerable physiological differences between populations of *C. curvispinum* at separated freshwater sites, in particular a lowering of permeability to Na^+, which would reduce passive ion loss requiring less energy to be expended by the animal on active uptake. It would be interesting to compare the permeability of *G. tigrinus* from different populations living in more and less saline waters, to discover if this species is also still adapting to freshwater life in a similar manner.

In terms of field situations, these observations may have

relevance if effluents consisting only of NaCl were to be released. Since coal-mine drainage water is chemically diverse and similar in composition to sea water, rivers affected by such effluents resemble brackish water systems. When fresh water species are eliminated by increased salinity in these disturbed environments, estuarine species are able to colonise the area. If NaCl was solely responsible for an increase in salinity, perhaps due to effluent from a chemical process, the potential brackish water colonists would be more sensitive than freshwater species, and would be unable to establish populations in the areas once freshwater community was eliminated.

CHAPTER 10

General Discussion

The principal aim of this study was to describe the effects of increased salinity on the distribution of freshwater invertebrate communities in the English East Midlands, and to explain the distributional patterns of species in saline areas using life history and physiological data. The ecological data were also modelled with the aim of predicting the effects of increased salinity, so that recommendations could be made concerning the disposal of future saline discharges to minimise the environmental impact of such discharges.

The river fauna of the Midlands is naturally less diverse that that of more upland areas (Wright *et al.*, 1984). Groups which are poorly represented include the stoneflies (Bird 1982), which are represented mainly by the Nemouridae (in particular *Amphinemura standfussi*), and two other species, *Isoperla gramatica* and *Taeniopteryx nebulosa*. There is also a reduction in the variety of mayfly species in these rivers as part of the natural distribution pattern, with families characteristic of cold upland waters such as Heptageneidae and Siphlonuridae absent from the Midlands. The loss of these families, which score highly on the B.M.W.P. system, reduces the maximum potential score in Midlands sites, a common fault in most scoring systems, which assign high scores to "upland" families and lower scores to "lowland" families such as Valvatidae and Viviparidae. However, river sites in the East Midlands should score approximately 150 on the B.M.W.P. system if

they were clean enough to support the "lowland" families of mayflies such as Ephemerellidae and Ephemeridae, and caddis such as Leptoceridae and Limnephilidae. It is in these groups that most sites are deficient in response to almost any type of pollution.

Increased levels of salinity were found to be very common in the rivers of the East Midlands, this additional stress acting upon invertebrate communities already under great pressure due to high levels of organic and industrial pollution. That the invertebrate fauna of the East Midlands is further depleted by pollution when compared to that of other regions, can be seen by studying the Biological Quality reports of the two Water Authorities at present responsible for most of this area, Severn-Trent and Yorkshire.

In the Chesterfield area, Yorkshire Water report long stretches of river as "Class 4" (grossly polluted) (Water Quality Report 1987), and discuss plans to improve some of these stretches, in which pollution is due to both domestic sewage and industrial wastes. However, the report cites problems in some lengths of river which are "affected by water from abandoned mines over which we have no control". The Authority does, however, report some improvement in the grossly polluted River Rother at Chesterfield, after British Coal was persuaded to route the discharge from its coking plant to the main sewer.

The two Water Authorities differ in their methods of assessing biological quality, but the resulting classifications are sufficiently compatible for comparisons to be made. Yorkshire Water do not directly publish the B.M.W.P scores for river sites, although the biological class to which they assign a site is based on this scoring system (pers. comm.). A site of Class B3,

for example, would have a B.M.W.P. score of below 50, and a Class B2 site a score of roughly 50-90. Most of the rivers in the mining area in the south of the Yorkshire Water area are in Classes 2, 3 and 4, while the majority of sites in the North of the region, north and west of York, with little mining and industrial input, are in Classes 1A and 1B, with scores greater than 150. Severn-Trent Water use the B.M.W.P system directly to classify their rivers, but their classification system involves six divisions, "Unsatisfactory" (score 0-10), "Poor" (score 10-25), "Moderate" (score 26-50), "Good" (score 51-100), "Very Good" (score 101-150) and "Excellent" (score above 150). Under this system, most of Millwood Brook, apart from the sites very close to the mine, would be classed as "Poor" or "Moderate", although some stretches the River Poulter would be "Good". The majority of sites within the Mansfield area would be "Moderate" in quality, or lower.

The low scores of the rivers in the River Idle catchment indicates that the natural capacity for "self-cleaning" (Hynes, 1970) of the rivers in the area has been exceeded. The dilution of the saline mine wastes is not sufficient to eliminate its effects, and the additional burden of sewage and industrial wastes results in a depressed fauna throughout the length of the catchment. Increased salinity can, in fact, be detected in the River Idle downstream to its tidal limit.

The present research has identified species level changes in some faunal groups which are the result of increased salinity, and a saline affected community has been described. The Tubificidae, generally described as pollution tolerant in relation to organic discharges, were found to be very susceptible to salinity, and were

eliminated completely at sites with a mean annual chloride concentration above 28mM chloride. This allows the highly saline tolerant enchytraeid *Lumbricillus rivalis* to colonise river sections where it would not normally be found. This can be explained directly from the laboratory investigation of the effects of salinity on the breeding of the two species. *Tubifex tubifex* had a reduced reproductive capacity above 28mM NaCl, with fewer young surviving than in lower salinities. *Lumbricillus rivalis*, in contrast, has an enhanced reproductive ability in salinities above 28mM NaCl, up to an optimum at about 56mM. This species is present in low levels in a normal river fauna, but is only able to dominate the oligochaete fauna when the reproduction of Tubificidae is reduced by salinity. If the salinity was reduced, the proportion of *L. rivalis* would decrease as the numbers of Tubificidae increased, and a normal fauna could be restored.

The mayfly *Baetis rhodani*, which is also considered pollution tolerant was replaced at quite low salinities by *Baetis vernus*, which was able to tolerate up to 28mM chloride. The chironomid *Prodiamesa olivacea*, also appeared to be encouraged by elevated salinity, especially when combined with some degree of organic input, and was found together with *Chironomus spp.* in these areas. It would be useful, in future work, to identify the species of Orthocladiinae found in saline areas, as species from the genera *Orthocladius* and *Cricotopus* were found at clean, slightly saline, and very saline sites, and it is likely that different species from these genera are found in these regions. Some species of *Cricotopus* are known to be brackish-water inhabitants (Cranston, 1987) and it would be intersting

to discover if these were the species found below the mine outfall at Creswell, since the brackish-water naidid *Paranais litoralis* was found in this area and at other very saline sites such as those on Vicar Water. There is now a key to some of the Orthoclad species available, (Cranston, 1987), and the identification of the species found at Creswell should be possible.

The gastropod fauna in saline areas was restricted to the ubiquitous *Potamopyrgus jenkinsi* (originally a brackish-water species), *Lymnaea peregra* and several species of Pisidium and Sphaerium. In less saline sites, other species such as *Bithynia tentaculata* also occur. The predominant caseless caddis at saline sites was *Hydropsyche angustipennis*, a species which is normally associated with the lower reaches of rivers. The other species common in the lower reaches of rivers, *H. siltali*, *H. pellucidula* and *H. contubernalis* (Eddington, 1981) were not found anywhere in the catchment where there was elevated salinity, although they were found in sites on the Meden where there was no salt intrusion.

In saline-affected areas *Gammarus pulex* was replaced by *G. tigrinus*, which now occurs widely in the Midlands in river systems other than the Idle catchment, for example in the River Trent at Beeston, Nottingham (pesonal observation.).

A typical species list from a mildly saline site (29-40mM chloride), therefore, would consist of very few tubificidae; large numbers of *Lumbricillus rivalis*; *Potamopyrgus jenkinsi; Lymnaea peregra* and possibly some Sphaeriidae; *Gammarus tigrinus; Asellus aquaticus; Baetis vernus; Hydropsyche angustipennis; Sigara concinna; Haliplidae* and other beetles; *Prodiamesa olivacea, Chironomus* spp, *Orthocladius* spp. and *Cricotopus* spp.;

and some other dipteran families such as Empididae and Tipulidae. This would give a B.M.W.P. score of between 30 and 40, Class 3 (Yorkshire Water) or "Moderate" (Severn-Trent Water).

The distribution and physiology of *Gammarus tigrinus* in the Midlands implied an organism which was adapting to freshwater habitats from its estuarine origins, as is suggested for the amphipod *Corophium curvispinum* (Taylor and Harris, 1986a). In experimental work the responses of *G. tigrinus* were nearly always between those of *G. pulex* and the estuarine *G. zaddachi*, and it was also able to breed in fresh water, although it has not yet been found in areas of the country which have no stretches of rivers with elevated salinity.

Another gammarid, *Gammarus duebeni*, is also able to live in both saline and fresh waters, and in Ireland is the dominant species in fresh water in the absence of *G. pulex*. There is some argument as to whether freshwater populations of *G. duebeni* are genetically distinct from brackish water populations, or whether the two are separate physiological races (Lockwood, 1971). There were no estuarine populations of *G. tigrinus* available to compare with the riverine populations examined, but it seems more likely that higher summer temperatures encourage *G. tigrinus*, rather than salinity alone, as the increased temperature enables this species to reach very high reproductive rates. This allows the build up of sufficient numbers to survive the winter and so many adults are available to begin breeding the next spring.

Gammarus tigrinus probably will not become common in upland areas due to low summer temperatures, unless salinity removes *G. pulex*, but it could well become the dominant species in the Midlands and possibly East

Anglia. At large river sites the two species may be found together, although for how long co-existence has occurred is not known.

If the classification system proposed by Dorgelo (1976) is used to describe the optimum range of *G. pulex*, *G. tigrinus* and *G. zaddachi*, *G. pulex* would be in group V, oligostenohaline (fresh water) species; *G. zaddachi* in group II or III, euryhaline or extremely euryhaline species which tolerate a wide range of salinities but have an optimum around that of sea water, and *G. tigrinus* in group IV, genuine brackish water species, which tolerate a wide range of salinities but have an optimum which lies below the salinity of full sea water but above that of fresh water. Several species, such as *G. duebeni* are described as "holeuryhaline" (existing equally well in fresh, brackish and sea-waters). It is these species which several authors (eg. Lockwood *et al.*, 1973; Pinkster *et al* 1970; Gledhill *et al.*, 1976) have suggested consist of separate physiological races existing in fresh and brackish water habitats. It is interesting to speculate that *G. tigrinus* might also develop separate races as it colonises freshwater habitats, since it is able to breed successfully at salinities of 1mM in England and also in estuarine conditions in its native North America.

It is likely that *G. tigrinus* will continue to spread in England, since many of the areas which it colonises are either unsuitable for *G. pulex* or only marginally suitable due to increased salinity. Indeed, in Germany *G. tigrinus* was introduced to rivers where the native *Gammarus* species had been eliminated through pollution, in order to provide food for fish (Bulnheim, 1972). Once established in a saline affected area, *G. tigrinus* would

not be eliminated by a reduction in salinity, and the presence of this species in the Midlands is likely to be permanent.

In laboratory experiments, *Gammarus tigrinus* did experience the same problems of ionic regulation in fresh water as did *G. zaddachi*, since when subjected to increasing NaCl without a parallel increase in potassium, animals of both species rapidly died. In contrast, the freshwater *G. pulex* was able to obtain sufficient potassium from the water to survive raised naCl concentrations, a surprising finding which would be worth further study.

The computer programs TWINSPAN and DECORANA have been used with increasing frequency in recent years in many ecological investigations, but the results of the analysis are frequently disappointing, with diffuse or overlapping TWINSPAN groups which are not adequately separated in two-dimensions on DECORANA axes. The problem seems to be that, when using small data sets, the analysis is only successful when there are one or two strong environmental gradients affecting the communities. In the present work, salinity and organic pollution provide two very strong gradients which spread the TWINSPAN groups along only two DECORANA axes. Successful modelling using the two programs has also been carried out by G J Bird (pers. comm) relating tanaidacean (crustacean) communities to ocean depth and by Terrell-Nield (1989) relating beetle species to woodland boundaries. In both these instances there were clear environmental gradients, and TWINSPAN produced separate groups on one or two DECORANA axes. If there are unclear gradients, the DECORANA ordination can produce four equally important axes, and the TWINSPAN groups then need

to be considered in three or four dimensions, which is very difficult. With large data sets such as that used by Wright *et al.* (1987) the effects of many variables and several axes can be considered.

The predictive mathematical model produced from the ecological data was tested on information from a range of sites in saline affected areas, and performed with a reasonable degree of accuracy over these sites. The model's database would benefit from enlargement by the addition of species from non-saline sites in the Midlands, and from the use of more environmental variables in the MDA (Multiple Discriminant) analysis. At present, mildly saline sites are poorly predicted, which could cause problems if the model was used to predict the effects of a new saline discharge which was calculated to produce only slight salinity in the receiving stream. Group 10 (which contains the mildly saline sites M17, M18 and M19), however, does produce species lists which describe mildly saline sites, although they are at present too long.

The predictive modelling and ecological investigations provide some guidelines for the level of future saline discharges which could be allowed into rivers. The maximum level at which a freshwater community could continue with no detectable effects is an annual mean of 14mM chloride, and a river in good condition (Class B1B, would remain in that category. A detectable, but not severe effect would be noticeable up to a mean of 28mM chloride, and this would downgrade a "good" quality site (Class B1B), to a moderate quality site (Class B2). Above this level the effects would be easily detectable, and would downgrade good quality sites to "polluted" classes (B3).

Saline effluents are released not only from working collieries, but from seepages and water from old seams which flood and cause excessive subsidence if they are not drained. Drainage water from old mine workings and tips is a recognised problem in South Wales (Scullion and Edwards, 1980). Here it was estimated that 60% of the rivers were of poor quality due to the coal industry, which in addition to acid and ferrugineous drainage from working and abandoned mines, also inputs coal particles from drift mines and preparation plants, and toxic discharges (NH_3, HCN, phenol) from coal carbonisation. The Welsh coal effluents are acid rather than saline in nature, but in other coalfields, such as Durham, saline effluents have been released for many years and Beadle and Cragg (1940) described a stream near the coast in Durham which was continually brackish due to mine effluent. In this coastal area, however, the saline effluents can be quickly discharged to sea, where the salinity is not a problem, although particulate coal waste has spoiled several beaches in County Durham.

The disposal of the effluent from Creswell and other collieries in a less polluting fashion presents serious problems to British Coal. The removal of salts from water is a very expensive process, requiring large amounts of energy, and although some Middle East countries use solar power in plants producing drinking water from sea water, the cost is still very high. The most frequently used method of de-salination is reverse osmosis (Pearce, 1987). In this process, pressure at the surface of a saline solution forces pure water through a membrane that is too dense to allow sodium or chloride ions through. The process produces two flows of water, one which may be

clean enough for irrigation or drinking, and the other which is very saline. This can then be dried or pumped to the coast. However, even in large plants using solar power, de-salination can cost £167 per 1000m^3. (If the mine effluent at Creswell, flowing at 100lsec-1, were to be de-salinated using this method, the cost would be £1432 per day). This is clearly impractical, since apart from the cost, this method merely concentrates the salt and small quantities of extremely concentrated brine remain for disposal, either by tanker or pipeline, or by drying in lagoons.

A more economic option open to British Coal appears to be to pump the saline water to a larger river, where dilution would reduce the effects of the salinity, and the chloride concentration of the river water could be held below 20mM throughout the year. If the effects of this type of increase in salinity are tested using the "Predict" program, this change moves sites from Group 11 (non-saline) to Group 10 (mildly saline) with the resulting change in fauna restricted to an alteration in the species of mayflies present, a different oligochaete fauna containing only *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, and a reduction in diversity of caddis, but still describes a reasonably diverse fauna at the site.

British Coal's proposals for the future disposal of the effluent from Creswell Colliery are to lay a larger pipeline through Creswell Crags and pump water from Creswell and Whitwell Collieries to Clumber Lake via old land drainage pipes across Welbeck Estate. There have been several objections to this proposal since it would involve digging up archaeological sites in the Creswell Crags area, an S.S.S.I, and it is unlikely that

permission would be obtained. In addition, Clumber Lake is a popular coarse fishery, and increasing the salinity of the water would affect its viability. It would also introduce ochre deposits into the sediment which would be aesthetically unacceptable to the users of the lake. This lake already suffers from mining subsidence which causes water to seep through cracks in the lake bed and lowers the level. It was drained in 1988 for repairs, but in view of the number and age of the workings underlying the area, further leakage seems likely. For all these reasons, British Coal's proposal was rejected, and they have yet to decide on an alternative scheme.

An alternative would be to route the mine effluent through the water treatment works at Creswell, which would involve little extra building work. Since this works produces an effluent of 1-5 Ml/day, (Severn-Trent Water Report, 1988) the dilution of the mine-water when mixed with the domestic sewage would be sufficient to allow the efficient operation of the filter bed (Yorkshire Water pers. comm.), and although the final effluent would be more saline than at present, it would be more dilute than the effluent at present entering upstream and would not have such a "shock" effect on the fauna. Alternatively, a pipeline could be built to divert the mine-water to a larger works such as those at Langwith, where British Coal already pump mine-water for discharge into the River Poulter every winter. The greater dilution available at a larger works would ameliorate the effects of the salinity. British Coal, would, however, have to pay the Water Authority for the disposal of effluent via any Water Reclamation Works, and this does not appear to have been considered at Creswell, although British Coal does divert the highly phenolic

waste from the coking plant near Chesterfield through the sewer there (Yorkshire Water 1988). However, the removal of the mine-water pipe would, paradoxically, result in problems in Millwood Brook below Crags Pond in summer, when there is very little flow from Crags Pond, and the river would quickly dry up in this area unless there was some increased flow from the dam. In summer, at present, most of the river flow in this area consists of mine-effluent. If this were removed and routed through the water treatment works, the bulk of the river flow would occur from the works, with very little dilution.

A further suggestion would be for British Coal to dilute the effluent themselves, using either mains water or water abstracted from Millwood Brook further upstream, and release larger quantities of less concentrated effluent. This would reduce the "shock" effect on the fauna, and also help to dilute the effluent from the Water Teatment Works further. This option, would, however, be expensive and involve several new consents being granted.

The Sewage Treatment Works at Creswell Crags has also been shown to cause problems in Milwood Brook, particularly due to its storm overflow, and there were plans to reduce the impact by increasing the capacity of the works and modernisation. However, the planned extension would have destroyed a very important archaeological site and objections were made together with a proposal to divert the effluent to another works. Finally, a compromise was reached, and improvements costing £250,000 were planned at the works. The Water Authority has now abandoned these plans and has at present no further scheme for improvements at the works, considering that the effluent is satisfactory in quality.

It seems likely, therefore, that the present problems at Creswell are set to continue, although Creswell Colliery has an estimated life of only 10-20 years. After the Colliery has shut down, water will still need to be pumped from the seams to prevent movement of rocks, but the quantity would be reduced over the years as the seams gradually subside and close. There will continue to be some saline water from the colliery for many years, and this problem will also occur in the other collieries in the Midlands, despite the reduction in coal mining in the area.

Salination of watercourses in coal-mining areas of Britain has been a problem for most of this century, and will continue to cause problems in the future both from new mines which are given permission to discharge. The new "super-pit" at Asfordby, for example, has been granted permission to discharge saline minewater at a rate of 3 litres per second (to an estimated maximum of 110m^3 per day) into the River Soar at Cotes Mill (pers. comm. Severn-Trent Water). The River Soar is at present of "Good" quality, and it is predicted that the degree of dilution available in this larger river will be sufficient to prevent its deterioration.

As coal mining progresses further east into the Vale of Belvoir, it is hoped that the disposal of saline waste water produced by the new collieries will be conducted in a manner designed to reduce environmental impact. The only practical method for safe disposal of saline wastes is dilution, and the information provided by this thesis gives guidelines for setting the levels of salinity which could be allowed in rivers without causing major ecological damage.

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APPENDIX A

	SITE												SITE										
	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
TAXA																								
Porifera																							+	
Hydroidea																			+					
TRICLADIDA																								
Planaria torva (Muller)										+														
Dugesia polychroa/lugubris																							+	
Polycelis nigra/tenuis		+	+			+	+															+	+	+
Polycelis felina (Dalyell)	+																							
Dendrocoelum lacteum (Muller)		+																					+	
GASTROPODA																								
Valvata piscinalis (Muller)																					+	+	+	
Potamopyrgus jenkinsi (Smith)	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		+	+	+	+	+
Bithynia tentaculata (L.)																								+
Lymnaea peregra (Muller)		+		+	+	+	+					+					+		+			+	+	+
Physa fontinalis (L.)																								+
Planorbis sp.																					+			+
Ancylus fluviatilis Muller																						+	+	

cont...

	SITE																					
	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
BIVALVIA																								
Sphaerium spp.																							+	+
Pisidium spp.	+	+	+	+		+														+	+	+	+	
OLIGOCHAETA																								
Paranais litoralis (Muller)								+	+	+	+	+	+	+	+	+								
Nais elinguis (Muller)		+	+	+	+	+	+		+	+	+	+	+	+	+	+		+	+	+	+		+	+
Stylaria lacustris (L.)																								+
Tubifex tubifex (Muller)		+	+		+	+	+														+		+	+
Psammoryctides barbatus (Grube)																							+	+
Limnodrilus hoffmeisteri Claparede		+	+			+														+				
Limnodrilus udekemianus Claparede						+																		
Potamothrix hammoniensis (Michaelsen)			+		+																+		+	+
Aulodrilus pluriseta Piguet																					+			
Tubificidae		+	+		+														+		+	+		+
Lumbricillus rivalis Levinsen			+				+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+
Lumbriculus variegatus (Muller)			+				+																	
Lumbricidae	+		+				+			+	+													
HIRUDINEA																								
Theromyzon tessulatum (Muller)							+														+	+	+	
Glossiphonia complanata (L.)		+	+			+	+			+	+										+			+

cont...

	SITE																						
	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
Helobdella stagnalis (L.)																								+
Erpobdella octoculata (L.)		+	+			+																		+
HYDRACARINA	+		+			+	+																+	
CRUSTACEA																								
Asellus aquaticus (L.)	+		+		+	+	+		+		+	+	+	+	+	+			+	+	+	+	+	+
Asellus meridianus Racovitza		+																						
Crangonyx pseudogracilis Bousfield																					+			
Gammarus pulex (L.)	+	+	+		+	+	+	+	+	+	+	+		+	+						+			
Gammarus tigrinus Sexton																+	+	+	+	+	+			+
EPHEMEROPTERA																								
Baetis vernus Curtis	+																		+		+	+	+	
Baetis rhodani (Pictet)	+	+	+			+	+			+									+		+			
Cloeon dipterum (L.)							+			+												+		+
Cloeon simile Eaton																					+			
Ephemerella ignita (Poda)	+		+																					+
Caenis sp.																							+	+

cont...

	SITE																					
	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																								
Amphinemura standfussi Ris	+																							
Nemoura cambrica (Stephens)	+																							
Isoperla grammatica (Poda)	+																							
HEMIPTERA																								
Velia caprai Tamanini	+																							
Sigara dorsalis (Leach)							+															+		+
Sigara concinna (Fieber)																								+
COLEOPTERA																								
Haliplus sp. (larva)				+			+			+													+	
Haliplus lineatocollis (Marsham)																					+	+		
Haliplus ruficollis (Degeer)																						+		
Deronectes depressus/elegans (Fabricius)							+															+		
Ilybius sp. (larva)																						+		
Helophorus brevipalpis Bedel	+			+																				
Helodes sp. (larva)	+																							
Elmis aenea (Muller)	+		+				+		+															
NEUROPTERA/MEGALOPTERA																								
Sialis lutaria (L.)																					+	+		
Sisyra sp.																							+	

cont...

	SITE																					
	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
TRICHOPTERA																								
Rhyacophila dorsalis (Curtis)	+																							
Glossosoma sp.	+																							
Agapetus sp.	+																							
Plectrocnemia conspersa (Curtis)	+																							
Sericostoma personatum (Spence)	+																							
Polycentropus flavomaculatus (Pictet)																						+		
Tinodes waeneri (L.)																							+	
Hydropsyche angustipennis (Curtis)																			+				+	+
Ithytrichia sp.																					+			
Hydroptila spp.						+	+														+	+	+	+
Limnephilus lunatus gp.	+	+	+		+	+	+			+											+			
Halesus/Potamophylax gp.	+		+		+																			
Athripsodes cinereus (Curtis)																							+	+
Mystacides sp.																								+
Mystacides nigra (L.)																								+
Mystacides longicornis (L.)																							+	
DIPTERA																								
TIPULIDAE																								
Tipula coukei/lateralis							+																	
Tipula montium gp.	+																							
Dicranota sp.																					+	+	+	

cont...

	SITE												SITE										
	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
Eloeophila sp.	+																							
Ormosia sp.																					+			
Molophilus sp.							+																	
DIXIDAE																								
Dixa sp.			+																					
Dixa nebulosa Meigen																								+
Dixa submaculata Edwards	+																							
CHIRONOMIDAE																								
Tanypodinae																								
Apsectrotanypus spp.													+								+			
Macropelopia spp.					+		+					+							+		+			
Procladius spp.				+	+							+	+			+	+	+			+	+	+	+
Thienemannimyia gp.					+		+												+		+	+	+	+
Orthocladiinae																								
Brillia longifurca Kieffer																		+						
Cricotopus spp.		+	+				+		+	+	+			+		+	+	+	+	+	+	+	+	+
Eukiefferiella spp.	+	+	+							+										+	+			
Eukiefferiella species D		+	+																					
Eukiefferiella claripennis (Lundbeck)			+																					

cont...

	SITE												SITE										
	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
Orthocladius spp.			+			+	+		+	+	+			+	+			+	+	+			+	+
Prodiamesa olivacea (Meigen)					+	+	+			+		+							+		+	+	+	+
Rheocricotopus spp.			+			+	+					+	+						+					
Camptocladius spp.													+											
Corynoneura spp.	+																							
Limnophyes spp.	+																							
Metriocnemus spp.							+			+														
Nanocladius spp.			+									+												
Chironominae																								
Chironomus gp.		+		+	+	+			+		+	+	+	+	+	+	+	+		+				+
Cryptochironomus spp.																						+	+	
Paracladopelma spp.						+																		
Paratendipes spp.																						+	+	
Penta/Polypedilum gp.	+																						+	+
Micropsectra spp.	+		+		+															+		+	+	
SIMULIDAE																								
Simulium costatum Friederichs	+																							
Simulium lundstromi Lundstrom	+																							
Simulium aureum gp.																					+			
Simulium equinum gp.																					+			

cont...

	SITE												SITE										
	1	2	3	A	B	4	5	6	7	C	8	9	10	11	12	13	14	15	D	16	E	17	18	19
Simulium reptans var. galeratum Edwards																							+	+
Simulium noelleri (Friederichs)																							+	+
Simulium erythrocephalum Den Geer																								+
Simulium ornatum Meigen		+	+				+																	
OTHER DIPTERA																								
Empididae							+		+		+												+	
Tabanidae				+																				
Ephydridae							+																	
Syrphidae							+																	
Pericoma spp.			+				+																	
Psychoda spp.							+		+	+				+		+								
Limnophora sp.							+			+														
Ceratopogonidae	+				+	+	+		+	+			+					+			+	+	+	+

APPENDIX B

Code, Location and Grid Reference of "Test" Sites

Code	Location	Grid Ref
ME1	River Meden (Mansfield)	SK558679
ME2	River Meden (Mansfield)	SK568686
ME3	River Meden (Mansfield)	SK594700
ME4	River Meden (Mansfield)	SK618702
ME5	River Meden (Mansfield)	SK652718
S1	Sookholme Bath (Mansfield)	SK541665
S2	Sookholme Bath (Mansfield)	SK543667
S3	Sookholme Bath (Mansfield)	SK544668
S4	Sookholme Bath (Mansfield)	SK544669
S5	Sookholme Bath (Mansfield)	SK545669
P1	Poolsbrook (Staveley)	SK438738
VW1	Vicar Water (New Clipstone)	SK600632
VW2	Vicar Water (New Clipstone)	SK606649
P3	Poolsbrook (Staveley)	SK445751
LA	River Lathkill (Lathkill Dale)	SK200063
CM	Crabmill Lane Flash (Cheshire)	SJ628994
RW1	Rainworth Water (Rainworth)	SK636604
RW2	Rainworth Water (Rainworth)	SK591586
L1	River Poulter at Nether Langwith	SK526702

APPENDIX B (Continued)

Environmental Measurements from "Test" Sites"

Site	Flow (m/sec)	Cl^- (mM)	Cond (mS/cm)	Width (m)	Depth (cm)	Substrate (1-5)
ME1	3	26	1.4	10	25	4
ME3	0.5	8	1.3	5	100	1
ME4	1.1	12.5	1.6	5	22	4
S2	0.9	16	6.4	2	13	2
S3	0.5	106	9	1	16	1
S4	0.6	636	5.6	1	8	3
S5	0.6	66	6.2	1	10	3
VW2	0.4	90	9	7	9	4
P3	1.0	80	3.5	10	12	1
LA	3	0.8	0.5	15	30	4
CM	0	24.5	2.13	40	50	1
RW1	0.9	20.5	2.7	5	20	3
RW2	0.9	40	3.06	3	12	4
L1	1.4	25	6	10	20	2

APPENDIX B (Continued)

Mean Environmental Measurements from Millwood Brook Sites

Site	FLOW (m/sec)	CHLORIDE (mM)	COND (mS/cm)	WIDTH (m)	DEPTH (cm)	SUBSTRATE (1-5)
M1	.142	1.73	.91	1.65	13	2
M2	.895	2.29	1.2	2.4	15	5
M3	.895	2.29	1.09	2.2	18	5
A	.407	45.24	5.29	1.8	37	1
B	.407	34.85	4.28	3.2	44	1.5
M4	.552	14.34	2.28	3.3	20	5
M5	1.57	15.52	2.44	4	7	5
M6	.71	393.2	36.19	3	7	5
M7	.506	199.17	18.33	4	25	3
C	.646	24.16	3.29	3.8	24	3
M8	.728	140.96	14.39	3.95	18	3
M9	.515	105.58	10.87	3.5	17	1
M10	.515	104.42	10.696	3.2	25	1
M11	1.127	99.44	10.84	2.8	29	3.5
M12	1.143	101.61	10.84	3	27	3
M13	.562	103.35	11	3.5	22	1.5
M14	.514	106.67	11.23	4	115	1
M15	.681	100.17	11.23	5	45	1.5
D	.334	11.03	1.54	1.1	11	3
M16	.567	62.64	7.57	3.5	24	3.5
E	1.08	13.61	2.66	6.9	66	1
M17	1.63	17.42	2.68	6	50	2.5
M18	.81	19.17	2.78	10.4	57	2.5
M19	3.38	21.47	2.76	10.8	28	3

APPENDIX C

TAXA	SITE																		
	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1
Porifera																			
Hydroidea																			
FLATWORMS																			
Planaria torva (Muller)																			
Dugesia polychroa/lugubris																			
Polycelis nigra/tenuis	+			+											+				
Polycelis felina (Dalyell)																			
Dendrocoelum lacteum (Muller)				+															
Crenobia alpina															+				
GASTROPODS																			
Valvata piscinalis (Muller)																			
Potamopyrgus jenkinsi (Smith)	+	+		+			+	+	+	+		+	+			+			
Bithynia tentaculata (L.)																			
Lymnaea peregra (Muller)	+	+		+			+					+		+	+	+	+	+	+
Physa fontinalis (L.)			+																+
Planorbis sp.																			
Ancylus fluviatilis Muller															+				

cont...

	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1
Sphaerium spp.																			
Pisidium spp.	+				+		+												+
OLIGOCHAETA																			
Paranais litoralis (Muller)								+		+									
Nais elinguis (Muller)	+	+	+	+	+	+			+	+		+	+						
Stylaria lacustris (L.)																+			
Tubifex tubifex (Muller)			+																
Psammoryctides barbatus (Grube)																			
Limnodrilus hoffmeisteri Claparede			+									+							+
Limnodrilus udekemianus Claparede																			
Potamothrix hammoniensis (Michaelsen)																+			
Aulodrilus pluriseta Piguet																			
Tubificidae					+			+				+							+
Lumbricillus rivalis Levinsen					+			+	+	+			+	+	+		+		
Lumbriculus variegatus (Muller)							+												+
Lumbricidae	+				+														
Rhyacodrilus coccineus																			+
LEECHES																			
Piscicola geometra																			+
Theromyzon tessulatum (Muller)																			
Glossiphonia complanata (L.)				+															

cont...

	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1
Helobdella stagnalis (L.)																+			
Erpobdella octoculata (L.)	+		+	+	+														
HYDRACARINA	+			+											+			+	+
CRUSTACEA																			
Asellus aquaticus (L.)	+	+	+	+	+							+				+		+	
Asellus meridianus Racovitza																			+
Crangonyx pseudogracilis Bousfield																			
Gammarus pulex (L.)	+	+		+											+				+
Gammarus tigrinus Sexton					+							+				+	+	+	
Gammarus duebeni																+			
MAYFLIES																			
Baetis vernus Curtis																			
Baetis rhodani (Pictet)	+	+		+											+				+
Cloeon dipterum (L.)																			
Cloeon simile Eaton																			
Ephemerella ignita (Poda)															+				+
Caenis sp.																			
Ecdyonurus sp.															+				

cont...

	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1
STONEFLIES																			
Amphinemura standfussi Ris																			
Nemoura cambrica (Stephens)																			+
Isoperla grammatica (Poda)															+				
Leuctra fusca															+				
Leuctra moselyi															+				
Diura bicaudata															+				
HEMIPTERA																			
Velia caprai Tamanini																			
Sigara dorsalis (Leach)												+							
Sigara concinna (Fieber)																+			
COLEOPTERA																			
Haliplus sp. (larva)				+			+					+							
Haliplus lineatocollis (Marsham)							+												
Haliplus ruficollis (Degeer)																			
Deronectes depressus/elegans (Fabricius)							+									+		+	
Ilybius sp. (larva)															+				
Helophorus brevipalpis Bedel																			
Helodes sp. (larva)																			
Elmis aenea (Muller)	+			+											+				+
Agabus (larva)						+						+			+				+
Platambus maculatus						+						+			+				+

cont...

NEUROPTERA/MEGALOPTERA	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1
Sialis lutaria (L.)	.	.	.	.	.	.	+	.	.	.	.	+	.	.	.	.	.	.	+
Sisyra sp.																			
TRICHOPTERA																			
Rhyacophila dorsalis (Curtis)	+			+											+				
Glossosoma sp.																			
Agapetus sp.																			
Plectrocnemia conspersa (Curtis)																			
Sericostoma personatum (Spence)																			
Polycentropus flavomaculatus (Pictet)																			
Tinodes waeneri (L.)																+			
Hydropsyche angustipennis (Curtis)	+			+													+		
Hydropsyche siltalai				+							+				+				+
Drusus annulatus															+				
Ithytrichia sp.																			+
Hydroptila spp.																			+
Limnephilus lunatus gp.																			+
Halesus/Potamophylax gp.						+									+	+			
Athripsodes cinereus (Curtis)																			
Mystacides sp.																			+
Mystacides nigra (L.)																			
Mystacides longicornis (L.)																			

cont...

	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1
TIPULIDAE																			
Tipula coukei/lateralis	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.
Tipula montium gp.						+				+									
Dicranota sp.																			
Eloeophila sp.																			
Ormosia sp.																			
Molophilus sp.																			
DIXIDAE																			
Dixa sp.																			
Dixa nebulosa Meigen																			
Dixa submaculata Edwards																			
Tanypodinae																			
Apsectrotanypus spp.																			+
Macropelopia spp.	+		+			+	+		+			+							+
Procladius spp.			+									+				+			+
Thienemannimyia gp.															+				
Orthocladiinae																			
Brillia longifurca Kieffer																			
Cricotopus spp.	+															+			
Eukiefferiella spp.		+		+			+					+			+				+
Eukiefferiella species D																			
Eukiefferiella claripennis (Lundbeck)																			

cont...

	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1
Orthocladius spp.	+		+												+			+	
Prodiamesa olivacea (Meigen)			+	+										+			+		+
Rheocricotopus spp.															+				
Camptocladius spp.																			
Corynoneura spp.																			
Limnophyes spp.																			
Metriocnemus spp.							+												
Nanocladius spp.																			
Thienemaniella spp.											+								
Chironominae																			+
Synorthocladius spp.															+				
Chironomus gp.					+							+		+		+	+		+
Cryptochironomus spp.																			
Paracladopelma spp.																			
Paratendipes spp.																			
Penta/Polypedilum gp.																			+
Micropsectra spp.			+		+	+				+					+		+		+
SIMULIDAE																			
Simulium costatum Friederichs																			
Simulium lundstromi Lundstrom																			
Simulium aureum gp.																			
Simulium equinum gp.					+														

cont...

	ME1	ME2	ME3	ME4	ME5	S1	S2	S3	S4	S5	P1	VW1	VW2	P3	LA	CM	RW1	RW2	L1
Simulium reptans var. galeratum Edwards																			
Simulium noelleri (Friederichs)																			
Simulium erythrocephalum Den Geer																			
Simulium ornatum Meigen	+			+	+	+													+
OTHER DIPTERA																			
Empididae						+							+		+				
Tabanidae																			
Ephydridae															+				
Syrphidae																			
Pericoma spp.	+					+													
Psychoda spp.																			
Limnophora sp.	+			+															
Ceratopogonidae					+			+		+			+		+				

APPENDIX D

Listing of the Computer Program "CLASS"

```
 10 CLS
 20PRINTCHR$(141);CHR$(131)"CLASSIFICATION PROGRAM"
 30 PRINT CHR$(141);CHR$(131)"CLASSIFICATION PROGRAM"
 40 PRINT:PRINT:PRINT CHR$(134)"SITENAME"
 50 INPUT A$
 60 PRINT:PRINT:PRINTCHR$(134)"TOTAL NUMBER OF SPECIES"
 70 INPUT A
 80 CLS
 90 B$="ARE THESE SPECIES ON YOUR LIST?"
100 PRINT:PRINT:PRINT:PRINT CHR$(136)CHR$(130)B$
110  DATA  Tubifex  tubifex,  Limnephilus  lunatus,
Prodiamesa olivacea, Polycelis nigra, Valvata piscinalis,
Lymnaea  peregra,  Pisidium  spp.,  Limnodrilus
hoffmeisteri,  Potamothrix  hammoniensis,  Theromyzon
tessulatum, Glossiphonia complanata
120  DATA  Erpobdella  octoculata,  Hydracarina  spp.,
Baetis  vernus,  Baetis  rhodani,  Cloeon  dipterum,
Ephemerella  ignita,  Sigara  dorsalis,  Elmis  aenea,
Hydropsyche  spp.,  Hydroptila  spp.,  Halesus/Potamophylax
gp., Dicranota spp., Macropelopia spp.
130  DATA  Thienemannimyia  gp.,  Eukiefferielaa  spp.,
Rheocricotopus spp., Penta/Polypedilum spp., Micropsectra
spp.
140 G=0
150 H=0
160 O=0
170 RESTORE 110
180 GOSUB 3440
190 FOR N=1 TO 29
```

```
  200 READ SPECIES$
  210 PRINT:PRINT:PRINT:PRINT:PRINT CHR$(133) SPECIES$
  220 PRINT:PRINT:PRINT"ANSWER Y OR N"
  230 INPUT P$
  240IF P$="Y" THEN G=G+1
  250 IF P$="N" THEN O=O+1
  260 IF P$="E" THEN GOTO 90
  270     IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 210
  280 CLS
  290 NEXT N
  300 PRINT:PRINT:PRINT CHR$(136)CHR$(130) B$
  310 RESTORE 330
  320 GOSUB 3440
  330  DATA  Paranais  litoralis,  Lumbricillus  rivalis,
Chironomus, Psychoda
  340 FOR N=1 TO 4
  350 READ SPECIES$
  360 PRINT:PRINT:PRINT:PRINT CHR$(131) SPECIES$
  370 PRINT:PRINT "ANSWER Y OR N"
  380 INPUT P$
  390 IF P$="Y" THEN H=H+1
  400 IF P$="N" THEN O=O+1
  410 IF P$="E" THEN GOTO 300
  420     IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 360
  430 CLS
  440 NEXT N
  450  GOSUB 3390
  460 IF G>H THEN PROC45
  470 IF H>G THEN PROC67
  480 DEFPROC45
  490 PRINT:PRINT CHR$(132)"END OF DIVISION 1"
```

```
  500 PRINT:PRINT:PRINT:PRINTCHR$(136)CHR$(130) B$
  510 RESTORE 520
520 DATA Polycelis felina, Lumbricidae, Hydracarina spp.,
Baetis vernus, Ephemerella ignita, Plecoptera, Velia
caprai, Helophorus spp., Helodes sp., Elmis aenea,
Rhyacophilidae, Plectrocnemidae, Sericostoma personatum,
Halesus/Potamophylax gp.
  530 DATA Tipula montium, Eloeophila spp., Dixa
submaculata, Eukiefferiella spp., Corynoneura spp.,
Limnophyes spp., Penta/Polypedilum gp., Micropsectra
spp., Simulium costatum, Simulium lundstroemi
  540 G=0
  550 H=0
  560 O=0
  570 GOSUB 3440
  580 FOR N=1 TO 24
  590 READ SPECIES$
  600 PRINT:PRINT:PRINT:PRINT:PRINTCHR$(133) SPECIES$
  610 PRINT:PRINT"ANSWER Y OR N"
  620 INPUT P$
  630 IF P$="Y" THEN G=G+1
  640IF P$="N" THEN O=O+1
  650 IF P$="E"THEN GOTO 500
    660   IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 600
  670 CLS
  680 NEXT N
  690 PRINT:PRINT:PRINTCHR$(136)CHR$(130)B$
  700 RESTORE 720
  710 GOSUB 3440
```

```
720 DATA Polycelis nigra, Dendrocoelum lacteum, Valvata
piscinalis, Lymnaea peregra, Planorbidae, Ancylus
fluviatilis, Sphaerium, Nais elinguis, Tubificidae,
Lumbricillus rivalis, Lumbriculus variegatus, Theromyzon
tessulatum, Glossiphonia complanata
  730 DATA Erpobdella octoculata, Gammarus tigrinus,
Cloeon dipterum, Caenis spp., Sigara dorsalis., Haliplus
spp., Sialis lutaria., Hydropsyche angustipennis.,
Hydroptilidae, Leptoceridae, Dytiscidae, Dicranota spp.,
Macropelopia., Procladius spp.
  740 DATA Thienemannimyia spp., Cricotopus spp.,
Eukiefferiella spp., Orthocladius spp., Prodiamesa
olivacea, Rheocricotopus spp., Chironomus spp.,
Cryptochironomus spp., Paratentipes spp., Simulium
reptans, Simulium argyreatum, Simulium ornatum
  750 DATA Empididae, Pericoma spp.
  760 FOR N=1 TO 41
  770 READ SPECIES$
  780 PRINT:PRINT:PRINT:PRINT:PRINT CHR$(131)SPECIES$
  790 PRINT:PRINT "ANSWER Y OR N"
  800 INPUT P$
  810 IF P$="Y" THEN H=H+1
  820 IF P$="N" THEN O=O+1
  830 IF P$="E"THEN GOTO 690
  840     IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 780
  850 CLS
  860 NEXT N
  870 IF A>=20 THEN G=G+1
  880 IF A<20 THEN H=H+1
  890
  900  IF G>H THEN PROC4
```

```
  910 IF H>G THEN PROC1011
  920 ENDPROC
  930 DEF PROC67
  940 PRINT:PRINT CHR$(132)"END OF DIVISION 1"
  950 PRINT:PRINT:PRINT:PRINTCHR$(136)CHR$(130) B$
  960 GOSUB 3440
   970 DATA Gammarus pulex, Paranais litoralis,
Lumbricidae, Glossiphonia complanata, Orthocladius spp.,
Empididae, Psychoda spp., Rheocricotopus spp.,
Camptocladius spp.
  980 G=0
  990H=0
 1000 O=0
 1010 RESTORE 970
 1020 FOR N=1 TO 9
 1030 READ SPECIES$
 1040 PRINT:PRINT:PRINT: PRINT:PRINT CHR$(133)SPECIES$
 1050 PRINT:PRINT "ANSWER Y OR N"
 1060 INPUT P$
 1070 IF P$="Y" THEN G=G+1
 1080 IF P$="N" THEN O=O+1
 1090 IF P$="E"THEN GOTO 950
 1100     IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 1040
 1110 CLS
 1120 NEXT N
 1130 PRINT:PRINT:PRINT:PRINTCHR$(136)CHR$(130) B$
 1140 GOSUB 3440
 1150  DATA Lymnaea peregra, Pisidium spp., Gammarus
tigrinus, Apsectrotanypus spp., Procladius spp.
 1160 RESTORE 1150
 1170 FOR N=1 TO 5
 1180 READ SPECIES$
```

```
 1190 PRINT:PRINT:PRINT:PRINT:PRINT CHR$(131)SPECIES$
 1200 PRINT:PRINT "ANSWER Y OR N"
 1210 INPUT P$
 1220 IF P$="Y"THEN H=H+1
 1230 IF P$="N" THEN O=O+1
 1240 IF P$="E" THEN GOTO 1130
 1250     IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 1190
 1260 CLS
 1270 NEXT N
 1280 IF G>H THEN PROC1213
 1290 IF H>G THEN PROC1415
 1300 ENDPROC
 1310 DEFPROC1011
 1320 PRINT:PRINT CHR$(132)"END OF DIVISION 2"
 1330 PRINT:PRINT:PRINT:PRINTCHR$(136)CHR$(130) B$
  1340 DATA Procladius spp., Porifera spp., Dugesia
polychroa,  Valvata piscinalis, Bithynia tentaculata,
Physa fontinalis, Planorbidae, Ancylus fluviatilis,
Sphaerium spp., Stylaria lacustris, Potamothrix
hammoniensis, Aulodrilus pluriseta
```

```
 1350 DATA Theromyzon tessulatum, Helobdella stagnalis,
Crangonyx pseudogracilis, Gammarus tigrinus, Baetis
vernus, Cloeon dipterum, Cloeon simile, Caenis spp.,
Sigara dorsalis, Sigara concinna, Haliplus spp.,
Dytiscidae, Sialis lutaria, Sisyra spp.
 1360 DATA Polycelis flavomaculatus, Tinodes waeneri,
Hydropsyche angustipennis, Ithytrichia spp., Hydroptila
spp., Athripsodes spp., Mystacides spp., Dicranota spp.,
Ormosia spp., Dixa nebulosa, Apsectrotanypus spp.,
Procladius spp.
 1370 DATA Thienemannimyia gp., Cryptochironomus spp.,
Paratendipes spp., Penta/Polypedilum, Micropsectra spp.,
Simulium aureum, Simulium equinum, Simulium reptans,
Simulium argyreatum, Simulium erythrocephalum,
Ceratopogonidae
 1380 RESTORE 1340
 1390 G=0
 1400 H=0
 1410 O=0
 1420 GOSUB 3440
 1430FOR N=1 TO 49
 1440 READ SPECIES$
 1450 PRINT:PRINT:PRINT:PRINT:PRINT CHR$(133)SPECIES$
 1460 PRINT:PRINT:PRINT"ANSWER Y OR N"
 1470 INPUT P$
 1480 IF P$="Y" THEN G=G+1
 1490 IF P$="N" THEN O=O+1
 1500 IF P$="E"THEN GOTO 1330
   1510   IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 1450
 1520 CLS
 1530 NEXT N
```

```
1540 PRINT:PRINT:PRINT:PRINTCHR$(136)CHR$(130) B$
1550 GOSUB 3440
1560 DATA Hydroidea, Polycelis nigra, Limnodrilus
hoffmeisteri, Limnodrilus udekemiensis, Lumbriculus
variegatus, Lumbricidae, Glossiphonia complanata,
Erpobdella octoculata, Hydracarina, Asellus aquaticus,
Gammarus pulex, Baetis rhodani, Elmis aenea
1570 DATA Halesus/Potamophylax gp., Tipula coulei,
Molophilus, Dixa spp., Eukiefferiella spp., Orthocladius
spp., Rheocricotopus spp., Metriocnemus spp., Nanocladius
spp., Paraclaopelma spp., Simulium ornatum, Ephydridae,
Syrphidae, Pericoma spp.
1580 DATA Psychoda spp., Limnophora spp.
1590 RESTORE 1560
1600 FOR N=1 TO 29
1610 READ SPECIES$
1620 PRINT:PRINT: PRINT:PRINT:PRINT CHR$(131)SPECIES$
1630 PRINT:PRINT "ANSWER Y OR N"
1640 INPUT P$
1650 IF P$="Y" THEN H=H+1
1660 IF P$="N" THEN O=O+1
1670 IFP$="E"THEN GOTO 1540
  1680  IF  (P$<>"N")AND  (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 1620
1690 CLS
1700 NEXT N
1710 IF G>H THEN PROCEND10
1720 IF H>G THEN PROCEND11
1730 ENDPROC
1740 DEFPROC1213
1750 PRINT:PRINT CHR$(132)"END OF DIVISION 2"
1760 PRINT:PRINT:PRINT:PRINTCHR$(136)CHR$(130) B$
1770 GOSUB 3440
```

```
1780 DATA Planaria torva, Lumbricidae, Glossiphonia
complanata, Baetis rhodani, Cloeon dipterum
1790 DATA Haliplidae, Limnephilus lunatus,
Eukiefferiella spp., Prodiamesa olivacea, Metriocnemus
spp., Psychoda spp., Limnophora spp., Ceratopogonidae
1800 G=0
1810 H=0
1820 O=0
1830 RESTORE 1780
1840 FOR N=1 TO 13
1850 READ SPECIES$
1860 PRINT:PRINT: PRINT:PRINT:PRINT CHR$(133)SPECIES$
1870 PRINT:PRINT:PRINT "ANSWER Y OR N"
1880 INPUT P$
1890 IF P$="Y" THEN G=G+1
1900 IF P$="N" THEN O=O+1
1910 IF P$="E"THEN GOTO 1760
1920 IF (P$<>"N")AND (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 1860
1930 CLS
1940 NEXT N
1950 PRINT:PRINT:PRINT:PRINTCHR$(136)CHR$(130) B$
1960 GOSUB 3440
1970 DATA Asellus aquaticus, Elmis aenea, Chironomus,
Empididae
1980 RESTORE 1970
1990 FOR N=1 TO 4
2000 READ SPECIES$
2010 PRINT:PRINT:PRINT:PRINT:PRINT CHR$(131)SPECIES$
2020 PRINT:PRINT "ANSWER Y OR N"
2030 INPUT P$
2040 IF P$="Y" THEN H=H+1
2050 IF P$="N" THEN O=O+1
```

```
 2060 IF P$="E" THEN GOTO 1950
   2070   IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 2010
 2080 CLS
 2090 NEXT N
 2100 IF A>16 THEN G=G+1
 2110 IF A<=16 THEN H=H+1
 2120 IF G>H THEN PROCEND12
 2130 IF H>G THEN PROCEND13
 2140 ENDPROC
 2150 DEFPROC1415
 2160 PRINT:PRINT CHR$(132)"END OF DIVISION 2"
 2170 PRINT:PRINT:PRINT:PRINTCHR$(136)CHR$(130) B$
 2180 GOSUB 3440
 2190   DATA Pisidium spp., Limnodrilus hoffmeisteri,
Gammarus tigrinus, Haliplus spp., Helophorus brevipalpis,
Brillia longifurca, Cricotopus spp., Eukiefferiella spp.,
Orthocladius spp., Micropsectra spp., Tabanidae
 2200 G=0
 2210 H=0
 2220 O=0
 2230 RESTORE 2190
 2240 FOR N=1 TO 11
 2250 READ SPECIES$
 2260 PRINT:PRINT:PRINT CHR$(133)SPECIES$
 2270 PRINT:PRINT "ANSWER Y OR N"
 2280 INPUT P$
 2290 IF P$="Y" THEN G=G+1
 2300 IF P$="N" THEN O=O+1
 2310 IF P$="E"THEN GOTO 2170
   2320   IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 2260
 2330 CLS
```

```
 2340 NEXT N
 2350 PRINT:PRINT:PRINT:PRINTCHR$(136)CHR$(130) B$
 2360 GOSUB 3440
 2370   DATA   Paranais   litoralis,   Asellus   aquaticus,
Apsectrotanypus spp., Rheocricotopus spp., Camptocladius
spp., Psychoda spp., Ceratopogonidae
 2380 RESTORE 2370
 2390 FOR N=1 TO 7
 2400 READ SPECIES$
 2410 PRINT:PRINT:PRINT:PRINT:PRINT CHR$(131)SPECIES$
 2420 PRINT:PRINT "ANSWER Y OR N"
 2430 INPUT P$
 2440 IF P$="Y" THEN H=H+1
 2450 IF P$="N" THEN O=O+1
 2460 IF P$="E" THEN GOTO 2350
 2470     IF    (P$<>"N")AND    (P$<>"Y")AND(P$<>"E")THEN
PRINT:PRINT "PLEASE ANSWER AGAIN":GOTO 2410
 2480 CLS
 2490 NEXT N
 2500 IF A>=11 THEN G=G+1
 2510 IF A<11 THEN H=H+1
 2520 IF G>H THEN PROCEND14
 2530 IF H>G THEN PROCEND15
 2540 ENDPROC
 2550 DEF PROC4
 2560 PRINT:PRINT:PRINT CHR$(141);CHR$(130)A$
 2570 PRINT CHR$(141);CHR$(130)A$
  2580PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)"YOUR
SITE IS IN GROUP 4"
 2590      PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"These sites have more than 30 species"
   2600   PRINTCHR$(132);CHR$(157);CHR$(131)"Conductivity
less than 1.2mS/cm"
```

```
 2610   PRINTCHR$(132);CHR$(157);CHR$(131)"Chloride  less
than 4.6 mM/l"
   2620   PRINTCHR$(132);CHR$(157);CHR$(131)"An   eroding
substrate"
 2630PRINTCHR$(132);CHR$(157);CHR$(131)"and   Depth  less
than 24 cm"
                                                    2640
PRINT:PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)"NO
CHLORIDE POLLUTION"
 2650 GOTO 3420
 2660 ENDPROC
 2670DEFPROCEND10
 2680PRINT:PRINT:PRINT:PRINT CHR$(141);CHR$(130)A$
 2690 PRINT CHR$(141);CHR$(130)A$
 2700PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131) "YOUR
SITE IS IN GROUP 10"
       2710PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"These sites have 20 or more species"
 2720     PRINTCHR$(132);CHR$(157);CHR$(131)"Conductivity
between 1.2 and 3.6mS"
 2730 PRINTCHR$(132);CHR$(157);CHR$(131)"Chloride between
4.6 and 20 mM"
 2740 PRINTCHR$(132);CHR$(157);CHR$(131)"A soft or  silty
substrate"
 2750     PRINTCHR$(132);CHR$(157);CHR$(131)"and    Depth
usually more than 24 cm"
 2760PRINT:PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"SLIGHT OR NO CHLORIDE POLLUTION"
 2770 GOTO 3420
 2780 ENDPROC
 2790 DEFPROCEND11
 2800 PRINT:PRINT:PRINT:PRINT CHR$(141);CHR$(130)A$
 2810PRINT CHR$(141);CHR$(130)A$
```

```
   2820     PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"YOUR SITE IS IN GROUP 11"
 2830       PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"These sites have 20 or more species"
   2840    PRINTCHR$(132);CHR$(157);CHR$(131)"Conductivity
between 1.2 and 3.6mS"
 2850PRINTCHR$(132);CHR$(157);CHR$(131)"Chloride  between
4.6 and 20 mM"
   2860    PRINTCHR$(132);CHR$(157);CHR$(131)"An   eroding
substrate"
 2870      PRINTCHR$(132);CHR$(157);CHR$(131)"and    Depth
usually less than 24 cm"
                                                      2880
PRINT:PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)"SLIG
HT OR NO CHLORIDE POLLUTION"
 2890 GOTO 3420
 2900 ENDPROC
 2910 DEFPROCEND12
 2920 PRINT:PRINT:PRINT:PRINT CHR$(141);CHR$(130)A$
 2930PRINT CHR$(141);CHR$(130)A$
 2940       PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"YOUR SITE IS IN GROUP 12"
   2950     PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"These sites have 16 to 20 species"
 2960      PRINTCHR$(132);CHR$(157);CHR$(131)"Conductivity
between 3.7 and 7 mS"
 2970 PRINTCHR$(132);CHR$(157);CHR$(131)"Chloride between
20 and 54 mS"
   2980    PRINTCHR$(132);CHR$(157);CHR$(131)"an   eroding
substrate"
 2990      PRINTCHR$(132);CHR$(157);CHR$(131)"and    Depth
usually less than 24 cm"
                                                      3000
```

```
PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)"MODERATE
TO HEAVY CHLORIDE POLLUTION"
 3010 GOTO 3420
 3020 ENDPROC
 3030 DEFPROCEND13
 3040 PRINT:PRINT:PRINT:PRINT CHR$(141);CHR$(130)A$
 3050 PRINT CHR$(141);CHR$(130)A$
   3060     PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"YOUR SITE IS IN GROUP 13"
 3070       PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"These sites have 0 to 16 species"
      3080PRINTCHR$(132);CHR$(157);CHR$(131)"Conductivity
above 7 mS"
 3090 PRINTCHR$(132);CHR$(157);CHR$(131)"Chloride between
54 and 225 mM"
 3100     PRINTCHR$(132);CHR$(157);CHR$(131)"An    eroding
substrate"
 3110PRINTCHR$(132);CHR$(157);CHR$(131)"and Depth usually
less than 24 cm"
                                                    3120
PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)"HEAVY   TO
EXTREME CHLORIDE POLLUTION"
 3130 GOTO 3420
 3140 ENDPROC
 3150 DEFPROCEND14
 3160 PRINT:PRINT:PRINT:PRINT CHR$(141);CHR$(130)A$
 3170 PRINT CHR$(141);CHR$(130)A$
   3180     PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"YOUR SITE IS IN GROUP 14"
 3190       PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"These sites have 11 to 20 species"
3200      PRINTCHR$(132);CHR$(157);CHR$(131)"Conductivity
between 3.6 and 13 mS"
```

```
 3210 PRINTCHR$(132);CHR$(157);CHR$(131)"Chloride between
20 and 100 mM"
 3220  PRINTCHR$(132);CHR$(157);CHR$(131)"A soft or silty
substrate"
   3230   PRINTCHR$(132);CHR$(157);CHR$(131)"and    Depth
usually more than 24 cm"
 3240
PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)"MODERATE
TO HEAVY CHLORIDE POLLUTION"
 3250 GOTO 3420
 3260 ENDPROC
 3270 DEFPROCEND15
 3280 PRINT:PRINT:PRINT:PRINT CHR$(141);CHR$(130)A$
 3290 PRINT CHR$(141);CHR$(130)A$
 3300      PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"YOUR SITE IS IN GROUP 15"
   3310    PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)
"These sites have 0 to 11 species"
 3320     PRINTCHR$(132);CHR$(157);CHR$(131)"Conductivity
above 13 mS"
 3330 PRINTCHR$(132);CHR$(157);CHR$(131)"Chloride between
100 to 250 mM"
 3340  PRINTCHR$(132);CHR$(157);CHR$(131)"A soft or silty
substrate"
   3350   PRINTCHR$(132);CHR$(157);CHR$(131)"and    DEPTH
usually more than 24 cm"
 3360
PRINT:PRINT:PRINTCHR$(132);CHR$(157);CHR$(131)"HEAVY   TO
EXTREME CHLORIDE POLLUTION "
 3370 GOTO 3420
 3380 ENDPROC
 3390 IF A>=20THEN G=G+1
 3400 IF A<20 THEN H=H+1
```

```
 3410 RETURN
 3420    PRINT:PRINT:PRINT  CHR$(131);CHR$(157);CHR$(132)"
FOR ANOTHER SITE TYPE RUN"
 3430 END
 3440 TIME=0
 3450 IF TIME=300 GOTO 3470
 3460 IF TIME<300 GOTO 3450
 3470 RETURN
 3480 PRINT CHR$(141);CHR$(130)A$
```

APPENDIX E

Method for Calculation of TWINSPAN
Group Membership from Environmental Data
The method is adapted from Moss (1988)
and is followed by the computer program
"PREDICT"

For each site to be classified:

1. The six functions F1-F6 are calculated.
eg
F1=.77958*A+.23317*B+.45764*C+.48187*D+.45915*E-1.1952*F

2. The function scores for the test site are compared with the mean scores for each group.

eg For group 4.
If mean function scores in group 4 are: F1=m14; F2=m24; F3=m34; F4=m44; F5=m54; F6=m64
Then Site Score (S)
$=(F1-m14)^2 + (F2-m24)^2 + (F3-m34)^2 + (F4-m44)^2 + (F5-m54)^2 + (F6-m64)^2$

3. The site is checked to see if it is within the scope of the classification.
To do this, the minimum group comparison score is used as a chi square vlaue with 5 (F6-1) degrees of freedom. If the minimum score is greater than the chi-squared value at the 1% level, the site is too far away from any of the group means to be reliably classified.

4. The probable group membership is calculated in four steps.

(i) The probability of membership is calculated for each group (P4-P15), using the following equation:-eg Probability for group (Pn)=N*exp(-s/2) where N=the number of sites in the group.

(ii) The total probability (PT) is calculated as P4+P10+P11+P12+P13+P14+P15

(iii) The Probable group membership PG=Pn/PT

(iv) The site is assigned to the group having the highest PG.

4. A predicted list of species for the assigned group is produced. This is the list of all species occuring in the original sites of that group. The 20 commonest species over all groups are assigned a probability of occurence of 1, while others have a probability of occurence calculated as the proportion of sites in a group in which the species occurs. These probabilities are multiplyed by PG to give the final probability of occurence.

APPENDIX F

Listing of the Computer Program "PREDICT"

```
10 VDU3
20 B1=0
30 Z$="FOR NEXT PAGE PRESS ANY KEY"
40 MODE 7
50 DIM B$(127)
```

60 DATA Porifera, Hydroidea, Planaria torva, Dugesia polychroa/lugubris, Polycelis nigra/tenuis, Polycelis felina, Dendrocoelum lacteum, Valvata piscinalis, Potamopyrgus jenkinsi, Bithynia tentaculata, Lymnaea peregra, Physa fontinalis, Planorbidae

70 DATA Ancylus fluviatilis, Sphaeriidae, Sphaeriidae, Paranais litoralis, Nais elinguis, Stylaria lacustris, Tubifex tubifex, Psammoryctides barbatus, Limnodrilus hoffmeisteri, Limnodrilus udekemianus, Potamothrix hammoniensis, Aulodrilus pluriseta

80 DATA Tubificidae, Lumbricillus rivalis, Lumbriculidae, Lumbricidae, Theromyzon tessultum, Glossiphonia complanata, Helobdella stagnalis, Erpobdella octoculata, Hydracarina, Asellus aquaticus, Asellus meridianus, Crangonyx pseudogracilis

90 DATA Gammarus pulex, Gammarus tigrinus, Baetis vernus, Baetis rhodani, Cloeon dipterum, Cloeon simile, Ephemerella ignita, Caenis spp., Amphinemura standfussi, Nemoura cambrica, Isoperla grammatica, Velia caprai, Sigara dorsalis, Sigara concinna

100 DATA Haliplidae, Haliplis lineatocollis, Haliplus ruficollis, Dytiscidae, Dytiscidae, Helophorus brevipalpis, Helodes spp., Elmis aenea, Sialis lutaria, Sisyra sp., Rhyacophila dorsalis, Glossosoma spp., Agapetus spp.

110 DATA Plectrocnemia spp., Sericostoma personatum, Polycentropus flavomaculatus, Tinodes waeneri, Hydropsyche angustipennis, Ithytrichia spp., Hydroptila spp., Limnephilus lunatus, Halesus/Potamophylax gp., Athripsodes cinereus, Mystacides spp.

120 DATA Mystacides nigra, Mystacides longicornis, Tipula spp., Tipula montium, Dicranota spp., Eloeophila spp., Tipula spp., Tipula spp., Dixa spp., Dixa nebulosa, Dixa submaculata, Apsectrotanypus spp., Macropelopia spp., Procladius spp.

130 DATA Thienemannimyia spp., Brillia longifurca, Cricotopus spp., Eukiefferiella spp., Eukiefferiella sp D, Eukiefferiella claripennis, Orthocladius spp., Prodiamesa olivacea, Rheocricotopus spp., Orthocladius sp., Corynoneura spp.

140 DATA Limnophyes spp., Metriocnemus spp., Nanocladius spp., Chironomus spp., Cryptochironomus spp., Paracladopelma spp., Paratendipes spp., Penta/Polypedilum gp., Micropsectra spp., Simulium costatum, Simulium lundstroemi, Simulium aureum

150 DATA Simulium equinum, Simulium reptans, Simulium argyreatum, Simulium erythrocephalum, Simulium ornatum, Empididae, Tabanidae, Ephydridae, Syrphidae, Pericoma spp., Psychoda spp., Limnophora spp., Ceratopogonidae, Piscicola geometra

```
160 DATA Hydropsyche spp.
170 FOR I=1 TO 127
180 READ S$
190 B$(I)=S$
200 NEXT I
210 CLS
220PRINTCHR$(141);CHR$(131);TAB(7);"PREDICTION PROGRAM"

  230    PRINT    CHR$(141);CHR$(131);TAB(7)"PREDICTION
PROGRAM"
240 PRINT:PRINT:PRINT CHR$(134)"SITENAME"
250 INPUT A$
260 PRINT:PRINT:PRINTCHR$(134)"FLOW RATE IN m/sec"
270 INPUT A
280 PRINT:PRINT:PRINTCHR$(134)"CHLORIDE IN mM/L"
290 INPUT B
300  PRINT:PRINT:PRINTCHR$(134)"CONDUCTIVITY IN mS"
310 INPUT C
320 PRINT:PRINT:PRINT CHR$(134)"WIDTH IN m"
330 INPUT D
340 PRINT:PRINT:PRINTCHR$(134)"DEPTH(cm)"
350 INPUT E
360  PRINT:PRINT:PRINTCHR$(134)"SUBSTRATE (1 TO 5)"
370 INPUT F
380  CLS
390 MODE 6
400  VDU19,1,6,0,0,0
410 C=LOGC
420 B=LOGB
430 D=LOGD
440 E=LOGE
```

```
  450  DIM F(15),FI(7)
  460 F1=0
  470
F1=.77958*A+.23317*B+.45764*C+.48187*D+.45915*E-1.1952*F
  480 F2=0
                                                          490
F2=-.18814*A-.2705*B+1.06*C-.46457*D-.09857*E+.42625*F
  500 F3=0
  510
F3=.31433*A+2.55266*B-2.11774*C-.21784*D+1.04736*E+.72261
*F
  520 F4=0
                                                          530
F4=-.23285*A-.0938*B+.11178*C+.95196*D-.80559*E+.14133*F
  540 F5=0
  550
F5=.28422*A-1.43383*B+1.35253*C+.03710*D+.37034*E+.09992*
F
  560 F6=0
                                                          570
F6=1.06497*A-.30865*B+.34046*C-.48998*D-.10349*E-.19946*F

  580 V4=0
  590 V10=0
  600 V11=0
  610 V12=0
  620 V13=0
  630 V14=0
  640 V15=0
  650 PROB4=0
  660 PROB10=0
```

```
670 PROB11=0
680 PROB12=0
690 PROB13=0
700 PROB14=0
710 PROB15=0
720 F(1)=0
730 F(2)=0
740 F(3)=0
750 F(4)=0
760 F(5)=0
770 F(6)=0
780 F(7)=0
790 A4=-2.822
800 B4=.933
810 C4=4.026
820 D4=-.32
830 E4=.364
840 F4=-.756
850 N4=1
860 A10=.3202
870 B10=.216
880 C10=5.8782
890 D10=-.6662
900 E10=1.246
910 F10=.314
920 N10=5
930 A11=-4.163
940 B11=1.614
950 C11=6.281
960 D11=-.055
970 E11=.553
```

```
 980 F11=-.273
 990 N11=3
1000 A12=-2.044
1010 B12=1.126
1020 C12=6.168
1030 D12=-.397
1040 E12=-.357
1050 F12=-.659
1060 N12=3
1070 A13=-1.203
1080 B13=1.5102
1090 C13=7.013
1100 D13=-.452
1110 E13=-.433
1120 F13=-.411
1130 N13=5
1140 A14=.763
1150 B14=.536
1160 C14=5.73
1170 D14=-.954
1180 E14=-.478
1190 F14=-.401
1200 N14=3
1210 A15=.827
1220 B15=.572
1230 C15=5.241
1240 D15=-.593
1250 E15=-.717
1260 F15=-.335
1270 N15=3
1280
```

```
V4=(F1-A4)^2+(F2-B4)^2+(F3-C4)^2+(F4-D4)^2+(F5-E4)^2+(F6-
F4)^2
                                                        1290
V10=(F1-A10)^2+(F2-B10)^2+(F3-C10)^2+(F4-D10)^2+(F5-E10)^
2+(F6-F10)^2
 1300
V11=(F1-A11)^2+(F2-B11)^2+(F3-C11)^2+(F4-D11)^2+(F5-E11)^
2+(F6-F11)^2
                                                        1310
V12=(F1-A12)^2+(F2-B12)^2+(F3-C12)^2+(F4-D12)^2+(F5-E12)^
2+(F6-F12)^2
 1320
V13=(F1-A13)^2+(F2-B13)^2+(F3-C13)^2+(F4-D13)^2+(F5-E13)^
2+(F6-F13)^2
                                                        1330
V14=(F1-A14)^2+(F2-B14)^2+(F3-C14)^2+(F4-D14)^2+(F5-E14)^
2+(F6-F14)^2
 1340
V15=(F1-A15)^2+(F2-B15)^2+(F3-C15)^2+(F4-D15)^2+(F5-E15)^
2+(F6-F15)^2
 1350 EX4=V4/2
 1360 EX10=V10/2
 1370 EX11=V11/2
 1380 EX12=V12/2
 1390 EX13=V13/2
 1400 EX14=V14/2
 1410 EX15=V15/2
 1420 PROB4=N4*EXP(-EX4)
 1430 PROB10=N10*EXP(-EX10)
 1440 PROB11=N11*EXP(-EX11)
 1450 PROB12=N12*EXP(-EX12)
```

```
 1460 PROB13=N13*EXP(-EX13)
 1470 PROB14=N14*EXP(-EX14)
 1480 PROB15=N15*EXP(-EX15)
                                                              1490
PROB=PROB4+PROB10+PROB11+PROB12+PROB13+PROB14+PROB15
 1500 F(1)=PROB4/PROB
 1510 F(2)=PROB10/PROB
 1520 F(3)=PROB11/PROB
 1530 F(4)=PROB12/PROB
 1540 F(5)=PROB13/PROB
 1550 F(6)=PROB14/PROB
 1560 F(7)=PROB15/PROB
 1570 FI(1)=F(1)
 1580 FOR I=1 TO 7
 1590 IF F(I)>FI(1)THEN FI(1)=F(I)
 1600 NEXT I
 1610 FOR I=1 TO 7
 1620 IF F(I)=FI(1) GOTO 1640
 1630 IF F(I)>FI(1)-.1 THEN FI(2)=F(I)
 1640 NEXT I
 1650 FI(3)=F(1)
 1660 FOR I=1 TO 7
 1670 IF F(1)<FI(3) THEN FI(3)=F(1)
 1680 NEXT I
 1690 IF FI(3)>0.872 THEN PROCRANGE
 1700 IF FI(2)>0 THEN GOSUB 4380
 1710 COLOUR 0
 1720 COLOUR 129
 1730 CLS
 1740  PRINT:PRINT:PRINT:PRINT:PRINT:PRINTTAB(5);"DO  YOU
WANT A PRINTOUT"
```

```
1750PRINT:PRINTTAB(7);"OF THE SPECIES LIST?"
1760 PRINT:PRINT:PRINT"     TYPE Y FOR YES"
1770 PRINT"     OR N FOR NO"
1780 INPUT G$
1790 IF G$="N" THEN GOTO 1810
1800 IF (G$<>"Y")AND(G$<>"N")THEN GOTO 1740
1810 IF FI(1)=F(1) THEN PROCGP4
1820 IF FI(1)=F(2) THEN PROCGP10
1830 IF FI(1)=F(3) THEN PROCGP11
1840 IF FI(1)=F(4) THEN PROCGP12
1850 IF FI(1)=F(5) THEN PROCGP13
1860 IF FI(1)=F(6) THEN PROCGP14
1870 IF FI(1)=F(7) THEN PROCGP15
1880 IF FI(2)=0 THEN GOSUB 4520
     1890          IF          FI(2)>0          THEN
CLS:PRINT:PRINT:PRINT:PRINT:PRINT:PRINT "SECOND GROUP"
1900 PRINT:PRINT:PRINT:PRINT:PRINT:PRINTZ$
1910 D$=GET$
1920 CLS
1930 IF FI(2)=F(1) THEN PROCGP4
1940IF FI(2)=F(2) THEN PROCGP10
1950IF FI(2)=F(3) THEN PROCGP11
1960IF FI(2)=F(4) THEN PROCGP12
1970IF FI(2)=F(5) THEN PROCGP13
1980IF FI(2)=F(6) THEN PROCGP14
1990 IF FI(2)=F(7) THEN PROCGP15
2000 GOSUB 4520
2010 END
2020 CLS
2030 DEFPROCGP4
2040 CLS
```

```
 2050 PRINT:PRINT:PRINT:PRINT TAB(10); "GROUP4"
 2060 PRINT:PRINT:PRINT TAB(7);"THESE SITES ARE FROM"
 2070 PRINT:PRINT TAB(7);"SMALL,CLEAN STREAMS"
 2080PRINT:PRINT:PRINT  TAB(1);"USUALLY  NEAR  THE  RIVER
SOURCE"
 2090 PRINT:PRINT TAB(1); "THEY HAVE MORE THAN 25 SPECIES
PRESENT"
                                                        2100
PRINT:PRINT:PRINT:PRINT:PRINT:PRINT:PRINT:PRINT:PRINTZ$

 2110 D$=GET$
 2120 GOSUB 4660
 2130 CLS
 2140 IF G$="Y" THEN VDU2
 2150 PRINTTAB(15);A$
 2160 PRINTTAB(5);"GROUP 4"
 2170 PRINTTAB(5);"TAXA"TAB(25);"PROBABILITY (%)"
 2180 DATA 9, 1, 16, 1, 35, 1, 38, 1, 41, 1, 72, 1,  125,
1, 6, 1, 29, 1, 34, 1, 40, 1, 44, 1, 46, 1, 47, 1, 48, 1,
49,  1,  57, 1, 58, 1, 59, 1, 62, 1, 65, 1, 66, 1, 73, 1,
79, 1, 81, 1, 86, 1, 93, 1, 100, 1, 101, 1, 108, 1,  109,
1, 110, 1, 111, 1
 2190 RESTORE 2180
 2200 FOR N=1 TO 20
 2210 READ K
 2220 READ L
 2230 B1=INT(F(1)*L*100)
 2240 PRINT TAB(5);B$(K)TAB(30);B1
 2250 NEXT N
 2260 IF G$="Y"THEN GOTO 2290
 2270 PRINT"FOR NEXT PAGE PRESS ANY KEY"
```

```
 2280 A$=GET$
 2290 FOR N=1 TO 13
 2300  READ K
 2310 READ L
 2320 B1=INT(F(1)*L*100)
 2330 PRINT TAB(5);B$(K)TAB(30);B1
 2340 NEXT N
 2350 IF G$="Y" THEN GOTO 2380
 2360 PRINT Z$
 2370 D$=GET$
 2380 VDU3
 2390 ENDPROC
 2400 DEFPROCGP10
 2410 CLS
 2420 PRINT:PRINT:PRINT TAB(15);"GROUP10"
 2430  PRINT:PRINT:PRINT:PRINT  TAB(5);"THESE  SITES  ARE
FROM WIDE RIVERS"
 2440 PRINT TAB(5);"WHICH MAY BE BELOW LAKES."
 2450 PRINT:PRINT:PRINT:PRINT"   THEY  HAVE  MAINLY  SOFT
SEDIMENTS"
 2460 PRINT:PRINT:PRINT TAB(5);"AND THEY HAVE SALINITY OF
"
 2470 PRINT TAB(10);"UP TO 20 mM/l"
 2480 PRINT:PRINT:PRINT:PRINT:PRINT:PRINTZ$
 2490 D$=GET$
 2500 CLS
 2510 GOSUB 4660
 2520 IF G$="Y" THEN VDU2
 2530 PRINT TAB(15);A$
 2540 PRINT TAB(5);"GROUP 10"
 2550 PRINT TAB(10);"TAXA"TAB(25);"PROBABILITY (%)"
```

```
 2560  DATA   1, .2, 4, .2, 5, .4, 7, 1, 8, .6, 9, 1, 10,
.2, 11, 1, 12, .2, 13, .4, 14, .4, 15, 1, 18, 1, 19,  .2,
20, 1, 21, .4, 22, 1, 24, .8, 25, .2, 26, .8, 27, .4, 30,
.6,  31,  .4, 32, .2, 33, .8, 126, .4, 34, .8, 35, 1, 37,
.2, 38, 1, 39, 1, 40, .6, 41, 1, 42, .4, 43, .2, 44,  .2,
45, .4, 50, .4, 51, .2, 52, .8
 2570  DATA  53,  .4, 54, .2, 55, .2, 56, .8, 59, .8, 60,
.4, 61, .2, 62, .4, 67, .2, 68, .2, 69, .4, 70,  .2,  71,
.6,  72,  1,  73, .2, 74, .4, 75, .2, 76, .2, 77, .2, 80,
.6, 82, .2, 85, .2, 87, .2, 88, .4, 89, 1, 90, 1, 92,  1,
96, 1, 97, 1, 104, 1, 105, .4, 107, .4, 108, .4, 109, .6,
112, .2, 113, .2, 114, .4
 2580 DATA 115, .4, 118, .2, 125, 1
 2590 RESTORE 2560
 2600 FOR S=1 TO 4
 2610 FOR N=1 TO 20
 2620 READ K
 2630 READ L
 2640 B1=INT(F(2)*L*100)
 2650 PRINT TAB(5);B$(K)TAB(30);B1
 2660 NEXT N
 2670 IF G$="Y"THEN GOTO 2700
 2680 PRINT "FOR NEXT PAGE PRESS ANY KEY"
 2690 P$=GET$
 2700 NEXT S
 2710 VDU3
 2720 ENDPROC
 2730 DEFPROCGP11
 2740 CLS
 2750 PRINT:PRINT:PRINT:PRINT:PRINTTAB(15); "GROUP 11"
 2760   PRINT:PRINT:PRINTTAB(5);"THESE   SITES  ARE  FROM
```

```
RIVERS WHICH "
 2770PRINTTAB(10);"ARE UP TO 8m WIDE"
 2780 PRINT:PRINT:PRINTTAB(3);"THEY HAVE LOW TO  MODERATE
SALINITY"
 2790 PRINT TAB(10);"(UP TO 20mM/1)"
 2800 PRINT:PRINT:PRINT:PRINT:PRINTZ$
 2810 D$=GET$
 2820 CLS
 2830 GOSUB 4660
 2840 IF G$="Y"THEN VDU2
 2850 PRINT TAB(15);A$
 2860 PRINT TAB(10);"GROUP 11"
 2870  DATA 5, 1, 7, 1, 9, 1, 11, 1, 16, 1, 18, 1, 20, 1,
22, .75, 23, 1, 24, 1, 26, .5, 27, .5, 28,  .5,  29,  .5,
30,  .25, 31, 1, 33, .75, 34, .75, 35, 1, 36, .25, 38, 1,
39, 1, 41, 1, 42, .25, 44, 1, 50, .25, 52,  1,  55,  .25,
59,  .8,  62,  .5, 71, .25, 72, 1, 73, 1, 78, .25, 80, 1,
83, .25, 84, .25, 88, .25, 90, .25
 2880 DATA 92, 1, 93, 1, 96, 1, 97,  .5,  98,  .75,  102,
.25, 103, .25, 104, 1, 106, .25, 109, .25, 117, .75, 118,
.25,  120,  .25,  121,  .25, 122, .5, 123, .25, 124, .25,
125, 1
 2890 RESTORE 2870
 2900 FOR S=1 TO 2
 2910FOR N=1 TO 20
 2920 READ K
 2930 READ L
 2940 B1=INT(F(3)*L*100)
 2950 PRINT TAB(5);B$(K)TAB(30);B1
 2960 NEXT N
 2970 IF G$="Y"THEN GOTO 3000
```

```
 2980 PRINT Z$
 2990 D$=GET$
 3000 NEXT S
 3010 FOR N=1 TO 17
 3020 READ K
 3030 READ L
 3040 B1=INT(F(3)*L*100)
 3050 PRINTTAB(5);B$(K)TAB(30);B1
 3060 NEXT N
 3070 IF G$="Y"THEN GOTO 3100
 3080 PRINTZ$
 3090 D$=GET$
 3100 VDU3
 3110 ENDPROC
 3120 DEFPROCGP12
 3130 CLS
 3140 PRINT:PRINT:PRINT:PRINTTAB(10);"GROUP 12"
  3150  PRINT:PRINT:PRINTTAB(5);"THESE  SITES  ARE  FROM
RIVERS"
 3160 PRINT TAB(10);"UP TO 8m WIDE"
 3170PRINT:PRINT:PRINTTAB(5)"THEY MAY RECEIVE DISCHARGES"

 3180 PRINTTAB(8);"WHICH ARE INTERMITTENT"
 3190  PRINT:PRINT:PRINT  TAB(5);"THEY  HAVE  MODERATE TO
HIGH CHLORIDE"
 3200 PRINTTAB(10);"(UP TO 60 mM/l)"
  3210  PRINT:PRINT:PRINT  TAB(3);"THERE  MAY  BE  SLIGHT
ORGANIC INPUT"
 3220 PRINT:PRINT:PRINT:PRINTZ$
 3230 D$=GET$
 3240 CLS
```

```
 3250 GOSUB 4660
 3260 IF G$="Y"THEN VDU2
 3270 PRINT TAB(15)A$
 3280 PRINT TAB(5);"GROUP 12"
 3290 PRINTTAB(15);"TAXA" TAB(25);"PROBABILITY (%)"
 3300  DATA  2, .33, 3, .33, 9, 1, 11, 1, 16, 1, 17, .33,
18, 1, 22, .33, 26, .33, 27, 1, 29, .33, 31, .33, 35,  1,
38,  1,  39, 1, 40, .33, 41, 1, 42, .33, 52, .8, 69, .33,
72, .8, 88, .33, 90, .8, 92, 1, 93, 1, 96, 1, 97, 1,  98,
.33, 102, .33, 104, 1, 109, .33, 123, .33, 124, .33
 3310 DATA 117, .8, 125, 1
 3320 RESTORE 3300
 3330 FOR N=1 TO 20
 3340 READ K
 3350 READ L
 3360 B1=INT(F(4)*L*100)
 3370 PRINT TAB(5);B$(K) TAB(30);B1
 3380 NEXT N
 3390 IF G$="Y"THEN GOTO 3420
 3400 PRINTZ$
 3410 D$=GET$
 3420 FOR N=1 TO 15
 3430 READ K
 3440 READ L
 3450 B1=INT(F(4)*L*100)
 3460 PRINT TAB(5);B$(K) TAB(30);B1
 3470 NEXT N
 3480 IF G$="Y"THEN GOTO 3500
 3490 PRINT:PRINT:PRINTZ$:D$=GET$
 3500 VDU3
 3510 ENDPROC
```

```
 3520 DEFPROCGP13
 3530 CLS
 3540 PRINT:PRINT:PRINTTAB(10);"GROUP 13"
 3550  PRINT:PRINT:PRINT  TAB(3);"THESE  SITES  ARE  FROM
RIVERS"
 3555PRINTTAB(10);"UP TO 8m WIDE"
  3560  PRINT:PRINT:PRINT:PRINTTAB(3);"THEY  HAVE  STONEY
SUBSTRATES"
 3570  PRINT:PRINT:PRINTTAB(3);"THEY  HAVE HIGH LEVELS OF
CHLORIDE"
 3575 PRINTTAB(10);"(ABOVE 20mM/1)"
 3590 PRINT:PRINT:PRINT:PRINT:PRINTZ$
 3600 D$=GET$
 3610 CLS
 3620 GOSUB 4660
 3630 IF G$="Y" THEN VDU2
 3640 PRINTTAB(15);A$
 3650 PRINT TAB(5);"GROUP 13"
 3660 PRINT TAB(5);"TAXA"TAB(25);"PROBABILITY (%)"
 3670 DATA 9, 1, 11, 1, 17, 1, 18, .8, 27, 1, 29, .2, 31,
.2, 35, 1, 38, .5, 39, .5, 59, .2, 89, .8,  92,  .6,  96,
.8, 104, .8, 118, 1, 123, .4, 125, .2
 3680 RESTORE 3670
 3690 FOR N=1 TO 18
 3700 READ K
 3710 READ L
 3720 B1=INT(F(5)*L*100)
 3730 PRINT TAB(5);B$(K) TAB(30);B1
 3740 NEXT N
 3750 IF G$="Y"THEN GOTO 3780
 3760 PRINT Z$
```

```
 3770 D$=GET$
 3780 VDU3
 3790 ENDPROC
 3800 DEFPROCGP14
 3810 CLS
 3820 PRINT:PRINT:PRINT TAB(10); "GROUP 14"
 3830   PRINT:PRINT:PRINT  TAB(3);"THESE  SITE  ARE  FROM
RIVERS"
 3840 PRINT TAB(3);"WITH SIGNIFICANT ORGANIC INPUT"
  3850PRINT:PRINT:PRINTTAB(5);"AND   MODERATE   TO   HIGH
CHLORIDE "
 3855PRINTTAB(10);"(20 TO 100mM/l)"
 3860    PRINT:PRINT:PRINT:PRINT"THEY    HAVE    A    SILTY
SUBSTRATE"
 3880 PRINT:PRINT:PRINTZ$
 3890 D$=GET$
 3900 CLS
 3910 GOSUB 4660
 3920 IF G$="Y"THEN VDU2
 3930 PRINTTAB(15);A$
 3940 PRINT TAB(5);"GROUP 14"
 3950 PRINT TAB(5);"TAXA"TAB(25);"PROBABILITY (%)"
 3960 DATA 9, 1, 11, 1, 16, 1, 18, .66, 27, 1, 35, 1, 39,
.66, 52, .8, 57, .33, 89, 1, 91, .33, 92, .66, 96, 1, 97,
1, 104, 1, 117, .8, 118, .8, 119, .33, 122, .5, 125,  .33

 3970 RESTORE 3960
 3980 FOR N= 1 TO 20
 3990 READ K
 4000 READ L
 4010 B1=INT(F(6)*L*100)
```

```
4020PRINT TAB(5);B$(K) TAB(30);B1
4030 NEXT N
4040 IF G$="Y" THEN GOTO 4070
4050 PRINT Z$
4060 D$=GET$
4070 VDU3
4080 ENDPROC
4090 DEFPROCGP15
4100 CLS
4110 PRINT:PRINT:PRINT TAB(10);"GROUP 15"
4120PRINT:PRINT:PRINT:PRINTTAB(3);"THESE  SITES ARE FROM
RIVERS WHICH"
 4130  PRINT  TAB(3);"HAVE  HIGH  LEVELS  OF  ORGANIC
POLLUTION"
4140   PRINT:PRINT:PRINT:PRINT   TAB(3);"AND  ALSO  HIGH
CHLORIDE LEVELS"
4145 PRINT TAB(10);"(MORE THAN 100 mM/l)"
4150 PRINT:PRINT:PRINT:PRINTTAB(3);"THEY  HAVE  A  SILTY
SUBSTRATE"
4170 PRINT:PRINT:PRINT:PRINT Z$
4180 D$=GET$
4190 CLS
4200 GOSUB 4660
4210 IF G$="Y" THEN VDU2
4220 PRINT TAB(15);A$
4230 PRINT TAB(5);"GROUP 15"
4240 PRINTTAB(5);"TAXA"TAB(25);"PROBABILITY (%)"
4250  DATA  9, 1, 11, 1, 17, 1, 18, 1, 27, 1, 35, 1, 39,
.8, 87, 1, 89, 1, 92, 1, 97, 1, 98, .66, 99, .66, 104, 1,
118, .66123, .33, 125, 1
4260 RESTORE 4250
```

```
 4270 FOR N=1 TO 16
 4280 READ K
 4290 READ L
 4300 B1=INT(F(7)*L*100)
 4310 PRINTTAB(5);B$(K) TAB(30);B1
 4320 NEXT N
 4330 IF G$="Y"THEN GOTO 4360
 4340 PRINTZ$
 4350 D$=GET$
 4360 VDU3
 4370 ENDPROC
 4380 PRINT:PRINT:PRINT"YOUR SITE HAS CHARACTERISTICS"
 4390 PRINT "OF TWO GROUPS"
 4400 PRINT "THE MOST LIKELY GROUP"
 4410 PRINT "WILL BE DISPLAYED FIRST"
 4420 PRINT "THE NEXT MOST LIKELY GROUP"
 4430 PRINT "WILL BE DISPLAYED SECOND"
 4440 RETURN
 4450DEFPROCRANGE
 4460 PRINT:PRINT:PRINT:PRINT:PRINT:PRINT:PRINT"YOUR SITE
IS NOT WITHIN THE RANGE"
 4470 PRINT"OF SITES IN THIS CLASSIFICATION"
 4480 PRINT:PRINT:PRINT"AND THE SPECIES PRESENT"
 4490 PRINT"CANNOT BE PREDICTED"
 4500 END
 4510 ENDPROC
 4520 VDU3
  4530  PRINT:PRINT:PRINT:PRINT:PRINT"ANOTHER   SITE   TO
CLASSIFY?"
 4540 PRINT"TYPE Y FOR YES OR N FOR NO"
 4550 P$=GET$
```

```
 4560 IF P$="Y" THEN CLS:PRINT:PRINT:PRINT:PRINT"TYPE RUN
TO CONTINUE":END
 4570 IF (P$<>"Y")AND (P$<>"N")THEN GOTO 4530
 4580 IF P$="N" THEN GOTO 4630
 4590 CLS
 4600 MODE 7
 4610 PRINT:PRINT:PRINT:PRINT:PRINT:PRINT"END"
 4620 END
 4630 MODE 7
 4640 PRINT:PRINT:PRINT:PRINT:PRINT:PRINT TAB(15);"END"
 4650 END
 4660 CLS
 4670 S$="THE PREDICTED NUMBER OF SPECIES"
 4680 S=INT(41-14.13*B)
 4690 PRINT:PRINT:PRINTS$
 4700 PRINT:PRINT TAB(10);"AT SITE    " A$
 4710 PRINT:PRINT TAB(10);"= "S
 4720 PRINT:PRINT:PRINT"IF YOU HAVE ONLY SAMPLED THE SITE
ONCE"
 4730 PRINT:PRINT:PRINT S$
 4740 S1=INT(S/3*2)
 4750 PRINT:PRINT TAB(10);"= "S1
 4760 PRINT:PRINT
```

APPENDIX G

Monthly Values of Temperature, Chloride ion and Conductivity measured at Sites M1, M3, M19 and OLL

SITE	M1			M3		
	Temp	Cond	Cl^-	Temp	Cond	Cl^-
	^0C	mS/cm	mM	^0C	mS/cm	mM
DATE						
3.87	7.5	0.94	2.4	7.0	1.1	3.0
4.87	10	0.9	2.1	10	0.9	2.2
5.87	11	0.96	2.5	10.5	0.98	4
6.87	10	0.86	1.0	11	0.8	1.5
7.87	13.5	0.96	1.0	14.0	1.03	2.4
8.87	15.5	1.0	1.4	16	1.0	2.0
9.87	13.25	1.0	2.2	14	1.7	2.2
10.87	5.5	0.8	1.6	8.0	0.8	1.8
11.87	4.5	0.86	1.8	6.75	.8	2
12.87	3	0.8	1.2	4	1.2	2.2
1.88	6.5	0.9	1.2	6.5	0.91	1.6
2.88	6.5	1.0	0	7.0	0.9	2
3.88	7.0	1.1	2.0	7.0	1.1	2.2
4.88	11.5	1.1	1.2	11.5	1.5	1.4

APPENDIX G (Continued)

Monthly Values of Temperature, Chloride ion and Conductivity measured at Sites M1, M3, M19 and OLL

	SITE	M19			OLL	
	Temp	Cond	Cl^-	Temp	Cond	Cl^-
DATE	^0C	mS/cm	mM	^0C	mS/cm	mM
3.87	8.5	2.7	28	8.0	3.4	24
4.87	16.2	2.15	15	11	3.1	18
5.87	13	2.0	13	11.5	3.8	31
6.87	12	2.2	15	12.5	3.2	30
7.87	16.5	2.16	13.5	16	3.16	29
8.87	22.5	2.3	17	22	2.74	24
9.87	18.5	2.1	17.5	18	3.3	35
10.87	9.0	2.4	18	10.0	2.2	14
11.87	6.0	3.0	17	6.25	3.24	20
12.87	2	2.9	20	3	3.0	21
1.88	5.0	2.6	20	5.0	2.8	20
2.88	5.5	2.3	20	6.0	1.4	18
3.88	7.5	2.69	26	7.5	2.9	23
4.88	14.7	3.4	17	16.5	3.4	16

APPENDIX H

Analysis of Filtered Nottingham Tap Water
used in Experimental and Culture Work
Analysis provided by Severn Trent Water

Parameter	Units	Mean	Standard Error
pH		7.77	+/- 0.02
Total Hardness	$mgl^{-1}CaCo_3$	208	+/- 3
Alkalinity	$mgl^{-1}CaCo_3$	122	+/- 2
Conductivity	uS/cm	562	+/- 13
Calcium	mM	1.65	+/- 0.02
Magnesium	mM	0.40	+/- 0.01
Sodium	mM	1.42	+/- 0.05
Potassium	mM	0.09	+/- 0.01
Chloride	mM	1.40	+/- 0.04
Sulphate	mM	0.78	+/- 0.02
Zinc	uM	<0.15	
Aluminium	uM	<2.40	
Cadmium	uM	<0.006	
Chromium	uM	<0.08	
Copper	uM	<0.08	
Iron	uM	<0.90	
Lead	uM	<0.005	
Manganese	uM	<0.18	
Nickel	uM	<0.034	

TWINSPAN SPECIES GROUPS FROM MILLWOOD BROOK