TOLERANCE DETERMINATION OF CERTAIN BENTHIC INVERTEBRATES AS INDICATORS IN RIVER WATER QUALITY MONITORING

by

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SUMMARY

River survey work which assessed biological and chemical status of over one hundred randomly selected rithron stations spanning four Water Authorities is documented. Water quality has been correlated with benthic macroinvertebrate distribution and alteration in community structure, with specific regard to caseless Trichopteran distribution recorded. Organic pollution, R. Tean, Staffordshire, and heavy metal pollution, R. Ystwyth and R. Rheidol, have been investigated in the field to further knowledge on the usefulness of caseless Trichopteran species as indicators and/or monitors of pollution.

The tolerance of the larvae of the two species, Hydropsyche angustipennis and Rhyacophila dorsalis, to increased organic load was studied by experimentally simulating three different water qualities. Mixtures of high quality river water with sewage effluent were prepared at the Aston Hydrobiology Field Station at Checkley (R. Tean) Staffordshire, providing intermediate field/laboratory conditions. Major factors influencing Trichopteran distribution included temperature, dissolved oxygen, pH and heavy metals.

Acute toxicity studies on H. angustipennis demonstrated the toxic effect of copper and zinc under variable conditions of water hardness, and the bioconcentration of zinc was demonstrated by the use of radioisotopes. Additional experiments investigated the acute toxicity of ammonia and two pesticides to the larvae.

The effect of temperature, dissolved oxygen and pH on the respiration rate of H. angustipennis was investigated using an apparatus specially developed in the laboratory. A modified Rank Electrode served as a respirometry chamber, whilst a further part of the system delivered test water at different oxygen and pH levels, other parameters being kept constant. Using this method, the effect of individual parameters involved in pollution was investigated.

Recommendations for the use of certain benthic macroinvertebrates for use in water quality monitoring are documented. Appendices contain data from field work investigations and relevant literature has been reviewed.

Key words: Caseless Trichoptera; Acute toxicity; Indicator species

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1. INTRODUCTION

1. INTRODUCTION

In the ecosphere, the hydrological cycle is extremely important - and for man, water and the freshwater ecosystem has always been a vital component of everyday life, determining cultural and economic development. Anthropogenic dependence upon freshwater has imposed enormous stresses on both lotic and lentic systems. Ever-increasing population and industrial activity and man's expectations of the multiple uses of essentially a finite resource requires careful and knowledgeable handling in order to prevent gross imbalance occurring.

In recent years, concern for water quality has increased, as a more environmentally aware 'Man' has realised the need to protect and maintain, at the highest quality possible, the aquatic ecosystem.

Efficient water management is necessary to integrate all of the activities on, in or requiring water, and in maintaining the quality of the resource for whatever function necessary.

The initial task of defining "water quality" and arriving at criteria is extremely difficult, but it has been agreed (Warren, 1971; Hawkes, 1974; Hellawell, 1978) that it should be defined pragmatically in respect of usage. Criteria and standards for any particular source of water may be assessed on legitimate use. Consequently water regarded as high quality for one purpose may be deemed unsuitable for another.

Examples of legitimate usage of water include abstraction for potable domestic use, industrial and agricultural purposes, navigation, and the discharge and disposal of domestic and industrial effluents.

A similar definition problem arises for water pollution. Mellanby (1980) speaking in general terms, points out that pollution may occur naturally, independent of any activity of man. Thus the vapours from a volcano may contain so much sulphur that plants cannot grow nearby. Rivers flowing through forests may become deoxygenated because so much natural organic matter (allonchthanous material) is de-

posited in them. The resultant decomposition leads to deoxygenation similar to contamination caused by man-made sewage being discharged into a river.

Holgate (1979) defined pollution as "something that is present at the wrong place at the wrong time in the wrong quantity". Hellawell (1978) points out that this definition will retrospectively apply to all pollutants. Further definition, from a legal standpoint, Wisdom (1956) states that pollution arises by the "addition of something to water which changes its natural qualities so that the riparian user does not get the natural water out of the stream transmitted to him". This definition relies on knowing what the 'natural qualities' of the 'natural water' are, as pointed out by Hawkes (1962) and Hynes (1960). Philips (1980) defines pollution as a man-induced detrimental alteration to the ecosphere. A closer definition listed by Thorpe (1981) states that "water pollution may be defined as any factor that changes the characteristics of the water so that any of its legitimate uses are impaired".

Again, it must be recognised that water considered polluted in relation to one use may be regarded as satisfactory for some other purpose.

Water pollution, however, initially achieved and to whatever degree, is essentially a biological problem. The repercussions are on wildlife, particularly fish and the aquatic invertebrates, also on man through contamination of water supplies or impairment of amenities. Therefore it seems strange that it has been treated as a chemical phenomenon, due historically to severe pollution being investigated and expressed in chemical terms.

There is an obvious need for regular and reliable biological and chemical hazard assessment and monitoring and surveillance to be carried out.

The terminology used here will be in line with that of Hellawell (1978) who defines the following words as:-

Survey: an exercise in which a set of standardised observations (or replicate samples) is taken from a station (or stations) within a short period of time to furnish qualitative and quantitative descriptive data.

Surveillance: a continued programme of surveys systematically undertaken to provide a series of observations in time.

Monitoring: surveillance undertaken to ensure that previously formulated standards are being met.

MONITORING AND WATER AUTHORITIES

Water authorities in Britain are responsible for monitoring the environment to ensure that water is available for multipurpose objectives. Such monitoring is bound to the Rivers (Prevention of Pollution) Acts 1951 and 1961, the Water Act 1973, the Salmon and Freshwater Fisheries Act 1975 and the control of Pollution Act 1974, Part II. Increasingly the 'water cycle' is no longer a straightforward hydrological cycle, because of the increasing demand water is now recycled. Thus waste water is no longer treated at a "sewage works" but at a "water reclamation works".

Monitoring ensures that the quality of river water is maintained and provides a consistent supply to the user. Abstractors are charged for the water they use according to the quantity, the use to which the water is put, the quality of the water abstracted and the time of year at which it is taken.

Regular sampling at frequent intervals down the course of a river enables the Water Authority to monitor the effect of one user upon another and see that effluents added upstream are not detrimental to abstractors downstream. Effluent standards (consents) are set in order to safeguard the downstream user. Consents are set and monitored

using the National Water Council Classification of rivers (Brewin and Hellawell, 1980). See Table 1.1.

Until recently, chemical sampling, which is very expensive and time consuming has been used to monitor water quality. Most river and much effluent sampling data are obtained by taking periodic 'snap' samples.

TABLE 1.1. N.W.C. Classification of River Water Quality

River Class	Class limiting criteria (95th percentile)	Average Concentration	River use
1A	DO >80% BOD ⩽ 3 mg/l Ammonia >0.4 mg/l	BOD ⩽ 1.5 mg/l	Potable supply Game fish High amenity
1B	DO > 60% BOD ⩽ 5 mg/l Ammonia >0.9 mg/l	BOD ⩽ 2.0 mg/l Ammonia ⩽0.5mg/l	As for 1A
2	DO > 40% BOD ⩽ 9 mg/l	BOD ⩽ 5 mg/l	Potable supply Good coarse fishery Moderate amenity
3	DO > 10% BOD ⩽ 17 mg/l		Low grade industrial
4	Quality inferior to class 3		
X	DO > 10%		Insignificant water course

DO - Dissolved oxygen as % saturation
BOD - 5 day carbonaceous BOD (ATU)
Ammonia - Expressed as NH_4

(From: Chemistry & Industry, 2 Aug. '80).

This may give misleading information, particularly if the sample is taken at the same time each day (a factory discharging its effluent may do so in the evening, causing severe pollutional problems but may not be revealed the following day by a chemical test because the slug has already travelled down river).

There is a practical economic restraint on intensive physico-chemical monitoring and although rationalised sampling programmes are carried out, thereby contributing towards an even flow of samples

through the laboratories, i.e. maximum use of expensive equipment and skilled analytical staff, there may still be some problems which the chemical monitoring does not pinpoint. For similar reasons only a limited number of parameters may be monitored.

BIOLOGICAL SURVEILLANCE

The implementation of routine invertebrate sampling, often in conjunction with physico-chemical sampling can make an important contribution to water resource protection. Unlike the 'snap' chemical samples, the benthic invertebrates are exposed to the water at all times and experience the entire variation in conditions - which chemical sampling may never record, e.g. diel O_2 changes, seasonal temperature changes etc. Consequently, biological sampling is valuable in detecting the presence of otherwise unknown or intermittent pollution which may be missed if unsuspected or not coinciding with the moment at which a chemical sample is taken (Brewin and Hellawell, 1980).

Biological sampling, in addition, is far less expensive and produces more rapid results than chemical sampling. Often a general assessment of water quality may be made instantly on site. Biological sampling in a simple "one-off" survey may be used to detect gross differences in water quality - or detailed surveillance may be required to detect more subtle environmental changes. The intrinsic complexity of biological systems means that data must be carefully interpreted and such factors as the heterogenous nature of freshwater habitats also taken into account.

For example, the substratum may vary in the river bed, resulting in a variety of microhabitats including areas of submerged and emergent weed, boulders, gravel, sand and mud. The water velocity will vary spatially and temporally, as may the temperature, turbidity and factors affecting the chemical quality.

All these factors may influence the composition of the benthic

community, before any pollutional stress is imposed. The stream bed community responds differently to different types of pollution, for example, particulate matter, organic effluents, heavy metals, warm water, radioactivity etc.

Much variation in sampling may be reduced by confining sampling to a single habitat, e.g. the riffle zone, or alternatively by sampling all the microhabitats. This is easy in the small, shallow upland streams, but does not lend itself to sampling and monitoring deep, slow flowing lowland rivers. In this situation an artificial substratum for colonisation by the animals provides uniformity of habitat (e.g. "saufu", standard aufuchs unit). The community of organisms may be "artificial" but may be useful indications of variations in water quality.

Biological surveillance may therefore be useful in assessing temporal and spatial changes in river systems, and may also prove useful in detecting or measuring the ecological effects of unexpected or intermittent pollutants. Another aspect is that it may also pick up likely sources of nuisance organisms, e.g. algae, A.aquaticus, diperan species.

Butcher (1946) recognised that a benthic community does give a good indication of change, whilst Hynes (1958) points out that although the chemist is usually called in to measure the amount of pollution, a biologist could also contribute as he has methods at his disposal which are in some ways more sensitive. Continuing, he pointed out that fish, being mobile, will move out of a polluted stretch of river when conditions become unfavourable. Benthic invertebrates and plants however are "subjected to the full rigours of local conditions at all times. They are therefore very good indicators of pollution".

The large amount of biological data which are generated, their interpretation and need to provide a comprehensive summary, has led to the development of a wide range of biotic indexes. These have been refined and adjusted over a number of years and incorporated as methods

of presenting data after biological surveillance has taken place. The data accumulated from macroinvertebrate sampling has proved extremely useful in monitoring water quality in two distinct ways.

INDICATORS

The "indicator species" concept is straightforward, but may be used in different contexts. Here definitions are once again required (after Hellawell, 1978).

Firstly indicators may be those individual organisms which readily accumulate toxic substances, so that analysis of their tissues provides an indication of environmental levels of the degree of exposure.

Secondly the "community" as a whole may be the indicator. Some species in the benthic community disappear on exposure to a given pollutant due to their sensitivity, others thrive and even increase in number in the presence of certain pollutants, or due to suppression of competition. Consequently the community structure demonstrates the degree of pollution.

Care must be taken in the application of indicator organisms, as Warren (1971) points out. There are ecological changes, both spatial and temporal that are a natural feature of aquatic ecosystems. Therefore pollutional load must be characterised and defined in impairment of legitimate use. Hawkes (1975) when describing longitudinal zonation points out natural seasonal changes too.

Several other pieces of terminology have also been employed including "key species", "critical species", "detectors" and "accumulators". Phillips (1980) states three basic methods to quantify pollutants in the aquatic environment; either the levels of the pollutant in water, in sediments or in a member of the indigenous biota. Hence 'indicator' is taken in the context of presence /absence and abundance provides an indication of the water quality. Consequently, any species may be an indicator, providing suffieient knowledge of the species' ecology and physiology is available in order to derive sens-

ible conclusions from the observations.

Whenever a survey is carried out to assess the condition of our rivers nationally, the more information available of the ecology, physiology, natural distribution, tolerance levels of each individual species found in the aquatic environment will contribute to the more accurate interpretation of the data.

The past decade has seen the increasing usage of biological data in the Water Authority national surveys, -possibly with the employment of hydrobiologists in the water authority and the D.O.E. requirement for some biological, in addition to chemical, classification. River surveys were carried out in 1970, 1975 and 1980, the objective being to provide an 'at a glance' picture of the state of our rivers. The managerial details are listed by Hinchcliffe (1980) but briefly the trend is towards a higher proportion of biological information being incorporated into the river data.

In the 1970 River Pollution Survey (D.O.E.1972) water quality was divided into four classes only (table 1.2). The classes were not rigidly defined and relied upon subjective judgement. Similarly the biological classification using A, B, C and D used in 1970 was unsatisfactory, relying on presence or absence of invertebrate groups as indicating different grades of water quality (Table 1.3). What was not recognised was the different nature of some different types of stream - having the same high water quality but completely different substrata. Girton (1980) states the inconsistencies arising when the slow flowing East Anglian rivers are sampled. Chemically they may be Class 1, but biologically they are lacking the presence of Plecoptera and/or Ephemeroptera, Trichoptera and Amphipoda. Such communities are not usually present in the sluggish muddy dykes, but are typical of an upland riffle community.

As most of the biotic indexes and scores have been developed for use in such areas, with eroding substratum, the methods produce meaningless

Table 1.2. Water Quality classification system used in the 1970 and 1975 River Pollution Surveys.

Class 1.	
Description.	Unpolluted and recovered from pollution.
Criteria.	1. All lengths of rivers whatever their composition, which are known to have received no significant polluting discharges. 2. All rivers which, though receiving some pollution, have a BOD less than 3 mg/l, are well oxygenated and are known to have received no significant discharges of toxic materials or of suspended matter which affects the condition of the river bed. 3. All rivers which are generally indistinguishable biologically from those in the area known to be quite unpolluted even though the BOD may be somewhat greater than 3 mg/l.
Class 2.	
Description.	Doubtful quality and needing improvement.
Criteria.	1. Rivers not in Class 1 on BOD grounds and which have a substantially reduced oxygen content at normal dry summer flows or at any other regular times. 2. Rivers, irrespective of BOD, which are known to have received significant toxic discharges which cannot be proved either to affect fish or to have been removed by natural processes. 3. Rivers which have received turbid discharges which have had an appreciable effect on the composition of the water or character of the bed but have had no great effect on the biology of the water. 4. Rivers which have been the subject of complaints which are not regarded as frivolous but which have not been substantiated.
Class 3.	
Description.	Poor quality requiring improvement as a matter of some urgency.
Criteria.	1. Rivers not in Class 4 on BOD grounds but which have a dissolved oxygen saturation, for considerable periods, below 50% 2. Rivers containing substances which are suspected of being actively toxic at times. 3. Rivers which have been changed in character by discharge of solids in suspension but which do not justify being placed in Class 4. 4. Rivers which have been the subject of serious complaint accepted as well-founded.

Table 1.2. continued.

Class 4.	
Description.	Grossly polluted.
Criteria.	1. All rivers having a BOD of 12 mg/l or more under average conditions. 2. All rivers known to be incapable of supporting fish life. 3. All rivers which are completely deoxygenated at any time, apart from times of exceptional drought. 4. All rivers which are the source of offensive smells. 5. All rivers which have an offensive appearance neglecting for these purposes any rivers which would be included in the class solely because of the presence of detergent foam.

Table 1.3. Water quality classification system to be used in the 1980 River Water Quality Survey.

River Class	Class limiting quality criteria (95 percentile)
1A	i. Dissolved oxygen saturation greater than 80% ii. Inhibited biological oxygen demand not greater than 3 mg/l iii. Ammonia not greater than 0.4 mg/l iv. Where the water is abstracted for drinking water, it complies with the requirements for A2* water. v. Non-toxic to fish in EIFAC terms (or best estimates if EIFAC figures not available.)
1B	i. Dissolved oxygen saturation greater than 60% ii. Inhibited biological oxygen demand not greater than 5 mg/l iii. Ammonia not greater than 0.9 mg/l iv. Where the water is abstracted for drinking water, it complies with the requirements for A2* water. v. Non toxic to fish in EIFAC terms (or best estimates if EIFAC figures not available).
2	i. Dissolved oxygen saturation greater than 40% ii. Inhibited biological oxygen demand not greater than 9 mg/l iii. Where water is abstracted for drinking water, it complies with the requirements for A3* water. iv. Non-toxic to fish in EIFAC terms (or best estimates if EIFAC figures not available).
3.	i. Dissolved oxygen saturation greater than 10% . ii. Not likely to be anaerobic. iii. Inhibited biological oxygen demand not greater than 17 mg/l.
4.	Waters which are inferior to Class 3 in terms of dissolved oxygen and are likely to be anaerobic at times.

results when applied to the potamon type rivers (lowland, slow and with a depositing substratum).

Reported from the 1970 Pollution Survey is that in the Wye catchment, 98% of the miles of river that were chemically class 1 were also biologically class A: in the Lincolnshire River Authority area approximately only 6% of the chemical class 1 rivers were biologically class A (27% were class B, 64% class C).

This example indicates care is required in the explanation and shows limitations for using benthic invertebrate communities for monitoring in all situations. Hawkes (1975) points out the importance of taking into account the river type when interpreting results from invertebrate sampling regimes.

In 1975 a biology survey was omitted from the Pollutant Survey but the intention was to develop a new classification for use in the 1980 River Water Quality Survey. Thus a Biotic Index Score system, based on the presence of selected invertebrate families was adopted (D.O.E. 1980). Although not very exacting classification, it fulfilled the requirements for a National survey. Refinements to this Score were made and even in its earliest drafts it recognised some inherent differences in the invertebrate communities of rithron and potamon zones (Table 1.4).

Later a further modification was made to combine eroding and depositing substratum scores. According to Girton (1980) the results of the two systems are very similar.

The work of a previous research team which contributed to the development of the Biotic Score for the 1980 survey, worked on ways of standardising biological methods for surveillance of river water quality.

The present study represents a continuation of the work investigating the use of specific indicator organisms (Trichoptera larvae)

in upland reaches of rivers - with a view to refining the present score system.

Table 1.4. The system of biological assessment proposed for the 1980 River Water Quality Survey.

Families	Score
Siphlonuridae Heptageniidae Leptophlebiidae Ephermerellidae Potamanthidea Ephemeridae Taeniopterygidae Leuctridae Capniidea Perlodidae Perlidae Chloroperlidae Aphelocheiridae Phryganeidae Molannidae Beraeidae Odontoceridea Leptoceridae Goeridae Lepidostomatidae Brachycentridae Sericostomatidae	10
Astacidae Lestidae Agriidae Gomphidae Cordulegasteridae Aeshnidae Corduliidae Libellulidae Psychomyiidae Philopotamidae	8
Caenidae Nemouridae Rhyacophilidae Polycentropodidae Limnephilidae	7
Neritidae Viviparidae Ancylidae Hydroptilidae Unionide Corophiidae Gammaridae Platycnemididae Coenagriidae	6
Mesovelidae Hydrometridae Gerridae Nepidae Naucoridae Notonectidae Pleidae Corixidae Haliplidae Hygrobiidae Dytiscidae Gyrinidae Hydrophilidae Clamnidae Helodidae Dryopidae Eliminthidae Chrysomelidae Curculionidae Hydropsychidae	5

Table 1.4. continued.

Families	Score
Tipulidae Simuliidae Planarildae Dendrocoelidae	5
Baetidae Sialidae Piscicolidae	4
Valvatidae Hydrobiidae Lymnaeidae Physidae Planorbidae Sphaeriidae Glossiphoniidae Hirudidae Erpobdellidae Asellidae	3
Chironomidae	2
Oligochaeta (whole class)	1

2. LITERATURE REVIEW

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2.1. HISTORICAL ASPECTS

Water quality in recent years has been assessed using benthic macroinvertebrate data in addition to chemical data.

All invertebrates may be incorporated in sampling exercises to determine the structure of the community in that particular water quality. Furthermore, any change in the structure and organisation of that community may be recorded in monitoring later, and thus the changes in numbers and abundance of organisms used to reflect any alteration. The interpretation of this information may be assisted by biological scores and diversity indexes.

Individual species in the benthic community may be utilised as indicator organisms. This methodology is becoming increasingly popular as more bioassay studies are being completed on invertebrate species. In the past fish and algae have been favoured as indicator systems, but algae are seasonal whilst fish are migratory, able to swim away from any stretch of water presenting unfavourable conditions. The benthic biota represents a continuously placed "probe" which experiences the entire range of water quality parameters at any one point in a river. One drawback of autecological studies is that the interpretation of the data must be put into perspective when applied to the field situation.

Historically, one of the earliest records in Britain of invertebrates being deliberately used as indicators of water quality was by Carpenter (1924). She examined the fauna of various Welsh rivers which received run-off from lead mine tailings. It was noted that the invertebrate fauna of these rivers was poorer than those with no lead pollution. From this work in the field she tried to find any experimental data to explain her findings, but was unable to uncover any work on invertebrates which had been subjected to tests with lead.

Looking back even further one finds that bioassays on invertebrate species really began with an upsurge of interest in physiology. From these preliminary experiments by zoologists our bioassay methods have developed. According to Anderson (1980), in his review paper, the earliest report in which aquatic invertebrates were involved in bioassay's was by Beudant in 1861. Beudant conducted a series of experiments in which he subjected 15 species of freshwater mollusc to 2% and 4% salt solutions. The results were that three species died in 4% saline whilst the other 12 species survived, but not as long as those in the control. He conducted further studies using saline solutions on 38 species of marine mollusc. Here he placed them either in freshwater or diluted sea water. Of the 38 species subjected to freshwater 18 had no survivors; the remaining 20 tolerated it well. Again, in the diluted sea water the individuals survived, but did not thrive as well as controls in pure sea water. Beudant's reason for these studies was to investigate adaptation and evolution, to see if marine and freshwater molluscs have a common origin, but it is interesting to note that he set about his bioassays in virtually the same way as present tolerance level tests are carried out.

Further studies were carried out by Kühne (1864) with protozoans and in 1871 Paul Bert told the Academy of Sciences in Paris of his observation on freshwater animals which he had immersed in sea water. These animals included Daphnia which died in 10 minutes, Cyclops, 20 minutes, Chironomus larvae, 1 hour, mayflies 2 hours and crayfish, 30 hours. In the same year Plateau recounted his work on all classes of invertebrates - but particularly arthropods.

He determined survival in saline solutions both more and less concentrated than sea water, finding that survival times of freshwater animals subjected to salt solutions varied with "thickness of skin".

Plateau also gradually acclimated Asellus to pure sea water, so

that they laid eggs in 20% - 80% seawater and produced a second generation. Ordinarily the Asellus died after being subjected to sea water within 5 hours.

Other workers of the 1870's - 1890's concentrated upon freshwater versus marine organisms, investigating osmotic effects and physiological problems.

In 1874 Ringer found that an excised frog heart could continue to function normally for a long period if perfused with a fluid containing a suitable content of sodium potassium and bicarbonate. Ringer and Buxton (1885) published a paper dealing with the effects of sodium bicarbonate and calcium chloride in distilled water to perfuse sections of the gills of freshwater mussels.

Oswald studied the nature of the toxicity of seawater on Gammarus by subjecting them to single salts in various concentrations and to combinations of salts (using sodium chloride, potassium chloride, calcium chloride, magnesium chloride and magnesium sulphate). The solutions of single salts were most toxic and survival was decreased with increasing concentrations.

In 1928, work by Gresens was published in which he attempted to establish the highest salinities that would permit reproduction and development, and also the highest levels to which Glossiphonia, Erpobdella, Asellus and Dendrocaelum would become acclimated. This study was prompted by a field situation in which he observed mixing of fresh and sea water. His results are listed in Table 2.1 below.

Table 2.1.

Species	Tolerance level % salt	Reproductive tolerance % salt	Acclimation % salt
Glossiphonia	3.5	-	5.25
Erpobdella	5.25	3.5	5.25
Asellus	5.25	5.25	15.00
Dendrocoelum	7.0	7.0	15.00

Although other workers continued macroinvertebrate bioassay studies, for example Breukelman, who found the killing rate varied with concentration of substance and with temperature, it was the work of Naumann (1933 and 1934) which really began bioassay work in earnest using Daphnia. He showed amazing forethought in his work, but due to publishing in a new journal received little attention. He dealt with D. magna as a suitable test animal, and could rear them satisfactorily in waters from many different sources. Naumann tested the toxicity of different materials, and made recommendations in handling D. magna with respect to experimental purposes. He noted:

a. size class - because young animals react differently to older ones,
b. colour change - since certain substances manifest their toxicity in this way,
c. individuals with broods - because a toxicant may affect reproduction,
d. constant temperature - 20°C for all tests,
e. diffused light conditions - to reduce swimming activities caused by intense light.

He also noted 'normal' behaviour in the test vessel, and the distribution of animals in the vessel. Mechanical irritation caused the animals to gather at the bottom, low concentrations of dissolved oxygen caused them to approach the top.

In following papers he observed acute and chronic toxicity, acute being defined as anything which happens in the first 24 hours. Furthermore he analysed the toxicity of materials used in the aquaria, air in the laboratory, error in toxicological research, problems with tap water and distilled water and finally the influences of chemicals, pH and free CO_2.

He speculated on the best way to carry out toxicity tests with D. magna and copper, if one should use single or several animals in each 50ml or 100 ml of test solution. He finally ran tests using distilled

water, and various soft and hard waters contaminated with copper. Those animals exposed to soft water containing humus survived higher concentrations of copper than those exposed in other waters. Presumably the humus acted as a chelating agent in the system. Therefore his final statement says that "the purer the water, the more toxic the copper". Further papers deal with sources of contamination in laboratory conditions, toxicities of zinc sulphate and lead chloride and work on the effect of organic wastes on D. magna. He found smaller animals died before larger ones and death occurred as the oxygen level was reduced to less than 1 mgl^{-1}. Further work on D. magna was carried out by Ellis (1937) and Anderson (1944).

In Britain the emphasis in bioassay studies with a view to recommending threshold levels of pollutants in rivers and lakes has been on fish. Much of the early investigative work and development of methods was done by the Water Pollution Research Laboratory, now the Water Research Centre (Stevenage), the Ministry of Agriculture, Food and Fisheries and the Freshwater Biological Association, all in conjunction with the regional Water Authorities. General requirements for collection and laboratory maintenance of animals for toxicity testing purposes have been described in detail by the American Public Health Authority (A.P.H.A), 1975 and the Environmental Protection Agency (E.P.A), 1975. The European Inland Fisheries Advisory Commission (E.I.F.A.C) documents, spanning 1970–1977 report on water quality criteria for freshwater fish and strive to standardise permitted levels of pollutants throughout Europe. Other more recent comprehensive documents on this topic are by Alabaster and Lloyd (1980) and further reports by the Department of the Environment (D.O.E), still in preparation.

Specific papers dealing with experimental work on Trichoptera will be dealt with in Section 2.8.

2.2. BIOLOGICAL DATA, THEIR USE IN POLLUTION SURVEILLANCE

The surveillance of benthic invertebrates has been recognised as a useful aid in the identification of water quality changes. From exclusive chemical sampling some years ago, biological sampling is now used and integrated into the system.

The Water Resources Act (1973) states that "Water Authorities shall have regard to the desirability of conserving flora and fauna of special interest, and shall take into account any effect which proposed schemes would have on the flora and fauna."

In the Control of Pollution Act (1974) it is stated "a Water Authority can review a consent if it appears that pollution injurious to the flora or fauna of a stream has been caused in consequences of discharges made by virtue of a consent given by the Authority". Thus Authorities have statutory duties to examine the flora and fauna of the water under their control.

Work over the last two decades has emphasised the importance of using macroinvertebrates (Hynes, 1960; Hawkes, 1962; Warren, 1971; Hellawell, 1978) and examples of this advice translated into action is recorded in papers on monitoring. For example in the U.K. these methods have been used by Bryce et al. (1978), Crossland (1979), Hamer and Soulsby (1980) and in the U.S.A. by Whipple and McIntosh (1979).

Thorpe (1981), reiterating Hawkes (1978) lists the principal changes of indicator value that have been shown to occur, often simultaneously, in aquatic communities in response to changes in water quality:

a. appearance or disappearance of particular taxa,

b. change in total numbers of taxa,

c. change in quantitative importance of taxa,

d. change in proportional quantitative importance of taxa.

Any one,or combination of these changes may influence the characteristics of a benthic community, thereby upsetting trophic levels and production.

The exploitation of these changes in the assessment of water quality and detection of pollution have been utilised. Specialisation and advocation of one particular taxon or group has been popularised by various workers in order to simplify the interpretation. Favoured aquatic groups have included bacteria, algae, macrophytes, invertebrates and fish. In this study it is intended to extend the information available on the group Trichoptera, and incorporate this into the score systems thus achieving a more refined and complete method of monitoring water quality.

2.3. SAMPLING BENTHIC INVERTEBRATES

In utilising benthic macroinvertebrates as indicators in water quality monitoring, one must have a reliable and reproducible system of sampling the rivers for these organisms.

Considerations:

There are numerous decisions to be made before one embarks upon a sampling programme. Firstly, each river poses individual problems and each sampling site will have different physical and environmental characteristics. Examples of such parameters include depth of water, velocity, nature of substratum, chemical nature of dissolved gases, salts, temperature, turbidity and amount of algae and littoral vegetation. It may be a rithron or potamon zone - easy to wade into or requiring a boat for access.

The heterogenieity of sampling sites lays open a multitude of appropriate, but different sampling methodologies. Consequently, different sites at which various sampling methods are employed then become incomparable when data analysis takes place.

2.3.1. ACTIVE v PASSIVE SAMPLES

There are numerous methods documented for sampling invertebrates in freshwater ecosystems. Hellawell (1978) categorises them into 'active' and 'passive' samplers.

Active:- requiring the active participation of an operator for the collection of the sample. These types include Hand Net, Kick Heel, Surbar, Cylinder and Grab samplers.

Passive:- Drift nets, artificial substrates and traps. The samplers are 'set' and then a collection made later.

Most sampling methods require active participation. Girton (1980) gives a comprehensive account of sampling methods. The two to be highlighted here will be kick-heel and cylinder sampling. As the sampling involved in this study was restricted essentially to upland reaches of rivers - generally riffle sections - a depth not usually exceeding 90 - 100 cm. was encountered. Wherever possible a quantitative sample using the Aston Cylinder Sampler was employed, as after much discussion it was agreed to be the most efficient method of taking samples for this particular study. Thus samples from different sites could be compared. On the few occasions where the use of the cylinder was impossible, then a kick-heel sample was taken.

2.3.1.1. The Cylinder Sampler

The cylinder sampler (H.M.S.O. 1980) essentially encompasses a known area of river bed and all animals within the confined area are collected, identified and counted. One sample can be compared against another as a standard procedure of collection is involved (see Methods Section, later).

2.3.1.2. Grab Samplers

The cylinder method has in many respects advantages over a grab-type sampler, such as the Ekman or Ponar. Any grab is designed to remove a standard sized portion of the substratum. The biting action theoretically scoops up the portion and any organisms contained therein. Success depends on the weight, penetration and action of the jaws of the grab. If the jaws fail to close completely due to a stone or stick wedged between the edges, then on removal, much of the sample meant to be contained within the grab is lost.

Modifications such as trigger mechanisms, weights etc. cause increased disturbance of water as the grab is employed - thus disturbing the sample area. Such drawbacks, in shallow riffle sections of rivers may be overcome effectively by use of the cylinder.

2.3.1.3. Kick-Heel Samples

In some circumstances, where the river is too deep, a cylinder sample may prove impossible, then a Kick-Heel sample may be employed. Essentially this method incorporates the use of a Standard Hand Net (H.M.S.O.1978) as developed by the Biological Working Party of D.O.E. The metal edged frame of the net is placed on the river bed, the handle being held vertically by the operator. By moving backwards, upstream and using vigorous little kick movements of the feet, the fauna are dislodged from the substratum. These are then swept into the net immediately downstream by the flow of the river.

This method may be employed for a set period of time, or dislodging animals from a given area in order to approach a consistent sample. This is a qualitative method, rather than a quantitative as in the cylinder method, but a useful tool when other sampling methods are precluded for various reasons.

2.4. BIOLOGICAL POLLUTION INDEXES

Inevitably work of this nature generates an enormous amount of data. In an attempt to summarise these data, from such river sampling exercises, various Biotic indexes have been developed.

Perhaps the earliest break-through with the concept of "biological indicators of pollution" and their incorporation into a recognised system was by Kolkwitz and Marsson (1908, 1909) in their Saprobic System. This system is based upon identification of different zones associated with organic enrichment, each of which is characterised by specific plant and animal species. Saprobity refers to the situation when sewage or other putrescible organic wastes are discharged into a

river. This leads to sequences of events in time and distance which create different environmental conditions. Thus different species composition is encountered in successive zones of the rivers. These zones were defined as (i) polysaprobic (predominantly with reduction processes taking place); (ii) α-mesosaprobic; (iii) β-mesosaprobic (these zones showing gradual change from reduction to oxidative processes); (iv) oligosaprobic (oxidation process only). "Saprobia" was the term introduced to express the dependence of the organisms on decomposing organic substances as sole 'source of food'. They also added a category of extremely pure water - but this is not generally used.

Persoone and de Pauw (1978), in their excellent review paper document other workers' investigations and classifications of zones of rivers receiving sewage. Essentially the systems were similar, differing only in nomenclature and the delimiting of zones.

Further work, with graphical presentation of the ecological characteristics and biological components of successive zones, to explain the succession of events during the biological self-purification of a river polluted by biodegradable wastes, provided a simplified explanation. This system was documented, according to Persoone and de Pauw (1978), by Dr. H.W. Jackson of the Environmental Protection Agency, using unpublished data. This was derived from C.M. Tarzwell (Robert A. Taft, Engineering Center, Cincinnati, Ohio) and a review paper of Bartsch and Ingram (1959). It has been based on the zonation first proposed by Suter & Moore (1922) and has been modified and used by Hynes (1960) and further developed by Hawkes.

The Saprobien system has been well used but has been criticised for lack of quantitative mathematical formulation. Fundamentally, each organism is considered and the expert uses his personal experience to evaluate the quality of the water. A second drawback of this system is the inapplicability to a river which is polluted by in-

organic industrial waste. The reliability of presence or absence of indicator species - as applied to organic pollution may be completely misleading in this context (Hawkes, 1962; Gaufin & Tarzwell, 1956).

Other workers have used modified Saprobian systems, for example Liebmann (1962) retained the four classical zones of Kolkwitz and Marsson, but called them "Wasserguteklassen" meaning "classes of water quality". These were ranked from I - IV (I = cleanest, IV = most polluted) . He also colour coded the I - IV for easy visualisation in water quality surveys. Liebmann relied heavily on the ciliates as "good indicators" of the state of pollution.

In 1964 Fjerdinstad further subdivided the four zones of the original saprobic system into nine categories with typical communities. He used only a few indicator species for delineating the zones.

Most recently Sladecek (1965) extended the original saprobic system to include new categories for highly polluted waters. This was referred to as eucaprobity - meaning waters highly polluted by biodegradable domestic and industrial wastes. It was divided into four subsections, and later introduces a further zone - Transsaprobic (meaning without bacterial breakdown) which was in turn subdivided into antisaprobic (where organisms are killed by poisons present) and radiosaprobic (a zone with radioactive contamination).

A further improvement by Sladecek was the allocation of physicochemical characteristics to each of the zones, and provides some rough quantitative estimation of the numbers of indicator species present.

In 1955 Pantle and Buck proposed the Saprobic Index, which takes into account the relative abundance of organisms used in the Saprobien System, where individual species are allocated a qualitative numerical value depending upon the saprobic zone to which the species belongs, and a numerical value representing the estimated quantities in which it occurred.

Simultaneously, in Europe and North America other systems using species were being developed. Differential tolerances, and alterations in numbers in each particular species had been the two criteria utilised in assessment of invertebrate data in the defining of water quality of rivers. Contrary to the saprobic system which focuses mainly on protozoa and algae, most of the other systems use benthic macroinvertebrates as major indicators. These included classification by Patrick (1949), Beck (1954) - who worked out a Biotic Index, Wurtz (1955) and Beak (1964). Mackenthun (1969) gives a visual representation of typical species classified as "sensitive","intermediate" and "tolerant" bottom dwelling macroinvertebrates. "Sensitive" species included members of the Plecoptera, Ephemeroptera, Trichoptera, "intermediate",the Simuliidae, Amphipoda, Isopoda, Gastropoda, Sphaeridae, Zygotera, Anisoptera, and Chironomidae and finally "tolerant "speices of Hirudinae, Tubificidae, Psychodidae and Tubifera.

It was a similar concept on which Woodiwiss (1964) based his Trent Biotic Index. With this method streams were classified according to the presence or absence of species or groups in the community - the best score being X for cleanest water and decreasing as reduction in community diversity, indicates the response to organic pollution. This system was extended by Woodiwiss (1976) to cover the range of water qualities 0 - XV. Again, as with the Saprobic System, Woodiwiss does not take into account the relative abundance of the organisms present.

This was semi-rectified by Chandler (1970) who devised a score system based upon organisms collected in a standardised and timed sample, into 5 levels of abundance. The sensitivity of the aquatic species was noted according to increasing tolerance of organic pollution, and in addition varying abundances of these groups were allocated a score.

All animals found in the sample are sorted, identified and counted, then the value looked up in a table to calculate the points scored. These are then totalled to give a final Chandler Score. The score increases with increasing abundance for clean water species, and decreases with increasing abundance for pollution tolerant species. Criticism of this system by Sladecek (1973) points out it is only a modification of the Saprobien system whilst Balloch et al. (1976) and Hawkes (1978) point out the weakness of subjective assessment in the allocation of scores to taxon and their abundance. A further classification was developed by Tuffery and Vernaux (1968) and is similar to the Trent Biotic Index. The modification is the sampling of "two facies" of a site in the rivers, i.e. the pool (lentic) and riffle (lotic) facies. The necessity of considering the two facies of a biotope is claimed to be important, since the benthic biocenoses in flowing water are different to those in the 'still' portions of the same locale. The authors claim that the idea is not to arrive at an exact taxonomic determination, but to identify the largest number of "systematic units" in each group of organisms.

In Britain the most recent development is the Biological Monitoring Working Party Score (BMWP Score), developed for use in the 1980 National River Survey (1981).

This Score system relies on identification of organisms to the family level. Each group is given a score, and the grand total then calculated according to what type of organisms are in the sample. To an extent it overcomes the problems of inherent differences in communities in rithron and potamon zones, as it includes many groups found in lowland rivers and ignored in previous classifications. Again, as with the Trent Biotic Index it relies upon presence or absence of species rather than an abundance rating.

These then are some of the 'tools' available to the hydrobiologist when assessing raw data from river samples.

2.5. DIVERSITY INDEXES

Here individual species give way to the utilisation of the community or species assemblage as indicators of water quality. The change in diversity in terms of numbers of species in a given situation may suggest pollutional stress in that environment. Personne and de Pauw (1978) quote "the reduction in the number of species, and proliferation of some remaining ones may occur as the result of the introduction of biodegradable wastes into rivers or lakes". A change in the value of an index thus relates to the intensity of pollution.

In order to assess diversity, an understanding of community structure is helpful and several mathematical models have been postulated to describe this. Hellawell (1978) reviews these models which include the Logarithmic Series Model, Lognormal Distribution and Broken-Stick Model, but these only provide a theoretical standard against which actual data may be compared and they may be too exaggerated.

Other methods of deriving a diversity index simply relate to numbers of species to total number of individuals. Various workers have produced their own index, but by simplifying and standardising the nomenclature it is possible to compare them.

Thus: I = Diversity Index

S = Total number of species present

N = Total number of individuals

n_i = Number of individuals in the i^{th} sample

Table 2.2. summarises some of the diversity indexes available.

Although a high diversity indicates high water quality, the reverse may not be quite true, since a reduction in diversity may not be attributable to polluted conditions. For example (Hawkes, 1978) in a torrential headstream the water quality may be exceptionally high, but the species diversity is restricted due to the severe

TABLE 2.2. DIVERSITY INDEXES (Nomenclature standardised as in Hellawell 1978 wherever possible)

Index Name	Formula	Comments
Menhinicks' Diversity Index	$I = S/\sqrt{N}$	Ref: Menhinick (1964)
Margalef's Community Diversity Index	$I = \frac{S - 1}{\log_e N}$	Ref: Margalef (1951). Does not account for number of individuals per species
Shannon-Weiner (Weaver) Community Diversity Index	$I = -\sum\left(\frac{n_i}{N}\right).\log_2\left(\frac{n_i}{N}\right)$	Ref: Wilhm & Dorris (1968) Calculation simplified by tables given in Hellawell or using a computer
Simpson's Index	$I = \sum \frac{n_i(n_i-1)}{N(N-1)}$	Ref: Simpson (1949) Values from 0 - 1 are inversely proportional to diversity of community
Modification	$I = 1-\sum \frac{n_i(n_i-1)}{N(N-1)}$	Ref. Pielou (1969) Subtracting the main term from unity makes the value proportional to diversification - thus similar to other indexes
Species - deficit	$F = \frac{A_1 - A_x}{A_1} \times 100$	Ref. Kothe (1962) The difference in number of species occurring upstream of a point of discharge (A_1) and downstream (A_x) is expressed as a % F = 0=no change F = 100=total suppression of community
Sequential Comparison In Index (S.C.I.	$SCI = \frac{N \text{ runs}}{N \text{ specimens}} \times \text{Taxa}$	Ref. Cairns et al. (1968, 1971). Theoretically individuals are compared in sequence and are either the same or different to the previous one examined. If it is similar it is part of the same run, if not it is a new run. The greater the number of runs per number of specimens examined, the greater the diversity

physical conditions. Thus, changes in diversity at one station over time is more significant than change along the length of the river.

Comparing the use of diversity indexes to Biotic Indexes and scores, they are more mathematically based and rely upon quantitative data; but unlike biotic indexes they do not utilise autoecological information, i.e. separate taxon responses.

It appears that although attempts are continually being made to reduce an enormous amount of biological data into a single figure, there are still many indexes and scores in which such abbreviations inevitably mean that much information is lost. Standardisation is a further problem, there existing almost as many score systems as there are numbers of biologists working in this field! Bartsch & Ingram (1966) concluded "In fact, one can well question whether a standard method is possible or even desirable."" Nevertheless, the advent of E.E.C. policies prompted international comparative studies on procedures and methods in various countries in an attempt to standardise biological water quality assessment. This included the Severn-Trent Water Authority participation in analysis of R. Trent and its tributaries (1976) co-ordinated by S.T.W.A., Nottingham. Other studies were in Koblenz on the R. Main and Parma where R. Parma, R. Stirone, and R. Po were studied. Knopp (1976) after the first study in Koblenz said "biological-ecological analysis cannot replace chemical, physical or biochemical analysis, but can indeed significantly compl-ement them". Only the complete spectrum of information provides an image of the quality conditions with respect to water management uses".

In this light perhaps we are being over-optimistic in simplifying everything to a one-figure value. Other workers are now of the opinion that alternative analyses methods may be useful.

2.6. CLUSTER ANALYSIS AND PRINCIPAL COMPONENT ANALYSIS

The promotion of the idea of a joint approach to water quality monitoring by both chemists and biologists by Hamer and Soulsby (1980) is to be highly recommended. In their work they advocate regular and adequate sampling by the Water Authorities as deficiencies in both chemical and biological data can lead to difficulties in analytical results at a later stage. Although sophisticated techniques for data analyses may be available, none can compensate for basic inadequacies in the sampling programme.

Assessment of biological data by Biotic and Diversity Indexes has already been discussed, but there are two multivariate techniques which may be of great potential use. Both methods of Cluster Analysis and Principal Component Analysis (P.C.A) were tested by Hamer and Soulsby (1980) in which they utilised data from surveys in three successive years from nine sites on different rivers. Each sample was replicated five times and used a 0.05 m^2 box sampler.

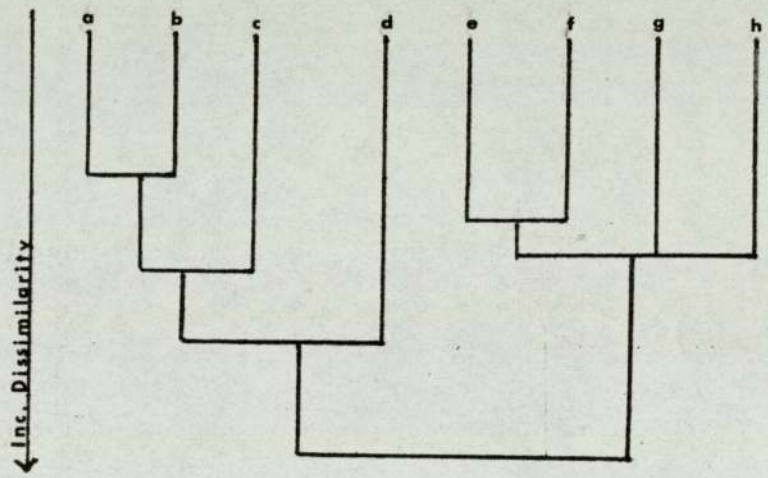
In the past P.C.A. was employed in terrestrial ecological work especially in botanical surveys such as Greig-Smith et al. (1967), Austin (1968) and Allen and Skagen (1973). An application in freshwater biology was by Erman & Helm (1971), when studying benthic invertebrates and by Taylor (1978) performing studies on fish catches in a reservoir and the way in which these were related to environmental variables. Mathematical bases for these techniques are thoroughly examined by Seal (1966) and Pielou (1969).

CLUSTER ANALYSIS

The Cluster Analysis technique which gives a simplified two-dimensional solution is less subtle than P.C.A. Essentially raw data are taken and a correlation matrix is calculated. Matrices of coefficients of similarity are then generated and this information is then displayed graphically. Clusters are depicted on a dendrogram. Parts and

clusters of parts are united with their nearest neighbour so that the sum of squares of the distances of parts from the centre of a new large cluster is minimised (Allen & Skagan, 1973). Thus some groups of samples (from stations on rivers) may have higher affinities than others and tend to group together, i.e. "cluster". Thus two small linkages form the first branches of the dendrogram and are very similar. This group, so formed, may then link at a lower level of similarity to another individual station or group. Such a process continues until all stations are grouped and linked at varying levels of similarity.

Fig.2. 1 Theoretical Dendrogram



Hellawell (1978) notes that since similarity coefficients are measured between pairs of samples it will become increasingly difficult to assign some samples to the appropriate cluster. This difficulty is especially acute with large matrices in which case it becomes essential to adopt a systematic procedure. There are various computer programmes available to perform cluster analyses and a number of clustering strategies available. The application of different clustering techniques may give dissimilar results but these may be of help when looking for objective classifications of data.

Dendrograms representing data of such a spectrum, when reduced to a two-dimensional representation cannot be expected to depict all

possible associations between all members of the groups.

2.6. PRINCIPAL COMPONENT ANALYSIS (P.C.A)

A method more commonly used in terrestrial ecology is a second method of data 'reduction' available. Firstly a correlation matrix is generated, eigen values and percentage variability calculated and attributed to a reduced number of 'factors'.

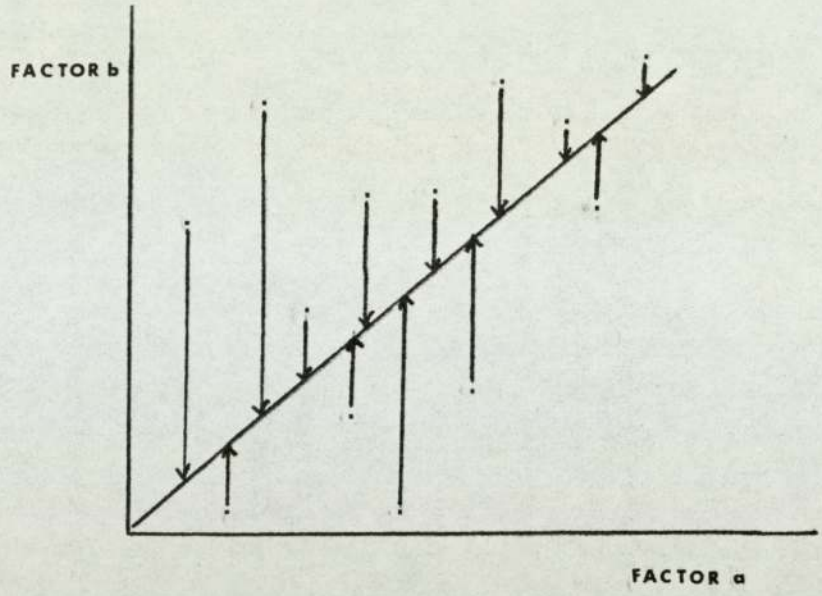
Factor analytic techniques enable one to detect any underlying pattern of relationship existing in the data. The data may then be 'rearranged' or 'reduced' to a smaller set of components. These may be regarded as source variables, the first three or four generated accounting for almost all of the variance in the data. The first component predicts as much of the variance of the original variables as possible. Often the first component is a gross value, the strict ecological information often being contained in the second or third component. All factor analytic applications are based on the data summarising capability of the method and are essentially in sections. (Ref. Statistical Package for Social Scientists Manual 1975, Update 1981).

a. Preparation of the correlation matrix.

b. Extraction of individual factors.

c. Rotation to terminal solution - the research for simple and interpretable factors.

Factors (or components) are extracted in such a way that one factor is independent from the other, i.e. orthogonal. P.C.A. is a method of transforming a set of variables into a new set of components which are orthogonal to each other. If all data points are plotted on to a two-dimensional scattergram to display similarity of sites, the best linear fit is then drawn in the line accounting for the maximum variation in the data, whilst losing least information, e.g.



Next points are rotated on to a straight line for ease of display and interpretation. This then is the 1st. Principal Component.

The 2nd. Principal Component is the second best linear combination of the variables, under the condition that the second is orthogonal to the first. In order to be orthogonal to the first the second P.C. must account for the proportion of the variance not accounted for by the first one. Therefore the 2nd. Principal Component is the linear combination of variables that accounts for the most residual variance after the effect of the first is removed from the data.
Further components are defined in this manner until all variance is exhausted. Each component is calculated as the eigen vector of the correlation matrix, the corresponding eigen value being the variance of the original variables accounted for by the component.

This type of analysis, as applied by Hamer and Soulsby enabled them to separate streams of poor from those of high water quality. These then provide reference points to which other sites may be compared. If data replicated over a period from the same site, the data may be used as a good baseline with which to compare future surveys.

Erman & Helm (1971) conclude from using ordination analysis on benthic invertebrates in the littoral zone of Bear Lake that "differences in species composition between communities are most often a re-

sult of underlying environmental differences".

2.7. TRICHOPTERA

2.7.1. TAXONOMY

As a group the Trichoptera (or caddis flies) may be broadly divided into cased and caseless types.

There are 14 recognised European families. The cased caddis flies belong to the following families:-

1. Limnephilidae
2. Phryganeidae
3. Odontoceridae
4. Leptoceridae
5. Molannidae
6. Glossosomatidae
7. Hydroptilidae
8. Sericostomatidae
9. Beraeidae

The caseless families include two types:

3 families of net spinners:

1. Hydropsychidae (10 species)
2. Philapotamidae (5 species)
3. Polycentropidae (13 species)

and the two 'free living' types:

4. Rhyacophilidae
5. Psychomyiidae

Trichoptera larvae are often referred to in the literature as 'caddis worms' or 'stick worms'. They are of great importance in freshwater ecology, being eaten in large numbers by both fish and water birds.

Consequently they are of great interest to the fisherman to whom they are known as sedge flies or rails (Ireland).

The families of caseless caddis to which special attention will be given in this study are the Hydropsyche spp. and Rhyacophila spp. Zoogeographically they span a wide range of conditions, but they do show habitat preferences for various reasons (Edington, 1968; Boon, 1978). In the literature they are relatively well documented (Moseley, 1939; Hickin, 1967). Their geographical distribution has had extensive work by Badcock and the British Records Centre. Taxonomic identification of both larvae and adults is relatively straightforward with the aid of Macan (1963), Hickin (1967), Mackereth (1954), Hildrew & Morgan (1974), Boon (1977) and Edington (1964 & 1980). A discrepancy in the taxonomy of the Hydropsychids caused a little confusion but the situation has now been clarified. Last century two adult caddis of different species were labelled with the same name Hydropsyche fluvipes. Only one H. fluvipes figured widely in Mosley's book, but H. fluvipes (Curtis) was consistently misidentified - consequently revealing that H. fluvipes has a very restricted distribution in Britain. A taxonomic revision of the Hydropsychidae was carried out by Döhler (1963), Neboiss (1963), Botosaneanu & Marinkovic-Gospodnetic (1966) and Tobias (1973).

Table 2.3. below summarises how the nomenclature now stands, as a new name had to be added to the British list, and for taxonomic connections a re-arrangement had to be made.

TABLE 2.3. OLD NAMES (Unchanged)

Diplectrona felix	- McLachlan 1878
Cheumotopsyche lepida	- Pictet 1834
Hydropsyche pellucidula	- Curtis 1834
Hydropsyche angustipennis	- Curtis 1834
Hydropsyche guttata	- Pictet 1834
Hydropsyche exocellata	- Dufour 1841
Hydropsyche saxonica	- McLachlan 1834
Hydropsyche contubernalis	- McLachlan 1865

TABLE 2.3. (continued)

Names Changed

OLD		NEW
Hydropsyche fluvipes (Curtis 1834)	->	Hydropsyche instabilis (Curtis, 1834)
Hydropsyche instabilis (Curtis 1834)	->	Hydropsyche siltalai (Döhler 1963)
		Hydropsyche fluvipes (Curtis 1834)

Older research papers use the old nomenclature so care is needed in reading to avoid confusion.

The more recent papers which use the new system include:

Badcock 1974, 1975, 1978a, 1978b

Hildrew and Morgan 1974

Hildrew 1978

Boon 1978a, 1978b

Hildrew & Edington 1979

Edington 1980

2.7.2. Life History

The females, after mating in flight with the male, enter the water and lay eggs in strips on hard surfaces (Badcock, 1953). Development of the eggs depends upon temperature, but given favourable conditions they may hatch in 10 - 24 days. Some species, or some broods may over-winter in the egg stage, or in an early larval form. There are 5 or 6 instars in the larval stages before the prepupal or resting stage. Pupation takes place under water, the cased varieties sealing off their cases, whilst the caseless types construct a silk cocoon. Some of the casemakers anchor the case to a support, and pupation generally takes between 14 - 21 days. Once again, it is possible for a larva to over-winter in the pupal stage, but more commonly the life cycle is complete is one year. Most imagines emerge between May - October and there may often by overlapping broods.

On emergence the caddis flies take to flight, different species displaying different behaviour in this respect. For example, some swarm during the day e.g. H. angustipennis swarms close by, and over water, the pattern being alternating forming and dispersing of the swarm. Other species practise only intermittent flight, and do not appear to have 'mass flights'. Light trap analysis reveals that some species fly predominantly after dusk, for example H. instabilis and R. dorsalis. Weather conditions also appear to affect the activity, as studies at Rothamsted have shown. An increase in the minimum temperature of 2.8°C doubled the annual nightly catches of caddis flies. Critchon (1957) found that his largest catches were made on rainy nights, although other factors, such as little or no wind and high barometric pressure sometimes affect emergence and swarming too.

In the Mississippi, Fremling (1960) reports on extremely abundant Hydropsychids, which create serious nuisance and health problems when they swarm around city lights and lighted store windows.

On contact with people setae are dislodged from wings and bodies and cause allergic reactions in hypersensitive people. Many others develop typical 'hay-fever' type symptoms. Other people are inconvenienced, as doors may not be left open and outdoor lighting is impractical. Similar nuisance problems have been reported from other parts of the world, and Fremling cites literature from New Zealand, Africa, and Canada. On the Mississippi the problem is of H. orris and Cheumatopsyche compyla taking advantage of the fast flowing sections of river in which to spin and attach their nets to catch food. At the Union Hydroelectric plant larvae form dense mats on cooling siphon gratings, thus impeding the flow of water to generators. Apparently this problem has also been encountered in Japan and California.

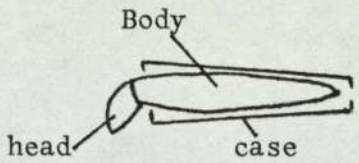
Oviposting females restrict themselves to the river, but males

invade the town. Measures to control them have included supplying lights of wave lengths 3,200 - 3,800 Å to attract flies to certain spots away from houses, shops and restaurants. Beneath the lights adult insects have been trapped and caught in pans of water containing sufficient detergent to 'wet' the insects. Measures receiving consideration to control the larvae included the use of DDT granules - using the filtering action of the Hydropsychids to advantage in catching the DDT in their nets. As the larvae regularly clean their nets they would be in contact with the DDT. The only drawback may be that an exterminated species may be replaced by a less desirable one, taking advantage of the new 'niche'. In the Miramachi river Ide (1957) reported proliferation of Chironomidae after spraying DDT to reduce caddis fly population. Fortunately, to date, such extreme nuisance problems have not yet been encountered and reported, but obviously autoecological studies are of value in assessing possible indicator value and/or biological control.

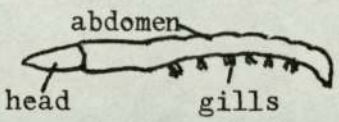
Returning to Trichopteran life cycles and physiology, it is useful to study various aspects with regard to improving the data available on these species. Some species are entirely riffle dwellers e.g. Hydropsyche, Rhyacophila sp. The hydropsychids require the flow of water to construct their nets, whilst Rhyacophila, being carnivores, prey on other riffle invertebrates. In slower flow conditions, other species may build cases and attach themselves to hard surfaces.

Respiratory adaptation often determines the habitat that particular species favours. Trichopteran larvae have an apneustic respiratory system, i.e. none of the spiracles functioning, but air entering the tracheal system by diffusion through the gills or general body surface (Imms Vol.1.).). Tracheal gills, filiform or lamellate are well supplied with trachae. These gills are usually borne on the abdomen, but are sometimes present on the thorax and rarely on the head. The

eruiform larva of Limnophil us, for example, with hypognathous head which emerges from the front of the case



renews oxygen around the abdominal gills by undulations of the abdomen, creating a current of water which is drawn through the case. In the campodeiform larva, with prognathous head, e.g. Hydropsyche spp.



there is no case, but undulations of the abdomen ensure a circulation of water around the gills. Living in riffle environments of fast flowing highly oxygenated water obviously assists the animal in respiration.

Finally, food of most trichopteran larvae may be varied as they are omnivorous. Only a few such as Rhyacophila are obligate predators.

2.8. TOXICITY TESTING

In order to integrate and fully understand the impact of pollutional stress , on both individual species and the whole benthic community, it is necessary to carry out intensive laboratory based toxicological studies. Results from such studies provide accurate data on the tolerant levels of specific pollutants which each species can withstand. Such data are valuable, only if the studies are carried out in carefully and controlled scientific manner, i.e. with good experimental design, parameters being monitored and results calculated and interpreted accurately. Furthermore, such results are only useful if they may subsequently be applied to the field situation and enhance the interpretation of results of surveys to monitor pollutional status.

Autecological studies are necessary to determine values for individual indicator species. Only when these data are complete will

it be possible to carry out thorough synecological studies.

In the broader view, it would be ideal to collect and collate data from the entire spectrum of organisms in the freshwater ecosystem, but as discussed earlier this is impractical. Consequently, only certain organisms are selected for use. Major components of the food web have been preferentially chosen for this purpose and benthic macroinvertebrates are popular organisms in this context.

Perhaps the most recent reference document on tolerance levels of macroinvertebrate species is the Murphy (1978) UWIST report; in which a large proportion of the data available for Trichoptera and all other species are collected. This is a well organised report, listing species, type and concentration of pollutant, stage of life cycle, experimental conditions and other available information.

A similar attempt to draw all such information together has been attempted in the U.S.A. by the Environmental Protection Agency (E.P.A. (1978) and Roback (1962) limits his summary to the tolerances for chemical factors caddis fly fauna in streams and rivers from stream survey reports. There are numerous other individual papers contributing snippets of information re caddis fly larvae to this overall information network. Unfortunately the drawback of many of these studies is of non-standardisation, i.e. incomparable results due to variations in species used as test organisms, test conditions etc.

Bioassay should theoretically be straightforward and simple, but there is often insufficient evidence based upon experimental or field observations to precisely locate organisms in a logical series, that being on gradation of most sensitive to most tolerant in relation to a pollutant and also in their placing in relation to other species. Daphnia and Gammarus appear far more widely documented than other species in this respect.

Although there are innumerable recorded field observations on the presence of caseless caddis species there remain large gaps in complim-

entary laboratory toxicity test data.

Various workers recognise the requirements of animals and experimental methodology, for example Murphy (1978), Phillips (1980). It is generally agreed that a test species must be:

a. plentiful
b. easy to collect and/or
c. easy to culture
d. simple to maintain in the laboratory
e. display suitable behavioural characteristics
f. be relatively sensitive to the pollutant in question.

The caseless caddis larvae Hydropsyche and Rhyacophila handsomely meet these requirements listed above, and may be of great potential use. To date the species in question have not been extensively studied, but a summary of toxicity tests to date is given in Table 2.4.

Drawbacks in toxicological work with invertebrates may be briefly listed as:

a. lack of comparable conditions
b. instar or stage of growth not recorded
c. parameters not recorded, e.g. temperature, pH, hardness, dissolved oxygen concentration
d. static or flow through conditions
e. if animals were fed during the experimental period.

With these kind of problems in mind the Environmental Protection Agency prepared a report to advise on standard procedure. This report, entitled Methods for Measuring the Acute Toxicity of Effluents to Aquatic organisms was geared to fish studies. Similarly in Britain a Standard Method has been described by M.A.F.F. a summary of which may be found in Alabaster & Lloyd (1980). In this work, guidelines have been adapted for use with macroinvertebrates, particularly the Hydropsychidae (a summary of this procedure will be given in the Methods section, Chapter 4).

TABLE 2.4. Summary of Toxicity tests on Trichopteran larvae

Species	Toxicity Unit	Concentration	Conditions	Reference
Hydropsyche sp.	7d LC50	>0.55 mgl^{-1} Chlorinated sewage effluent	30 - 50% Sat.	Arthur, 1975
H.angustipennis	50% mort. 96hr	1.0 mgl^{-1} O_2	FT 20°C	Davis, 1971
R. dorsalis	50% mort 10hr	1.0 mgl^{-1} O_2	FT 20°C	Davis, 1971
Trichoptera sp.	96hr LC50	1.7-3.8$mgl^{-1}O_2$ Range for 7 species	135 ppm $CaCO_3$ pH 7.8	Gaufin, 1973
H. bettini	30d LC50	3.38 mgl^{-1} H_2SO_4	FT 18.5°C	Bell, 1971
Hydropsyche sp.	96h LC50	3.34 mgl^{-1} H_2SO_4	FT 9.5°C	Gaufin, 1973
H. bettini	50% mort.10d	32mgl^{-1} $CdSO_4$	S 18°C pH 7.1	Wernick & Bell, 1969
	- do -	32mgl^{-1} $CuSO_4$	- do -	
	50% mort.7d	16mgl^{-1} $FeSO_4$	S 18.5°C" 7.7	"
	50% alive:>14d	64mgl^{-1} $NiSO_4$	S 18°C " 7.1	"
	50% mort.11d	16mgl^{-1} $ZnSO_4$	S 18°C " 7.4	"
Trichoptera sp.	84h LC50	5.1mgl^{-1} $CdCl_2$	S 50ppm $CaCO_3$ pH 7.6 DO 6.2 17°C	Rehwoldt, 1973
	96h LC50	3.4mgl^{-1} $CdCl_2$		
	24h LC50	58 mgl^{-1} $CrCl_3$		
	96h LC50	50 mgl^{-1} $CrCl_3$	"	"
	24h LC50	12.1mgl^{-1} $CuCl_2$	"	"
	96h LC50	6.2mgl^{-1} $CuCl_2$		
	24h LC50	5.6mgl^{-1} $HgCl_2$	"	"
	96h LC50	1.2mgl^{-1} $HgCl_2$		
	24h LC50	48.4mgl^{-1} $NiCl_2$	"	"
	96h LC50	30.2mgl^{-1} $NiCl_2$		
	24h LC50	62.6mgl^{-1} $ZnCl_2$	"	"
	96h LC50	58.1mgl^{-1} $ZnCl_2$		
Rhyacophila sp. Hydropsyche sp. Polycentropid sp.	100% survival >20 days	20 mgl^{-1} Zn		Herbst 1967

S = Static Test
F T = Flow Through

2.9. ALTERNATIVE APPROACHES

Toxicological studies, analysing single pollutants are obviously of great benefit, but alternative approaches utilising other factors affecting species distribution may be extremely useful.

Many workers have investigated the distribution of invertebrate fauna, and that of caddis flies in particular. In Britain such workers include Scott (1958), Badcock (1974) and Boon (1976). In Europe, Decamps (1967, 1968) mapped Trichoptera distribution in the Pyrenees, whilst Marinkovic-Gospodnetic (1966) documented the distribution in a small mountain stream in Yugoslavia. Schumacher & Schremmer (1970) carried out work on Trichoptera as ecological indicators in a small German stream, whilst a Spanish team sponsored by Ministry of Agriculture continue a distribution survey of rivers (del Tenago and de Jalon, 1981 pers. comm.), Dr. de Jalon specialising in identifying and mapping caddis fly distribution in Spanish rivers.

Environmental parameters affect the distribution of caddis fly larvae and some workers have investigated certain aspects of their ecology and physiology. Philipson (1954) investigated the respiratory physiology on net-spinning behaviour of several Trichopteran species in relation to water flow and oxygen concentration. Later in 1969, he looked at factors affecting net-spinning in H. instabilis, finding that the percentage of fifth instar larvae which will spin nets decreases with flow rate, also an increase in the temperature up to 12°C, produces an increase in the number of nets spun. Any further increase in temperature decreases their activity. Continuing laboratory work by Philipson and Moorhouse (1974) demonstrated changes in net-spinning and ventilation in three species of Hydropsychidae. Net-spinning was found to be influenced by temperature, current and availability of food, whereas ventilation rate depends upon flow rate and the amount of dissolved oxygen (Edington and Hildrew, 1973).

Ambühl (1959) made observations on current velocities and Edington (1968) in a combined field and laboratory programme showed species of net-spinning caddis have a sequential replacement downstream and also marked preferences for pools and riffles depending on whether they are "low velocity species" e.g. P. conspersa or "high velocity species" e.g. H. instabilis. Throughout a normal river profile, there is a sequential distribution of benthic fauna well documented by Hawkes (1975).

Hynes (1960) has previously written of caddis fly tolerances and habitat preferences generally, but it is now well known that a longitudinal distribution of Trichopteran species exists along a river. This pattern is quite rigid, providing there are no adverse factors, e.g. point sources of effluent to modify the distribution. Edington (1981, pers. comm.) often noted overlap in species on R. Usk, but there appear to be set pairs of species co-existing in these zones.

Scott (1968) supports these distributions by field observations on R. Dean. The caseless caddis species he encountered show marked preference for a particular current velocity, also for size of stone in the substratum and other factors such as the presence or absence of moss on the stones. Another factor affecting, and interacting in the aquatic environment to dictate the distribution of caddis fly larvae is temperature. In fast flowing, well aerated head waters the conditions and temperature regimes are much different to the sluggish, warmer lowland stretches. Extensive investigations into temperature change along the length of a river was carried out by Edington (1966) and Boon & Shires (1976) used a similar temperature recording technique to relate the distribution of caseless caddis down the R. North Tyne in N.E.England. Later, Hildrew & Edington (1979) looked at factors facilitating co-existence of Hydropsychid larvae in R. Usk, S. Wales. A downstream sequence of species was revealed, being de-

pendent upon water temperature, velocity, differences in life cycles and feeding habits.

From the information compiled above, a data base has been generated in relation to water velocity, the effect of flow rate on oxygen consumption, ventilation rate and respiration.

Davies (1971) quotes 50% mortality in R. dorsalis and H. angustipennis at 10 hours and 96 hours respectively, in a flow through experimental arrangement at 20°C. Respiratory measurements, to establish the tolerance levels and oxygen requirements of Hydropsychids would be of ecological value. Such investigations determine at what concentration oxygen becomes a limiting factor. Early studies involved measuring undulatory activity under various conditions. Oxygen uptake was measured either by the Winkler Method, a Warburg respirometer or some type of Oxygen Electrode.

Fox & Simmonds (1932) performed experiments with benthic macroinvertebrates including Hydropsyche and Molanna spp. Large numbers of animals in a water filled stoppered bottle were used, samples of water to assess oxygen uptake being extracted at regular intervals and assessed by the Winkler Method. Hydropsyche, the riffle species was found to use 1.5 times more oxygen than Molanna. It was also found to be more sensitive to lack of oxygen than Molanna.

Using Phryganea grandis L. , removed from its case, Van Dam (1938) found that rates of undulation were affected by a rise in temperature, or decrease in oxygen. Under these conditions the 'undulation-pause' pattern was disrupted, the pause periods becoming shorter and finally non-existent as the stress required continued undulations. If aerated water was then passed over the animal the normal sequence was regained.

An increase in carbon dioxide concentration caused no acceleration in the number of undulations.

Similar results were recorded by Fox & Sidney (1952) when Limno-

philus flavicornis was placed in oxygen saturated and poorly aerated water. The amplitude of the undulations increased with stress and special movements were also observed after defaecation.

Work by Philipson (1953) recorded the number of undulations per 5 minutes for four species of caddis larvae, H. instabilis, P. flavomaculatus, A. nervosa and S. stellatus at different rates of stirring. The minimum oxygen concentration was calculated, i.e. the O_2 level at which movement ceased. This is often referred to as the 'incipient limiting point', the lowest O_2 concentration at which the animal can maintain itself, often described as an autoecologically significant factor in the distribution of the species.

In 1970 Feldmeth carried out studies on respiration with respect to current velocity. He found that larvae acclimated to whatever velocity they were subjected to.

Further work by Philipson and Moorhouse (1976) recorded the respiratory behaviour of four species of Polycentropids. From the results, their distribution in streams was established due to differential tolerance of low oxygen levels. For H. pellucidula at 10°C, 100% saturation O_2 an uptake of 0.75 ± 0.02 mg/g/hr was recorded, with an increase to 1.98 ± 0.15 mg/g/hr at 25°C (Philipson, 1977). In 1979, McCullough and Minshall, using H. occidentalis recorded an oxygen uptake of 0.23 mgl^{-1}/g/hr at 20°C oxygen saturation.

Using a Radiometer ES046 oxygen electrode Greenwood 1980, pers. comm.) reported Polycentropids to use between 0.1 - 3.0 $mgl^{-1}O_2$/g dry wt./hr at temperatures between 5° - 25°C. Table 2.5 summarises results of respiratory measurements made on caseless caddis.

These types of investigations provide a useful and alternative 'tool' in investigating reasons for species distribution in rivers. They may provide an effective means of investigating pollutional stress and explain changes in benthic community structure.

3. PROGRAMME OF RESEARCH

CHAPTER 3

PROGRAMME OF RESEARCH

The rationale behind this study was to ascertain the significance of the Trichoptera in the benthic community, firstly by plotting their distribution over a range of water qualities, next to assess their tolerance to a variety of pollutants and subsequently to draw conclusions as to their value as indicator species in monitoring river water quality. This involved the following investigations:

The collection of biological data from a large number of sites of differing water qualities to facilitate the analyses of the distribution of the Trichoptera.

Synecological studies contribute knowledge to changes in community structure as a result of pollutional stress, but in order to understand the interactions the autecology of individual species must be known. Thus, intensive laboratory studies to assist in characterising the factors affecting some species, by toxicity, bioconcentration and respirometric studies were undertaken.

The integration of field and laboratory findings was considered important, so "intermediate" studies were carried out at the Checkley Research station where experimental channels adjacent to the River Tean provided an excellent environment for these investigations. Additional sampling on specific rivers also helped to link laboratory and field studies.

3.1. CONSIDERATIONS

Benthic invertebrates are the most commonly used taxa for monitoring river water quality. Some species are poor indicators, perhaps because they are extremely tolerant and ubiquitous or extremely sensitive and restricted to very few sites i.e. a narrow ecological range. Others are too small to be seen at a glance, therefore rapid assessment on a river bank is impossible, or they are difficult to transport from river

to laboratory becoming damaged and unrecognisable during transit. Other species fail because they become difficult to identify after preservation in formalin or alcohol.

Consideration of some of these difficulties led to the decision of taking caseless caddis larvae as the study organisms. They are relatively easy to sample, and can at least be recognised in a raw field sample without the aid of a microscope for identification. The families of caseless caddis flies Hydropsychidae, Polycentropidae, Philopotamidae, Psychomyiidae and Rhyacophilidae are spread over a wide range of conditions, but they do show habitat preferences for one reason or another (Edington 1966; Boon, 1978).

In the literature they are relatively well documented by Mosely (1939) and Hickin (1937). Their geographical distribution has had extensive work by Badcock and the British Records Centre and taxonomically identification of both larvae and adults is quite straightforward with the aid of Macan (1973), Hickin (1967), Mackereth (1954), Hildrew and Morgan (1974), Boon (1977), Edington (1964, 1980) and most recently an F.B.A.key, Caseless Caddis Larvae of the British Isles (Edington and Hildrew, 1981) has been produced.

In the laboratory they are relatively easy to maintain in large numbers and lend themselves to a fair amount of handling.

4. FIELD WORK

CHAPTER 4.

FIELD WORK

The field work section comprised a generalised national survey of rivers to investigate macroinvertebrate distribution in differing water qualities with special attention being given to the caseless Trichopteran larvae. In addition four individual river studies were carried out.

The River Churnet work sought to investigate the response of benthic invertebrates and in particular caseless trichopteran species to differing water qualities. Sequential separation of trichopteran species down the course of the river was also studied.

Similarly, the River Blythe study investigated the distribution of the caseless caddis species in this river system.

A further study on the River Tean, compared two stations above and below an organic effluent, its effect on the macroinvertebrate communities with particular consideration of Hydropsyche and Rhyacophila occurrence.

The fourth study on two Welsh rivers, the R. Ystwyth and R. Rheidol investigated the tolerance of the benthos to heavy metals with special attention to zinc.

4.1. METHODS AND MATERIALS

With the co-operation of Severn-Trent, Anglian, Yorkshire and North West Water Authorities who supplied Grid References for their regular sampling sites, one hundred sites were chosen using random number tables to give an unbiased set of sites for use over all the water qualities. As a result, their geographical distribution fell anywhere within 100 mile radius of Birmingham. These sites were generally restricted to rithron zones and sampling was to be carried out in the riffle sections where depth seldom exceeded 90 - 100 cm. It was decided that an appropriate method for sampling was the Aston Cylinder Sampler, and that three

random quantitative samples would be taken at each site.

The survey sites, together with their respective Grid References and chemical status (according to the 1975 Water Pollution Survey) are given in Tables 4.1 to 4.4. Sketch Maps 1, 2, 3 and 4 illustrate the points at which sampling occurred within each Water Authority Area. There are in total 30 sites of Class 1 water quality, 24 Class 2, 25 Class 3 and 20 Class 4 giving a reasonable spread across water qualities. (In calculations data from samplings sites 20 to 27 inclusive are not usually incorporated as data were incomplete.)

4.1.1. The Cylinder Sampler

Description

The cylinder sampler of Aston design may be compared to earlier models of the simpler Hynes (1971) type, and the more elaborate Neill (1938) version. The Aston Sampler (Photograph 1) has now been recommended as a standard sampler by the Department of the Environment (HMSO, 1980). It is an open ended cylinder made from 18 gauge stainless steel, which encloses an area of substratum $0.05m^2$. A serrated lower edge, consisting of 38 teeth 1 cm deep facilitates pushing the cylinder firmly into the substratum using the laterally positioned handles in a turning movement. This then encloses a given area of river bed. Water enters the sampler through an oval metal mesh covered aperture (to prevent the entry of drift organisms) which is on the upstream side of the sampler. Directly opposite is a second metal collared aperture on to which the collecting net is fitted. The net, made of 15 mesh/cm nylon bolting cloth, is fitted with a canvas collar with drawstring attachment. Thus the sampling area is confined so that the sampling procedure may be carried out.

The Sampling Procedure

Ensuring that the cylinder is pushed as far into the substratum as possible, with the water inlet aperture facing upstream, the sampler is

TABLE 4.1

KEY TO SAMPLING SITES IN SURVEY YORKSHIRE WATER AUTHORITY

No.	RIVER	SITE (CHEMICAL CLASS)	GRID. REF.
1	Calder	Hebden Bridge (1)	SD 995 269
2	Calder	Sowerby Bridge (2)	SE 059 235
3	Calder	Clifton Beck, Brighouse (2)	SE 149 227
4	Calder	Mirfield (3)	SE 189 205
5	Calder	Huddersfield, R. Colne (3)	SE 178 201
6	Calder	Dewsbury (4)	SE 251 205
7	Calder	Horbury (4)	SE 304 174
8	Dearne	u/s Barnsley (2)	SE 347 079
9	Dearne	d/s Barnsley (3)	SE 367 060
10	Dove	Darfield (4)	SE 407 038
11	Dearne	Broomhill (3)	SE 420 031
12	Dearne	u/s R. Don (3)	SE 499 012
13	Don	Kilnhurst (4)	SK 467 974
14	Don	Sheffield (3)	SK 333 906
15	Loxley	R. Don trib. (2)	SK 338 897
16	Drone	R. Rother trib. (4)	SK 373 751
17	Whitting	Whittington (4)	SK 383 743
18	Blackburn Brk.	R. Don trib. (4)	SK 389 921
19	R. Don	Oughtibridge (3)	SK 308 934
20	Ewden Beck	(1)	SK 297 955
21	R. Severn	(1)	SE 745 791
22	Wharfe	Tadcaster (2)	SE 448 433
23	Wharfe	Boston Spa (1)	SE 432 458
24	Wharfe	Harewood (1)	SE 312 461
25	Wharfe	Bolton Bridge (1)	SE 071 525
26	Wharfe	Burnsall (1)	SE 032 611
27	Washburn	Leathley Bridge (1)	SE 232 465

MAP 1 Sketch Map showing Sampling Sites in Yorkshire Water Authority Area.

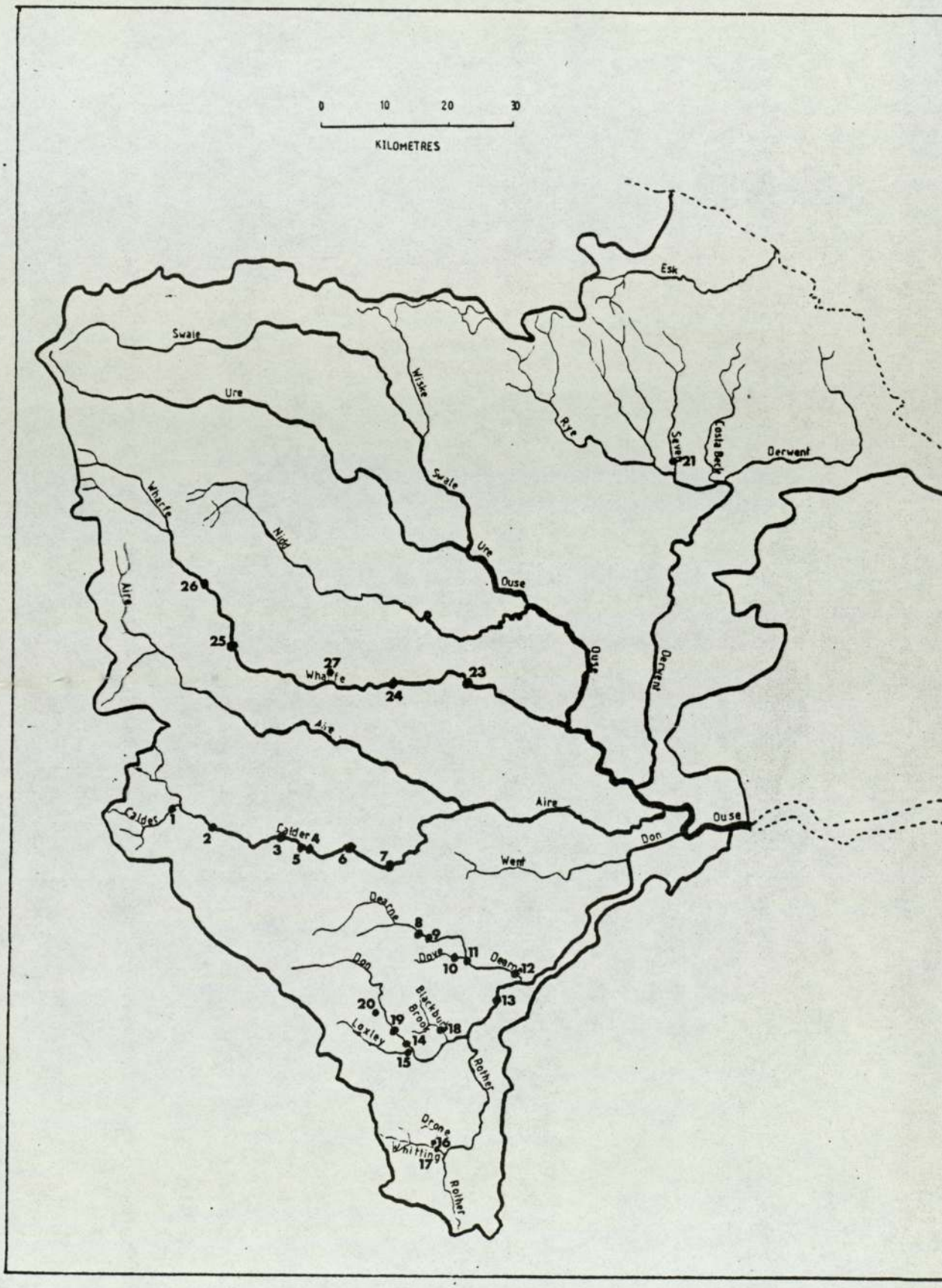


TABLE 4.2

KEY TO SAMPLING SITES IN SURVEY SEVERN-TRENT WATER AUTHORITY

No.	RIVER	SITE (CHEMICAL CLASS)	GRID. REF.
28	Dowles Brook	R. Severn Trib. (2)	SO 755 773
29	Stour	Dog Kennel Lane (4)	SO 969 831
30	Stour	d/s Coombeswood Brook (4)	SO 963 850
31	Devon	Hawton (1)	SK 786 511
32	Poulter	Elkesley (2)	SK 699 752
33	Maun	Mansfield A615 (3)	SK 546 616
34	Maun	Clipstone (3)	SK 601 649
35	Maun	Edwinstowe (3)	SK 647 655
36	Rainworth Water	Rainworth (3)	SK 480 580
37	Rainworth Water	Ollerton (3)	SK 648 645
38	Erewash	Pinxton (3)	SK 464 546
39	Bagthorpe Brook	Erewash trib. (4)	SK 450 510
40	Beauvale Brook	Nether Green (3)	SK 465 474
41	Churnet	Upper Hulme (1)	SK 012 610
42	Churnet	Bridgend (4)	SJ 978 550
43	Churnet	Abbey Green Road (4)	SJ 979 573
44	Churnet	d/s Wardles Works (4)	SJ 982 578
45	Churnet	Cheddleton Stn. (4)	SJ 975 520
46	Churnet	Consall (4)	SK 002 487
47	Churnet	Froghall (3)	SK 025 472
48	Churnet	Oakamoor (3)	SK 053 448
49	Churnet	Alton (2)	SK 072 426
50	Tean	Checkleybank (3)	SK 025 380
51	Tean	Beamhurst (3)	SK 060 350
52	Blythe	Cheswick Green (2)	SP 124 756
53	Blythe	Henwood Mill (2)	SP 181 794
54	Blythe	Temple Balsall (2)	SP 209 764
55	Blythe	u/s. Eastcote Brook (1)	SP 213 801
56	Blythe	Stonebridge (1)	SP 214 831
57	Langley Brook	u/s Middleton Works (2)	SK 183 981
58	Bourne	Over Whitacre (1)	SP 240 914
59	Bourne	d/s Fillongley (1)	SP 275 888
60	Bourne	Daw Mill Bridge (1)	SP 258 898
61	Anker	Atherstone (3)	SP 317 985
62	Anker	Witherley (3)	SP 325 971
63	Anker	Leathermill Bridge (3)	SP 339 956
64	Tame	u/s. Blue Billy Tip (4)	SO 989 887
65	Tame	West Bromwich (4)	SO 994 902
66	Tame	Great Bridge (4)	SO 976 922
67	Tame	Bescot (4)	SP 006 962
68	Ford Brook	Clayhanger (4)	SK 042 049

MAP 2   Sketch Map showing Sampling Sites in Severn-Trent Water Authority Area.

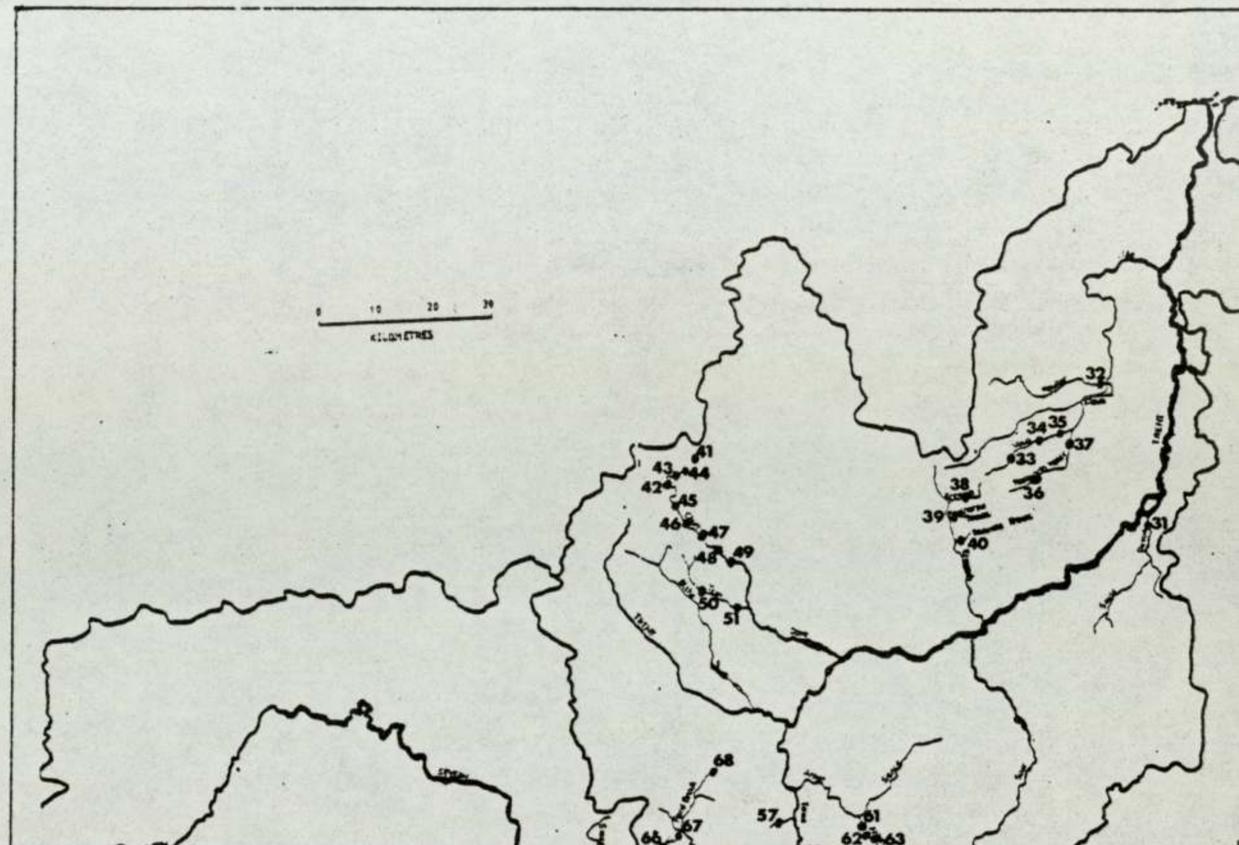


TABLE 4.3

KEY TO SAMPLING SITES IN SURVEY ANGLIAN WATER AUTHORITY

No.	RIVER	SITE (CHEMICAL CLASS)	GRID. REF.
69	Ouse	Fulwell Br. Nr. Westbury (1)	SP 626 346
70	Trib. R. Tove	Rd. Br. Sth. Preston Capes (1)	SP 577 534
71	Ouse	Pssenham (1)	SP 780 393
72	Tove	Br. Bozenham Mill (1)	SP 766 483
73	Tove	Cappenham Br. Towcester (1)	SP 715 488
74	Ascott Brook	B488 Rd. Br. Grove Lock (1)	SP 914 229
75	Clipstone Brook	A5 Rd. Br. Hockliffe (1)	SP 971 269
76	Ouse	No thr. Rd. Milton Ernest (1)	TL 015 558
77	Claydon Brook	A413 R. Br. Winslow (2)	SP 778 269
78	Claydon Brook	The White Br. Padbury (2)	SP 715 290
79	Claydon Brook	Rd/Rail Br. (2)	SP 745 276
80	Ouzel	Rd. Br. Stanbridge Ford (2)	SP 970 230
81	Ouse	A509 Rd. Br. Olney (2)	SP 888 509
82	Ouse	Ravenstone Mill (2)	SP 854 486
83	Ouzel	Caldecote Mill (2)	SP 886 428
84	Ouzel	Willen Road Br. Willen (2)	SP 882 409
85	Ouzel	Simpson Rd. Br. (2)	SP 885 361

MAP 3 Sketch Map showing Sampling Sites in Anglian Water Authority Area.

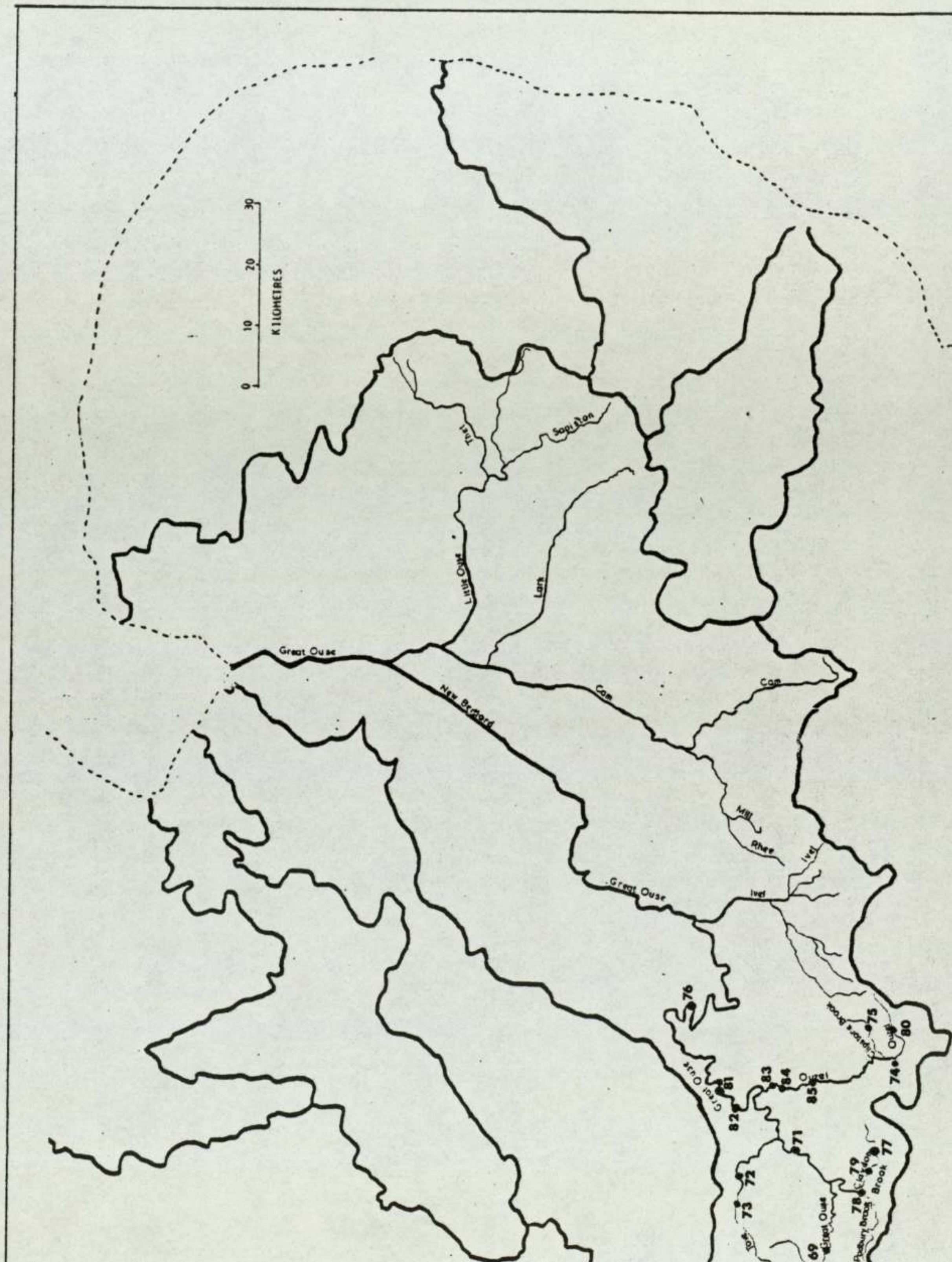
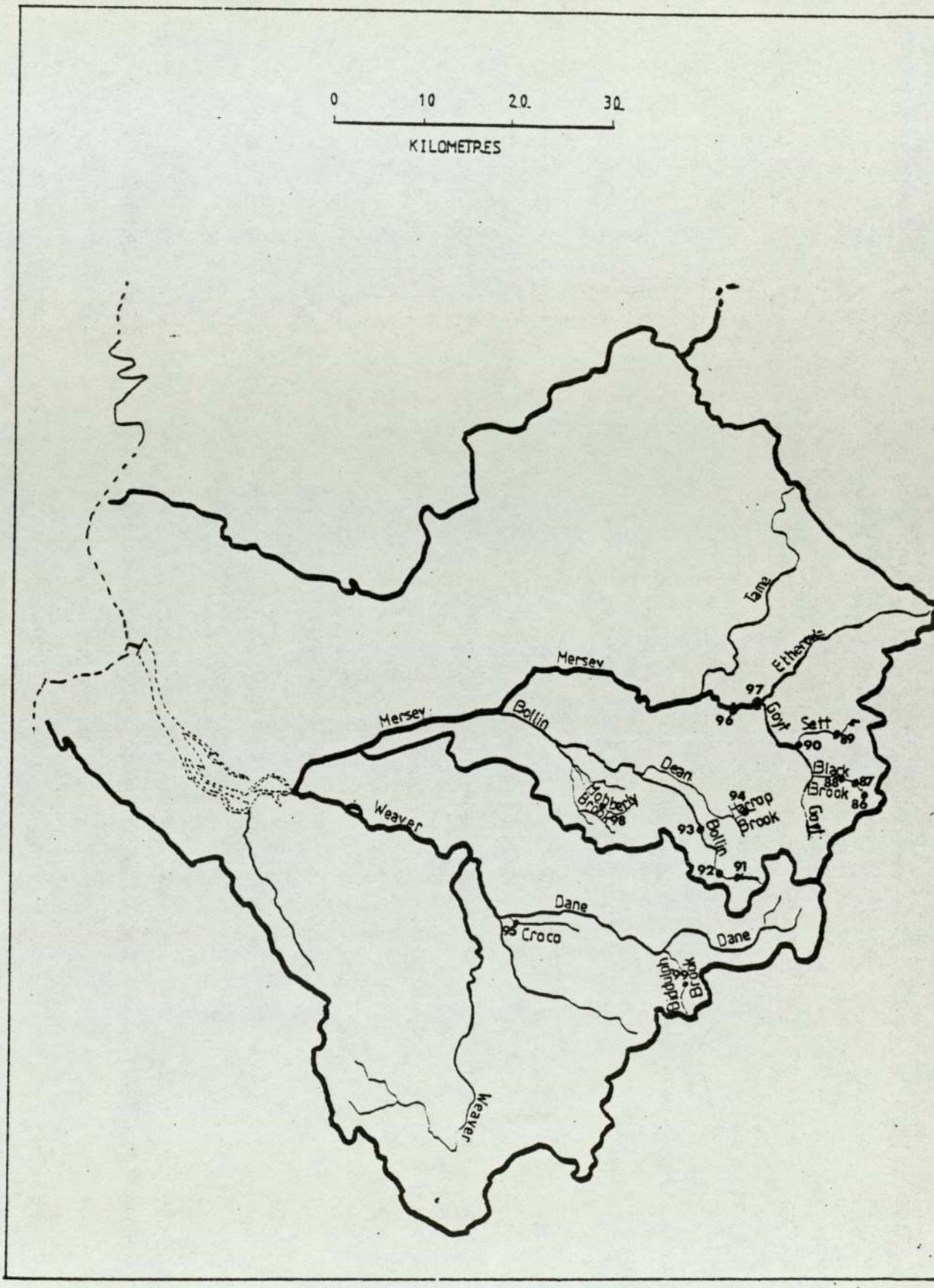


TABLE 4 .4

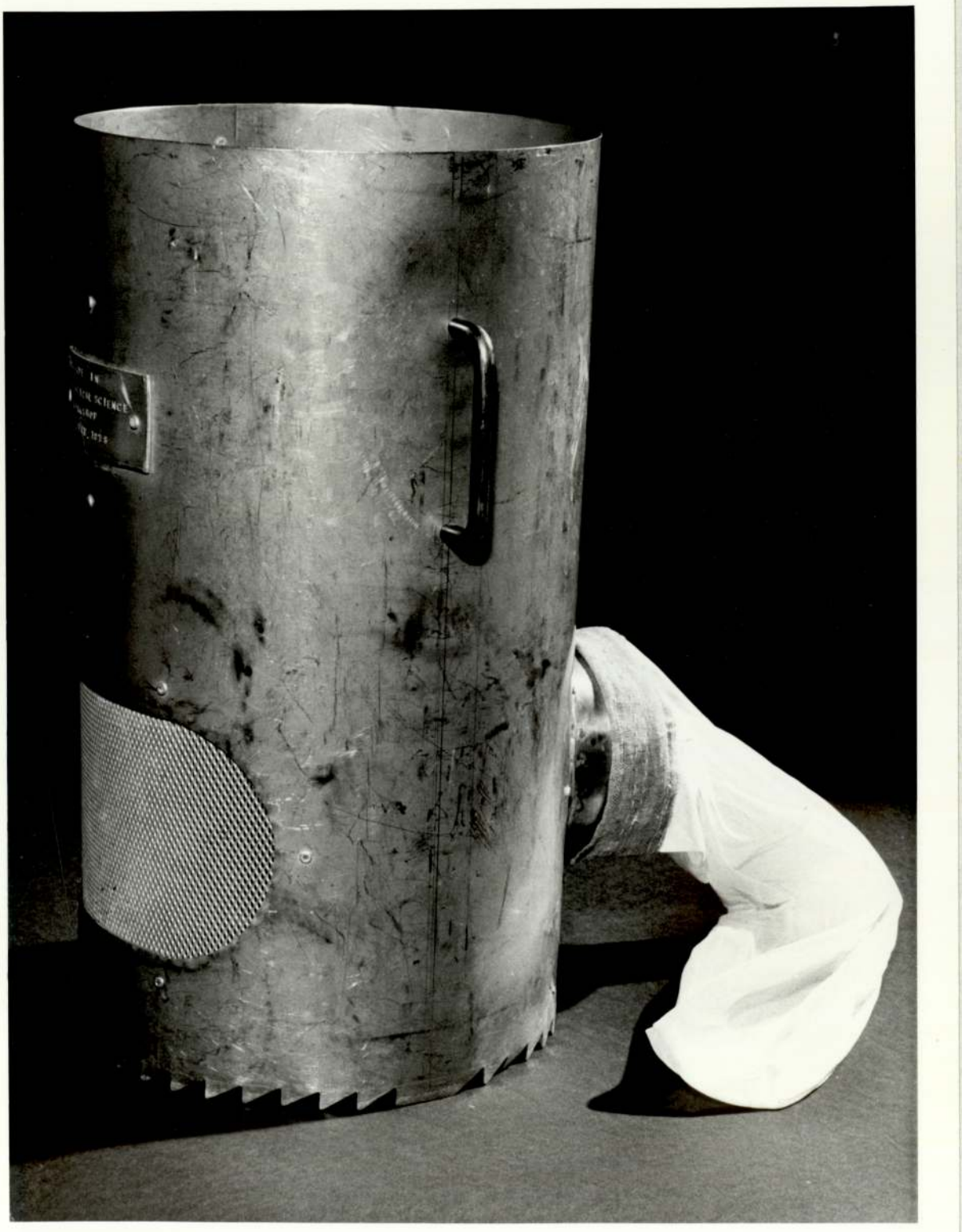
KEY TO SAMPLING SITES IN SURVEY NORTH WEST WATER AUTHORITY

No.	RIVER	SITE (CHEMICAL CLASS)	GRID. REF.
86	Warm Brk.	u/s Chapel-en-le-Frith(1)	SK 062 804
87	Warm Brk.	d/s Chapel-en-le-Frith (1)	SK 060 810
88	Roych Brook	u/s Black Brook (1)	SK 058 818
89	River Sett	Hayfield (1)	SK 037 839
90	River Sett	u/s R. Goyt (1)	SK 002 859
91	River Bollin	Langley (1)	SJ 945 717
92	River Bollin	Jarmin (1)	SJ 928 715
93	River Bollin	Beech Bridge (2)	SJ 915 746
94	Harrop Brook	u/s Bollington (2)	SJ 941 779
95	River Croco	u/s R. Dove (2)	SJ 708 660
96	River Goyt	Otterspool Bridge (3)	SJ 937 895
97	River Goyt	Iron Br. Compstall (3)	SJ 963 908
98	River Bollin	Mobberley Brk. Warford Lane (3)	SJ 816 782
99	River Dane	Biddulph Brk. d/s ETW (3)	SJ 890 595

MAP 4 Sketch Map showing Sampling Sites in North-West Water Authority Area.



Photograph 1. The Aston Cylinder Sampler



placed in the riverbed. Checking that the flow of water is unimpeded the area of substratum contained within the sampler is disturbed so as to dislodge the animals in the enclosed river bed. Large stones are washed clean of any attached invertebrates and these are swilled into the net by the flow of water through the sampler. Any attached algae are included as there may be organisms within the mass. Using a set number of rotations, standardisation of the sampling time is achieved (30 rotations in this example). Water is allowed to wash through the sampler until all organisms are transferred to the collection net. Animals may then be inspected immediately on a white tray, or transferred to a labelled screw top bottle or polythene bag. In this way samples may be returned to the laboratory and sorted whilst fresh or preserved.

4.1.2. The Kick-Heel Sample

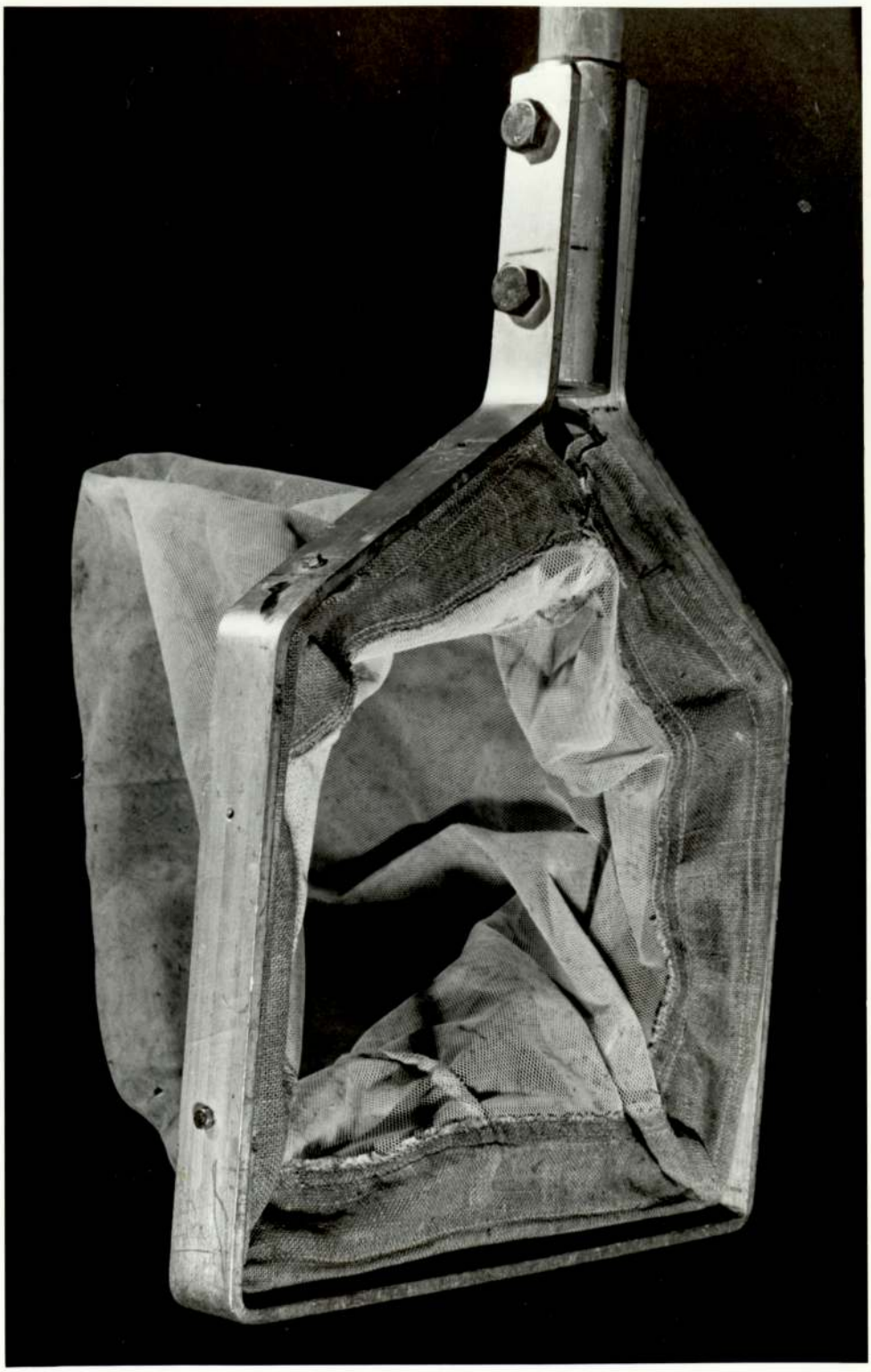
If for reasons of depth or flow a cylinder sample could not be taken, then a kick-heel sample using a standard hand net (HMSO, 1978) was used to obtain a qualitative sample on a few occasions.

Collecting the sample involves standing in the river with one's back facing upstream. The handnet (see Photograph 2) is held vertically so that the base metal frame of the handnet is resting on the riverbed slightly in front of the feet. Next, the sampler moves slowly backwards using repeated twists of the heels, in a zig-zag fashion, to dislodge the substratum. The flow of water downstream carries any dislodged invertebrates into the net for collection. This sampling method may be standardised by either timing the collection period, or dislodging the animals from a known area of riverbed.

4.1.3. Sample Processing

In the laboratory each sample was sorted, counted and individuals of all taxa identified using an Olympus Binocular Microscope and avail-

Photograph 2. The Standard Handnet



able keys. Individual Trichopteran specimens were preserved in 70% alcohol in individually labelled bottles. A species list was compiled according to the Coded Check List of Animals Occurring in Fresh Water in the British Isles (Maitland, 1977) for each sample, and recorded on data sheets. Biotic Indexes and scores were calculated for each site and recorded on the same sheets.

4.1.4. Physico-Chemical Parameters

Samples for physico-chemical parameters were taken at the time of biological sampling. Parameters measured were:-

a. Temperature - to the nearest $0.1^{\circ}C$

b. pH - Vibret Model 46A pH Meter within 1.0 unit

c. Dissolved Oxygen - Winkler Method mgl^{-1}

d. BOD - dilution where necessary mgl^{-1}

e. Total Hardness mgl^{-1} $CaCO_3$

f. Calcium Hardness mgl^{-1} $CaCO_3$

g. Magnesium Hardness mgl^{-1} $CaCO_3$

h. Phenolthalein Alkalinity $CaCO_3$

i. Total Alkalinity mgl^{-1} $CaCO_3$

j. Chloride mgl^{-1}

k. Nitrate $mgN\ l^{-1}$)
l. Ammonia $mgN\ l^{-1}$) Technicon Auto Analyser
m. Phosphate $mg\ l^{-1}$)

n. Suspended solids ($105^{\circ}C$) mgl^{-1}

o. Copper)
p. Cadmium) Perkin Elmer Model 306
q. Lead) Atomic Absorption Spectrophotometer
r. Chromium)
s. Nickel)
t. Iron)
u. Zinc)

4.2. RESULTS

The biological data from the three cylinder samples is recorded in Appendix 1a, and for comparative purposes the biological raw data from the Water Authorities is given in Appendix 1b. Similarly, chemical data from this survey are presented in Appendix 2a, together with Water Authority annual mean values recorded in Appendix 2b.

4.2.1. An Overview of Results

By taking a general view of the distribution of major groups of organisms in this survey we may see that the caseless caddis were found to compose 7.2% of the total numbers of individual invertebrates found in all of the samples in the survey. This compares with data from Roback (1962) who, in a study of the fauna of rivers in the United States, found between 8 - 13% of organisms were Trichoptera.

The figure of 7.2% is therefore of a similar order of magnitude, and shows that the caseless caddis species are obviously important representatives in benthic communities and as such should be useful tools as indicators. In this survey, six species of Hydropsyche were found, namely H. angustipennis, H. siltalai, H. pellucidula, H. instabilis, H. contubernalis and H. fluvipes; the first four species being quite common, the latter two only occasionally sampled. The other caseless caddis collected were Rhyacophila dorsalis, Polycentropus flavomaculatus, Plectrocnemia conspersa and occasionally a Psychomyid sp..

As the Hydropsyche spp. were far more numerous, special attention has been paid to their distribution, limitations and tolerances. Tables 4.5 and 4.6 summarise respectively the chemical data which were collected personally, and by the Water Authorities at all the sites where the Hydropsychids were found. These show comparable data for many similar sites within the survey. Individual values for chemical parameters are slightly different as Table 4.5 are individual observations as opposed to Table 4.6 where annual means are recorded.

TABLE 4.5

SITES OF OCCURRENCE OF *Hydropsyche* spp. IN DISTRIBUTION SURVEY

(Own data collected at time of biological sampling)

RIVER : SITE	Temp. °C.	p.H	D.O. mgl^{-1}	5 Day BOD mgl^{-1}	Total Hardness $CaCO_3$ mgl^{-1}	Ca Hardness	Mg Hardness	Total Alk.	Cl^- mgl^{-1}	$N\text{-}NO_3$ mgl^{-1}	$N\text{-}NH_3$ mgl^{-1}	$P\text{-}PO_4$ mgl^{-1}	S.S. 1050 mgl^{-1}	Cu ppm	Cd ppm	Pb ppm	Cr ppm	Ni ppm	Fe ppm	Zn ppm
Calder Hebden Bridge	14.0	6.5	10.1	1.1	39.0	23.0	16.0	10.0	16.3		0.1	0.3	4.0	0.05	0.0	0.1	0.01	0.0	3.3	0.01
Dove, Darfield	15.0	7.15	8.25	5.3	391.0	190.0	200.0	180.0	148.0	3.75	0.6	1.5		0.06	0.015	0.35	0.04	0.175	5.8	0.06
Dearne, Broomhill	15.0	6.75	8.55	7.4	312.0	177.0	135.0	140.0	156.0	5.85	2.2	1.4		0.87	0.02	0.3	0.06	0.23	22.0	0.035
Dowles Brook	9.5	7.56	10.9	1.3	200.0	130.0	70.0	140.0	34.0	2.6	0.1	0.0	0.5	0.05	0.005	0.15	0.0	0.05	1.7	0.01
Rainworth Water	10.0	7.9	11.0	6.9	224.0	132.0	92.0	105.0	48.0	12.2	0.4	5.5	11.0	0.04	0.0	0.2	0.02	0.05	4.05	0.02
Bagthorpe Brook	13.0	7.5	9.15	4.45	270.0	151.0	119.0	160.0	34.0	2.0	0.1	0.5	12.5	0.07	0.01	0.2	0.03	0.07	2.4	0.01
Churnet, Upper Hulme	7.2	6.8	12.1	1.9	49.0	38.0	11.0	175.0	15.5	2.9	0.4									
Churnet, Bridgend	8.0	7.0	11.8	2.2	74.0			41.0	29.0	2.0	0.9		15.0							
Churnet, Abbey Green Rd	8.5	6.8		2.0	71.0	55.0	16.0	30.0	20.0	0.9	0.4	1.0	1.0							
Churnet, d/s Wardles Wks.	7.0	7.0	9.8	8.0	133.0			59.0	66.0	2.3	1.2		13.0							
Churnet, Consall	13.0	7.2	9.0	2.3	123.0			80.0	23.0	4.0	0.2		9.0							
Churnet, Froghall	14.0	6.95	9.3	4.0	158.0	118.0	40.0	70.0	24.8	4.0	0.4	0.6	12.5	0.19	0.01	0.1	0.07	0.07	5.65	0.02
Churnet, Oakamoor	12.0	6.8	10.0	3.6	172.0	124.0	48.0	60.0	22.7	3.2	0.1	0.0	13.0	0.15	0.01	0.15	0.06	0.1	4.7	0.02
Churnet, Alton	11.8	7.1	12.55	2.15	159.0	128.0	31.0	115.0	43.0	3.4	0.5	0.8	7.5							
Blythe, Cheswick Green	12.5	7.25	8.65	2.85	237.0	133.0	104.0	160.0	36.2	5.6	0.2	3.6	7.0	0.09	0.01	0.15	0.04	0.07	2.0	0.015
Blythe, Temple Balsall	16.0	8.4	14.7	4.2	306.0	156.0	150.0	185.0	40.4	3.2	0.3	0.5	7.0	0.1	0.015	0.2	0.03	0.1	1.5	0.01
Blythe, u/s Eastcote Brook	13.0	7.3	8.1	3.0	284.0	161.0	123.0	170.0	39.0	4.35	0.2	2.8	7.3	0.08	0.01	0.15	0.02	0.07	1.35	0.01
Blythe, Stonebridge	13.5	7.0	5.8	4.8	244.0	140.0	104.0	125.0	46.8	9.0	0.8	4.8		0.11	0.01	0.1	0.03	0.05	1.0	0.02
Langley Brook	12.0	7.3	9,6	0.9	349.0	215.0	134.0	190.0	41.8	3.55	0.2	2.8	6.5	0.07	0.01	0.2	0.04	0.05	1.05	0.01
Bourne, Over Whitacre	14.0	7.9	10.1	2.6	251.0	204.0	47.0	211.0		11.2			25.0	0.02	0.003	0.03		0.03		0.01
Bourne, Daw Mill Bridge	12.5	7.55	12.4	2.8	368.0	296.0	72.0	210.0	41.8	11.7	0.1	1.1	5.5	0.07	0.02	0.25	0.03	0.1	4.9	0.01
Anker, Leathermill Bridge	16.0	7.42	10.3	5.65	323.0	216.0	107.0	250.0	320.0	5.8	13.8	6.3	15.0	0.09	0.04	0.3	0.11	0.27	0.9	0.04
Ouse, Fullwell Bridge	9.0	8.0	17.7	2.1	184.0	171.0	13.0	290.0	33.0		0.2	0.2	4.0	0.18	<0.01	<0.01	<0.01	<0.01		0.01
Tove, Bozenham Mill	9.5	7.9	16.25	0.5	357.0	305.0	52.0	220.0	34.2	7.0	0.5	0.0	27.0	0.12	<0.01	<0.01	<0.01	<0.01		0.01
Tove, Coppenham Bridge	9.5	8.0	13.2	0.1	342.0	316.0	26.0	250.0	31.0	7.0	0.4	1.0	4.0	0.06	<0.01	0.015	0.01	<0.01		0.01
Clipstone Brook	9;0	7.8	13.3	0.9	197.0	175.0	22.0	225.0	40.0		0.3	0.8	7.0	0.06						
Ouse, Milton Ernest	9.8	7.85	10.5	2.25	178.0	166.0	12.0	210.0	48.0		0.4	0.2	5.0	0.1						
Claydon Brook, Winslow	10.0	7.8	11.2	0.6	188.0	173.0	15.0	290.0	41.0		0.3	0.4	5.0	0.06						
Claydon Brook, Padbury	11.0	8.2	15.8	2.7	196.0	181.0	15.0	235.0	45.0		0.1	0.2	1.0	0.14	<0.01	0.02	<0.01	<0.01		0.02
Ouzel, Standbridge Ford	10.5	7.65	13.5	4.3	176.0	167.0	9.0	290.0	82.5		1.6	4.4	15.0	0.12	<0.01	<0.01	0.01	0.02		0.05
Ouse, Olney	10.0	7.6	10.9	1.75	348.0	318.0	30.0	215.0	47.5	7.7	0.4	1.3	33.5	0.06	0.0	0.2	0.01	0.006		0.02
Ouse, Ravenstone Mill	9.5	7.8	10.65	2.5	346.0	322.0	24.0	230.0	46.5	8.2	0.6	1.3	3.0	0.05						
Ouzel, Caldecote Mill	8.0	7.5	10.15	1.95	334.0	298.0	36.0	165.0	63.5	9.8	1.9	3.0	24.0	0.08						
Ouzel, Willen	12.0	8.12	9.53						58.7	9.0	0.32	1.56		0.01	<0.01	0.01	<0.01	<0.01		0.01
Ouzel, Simpson	9.0	8.0	14.1	1.95	Inter-ference	296.0		225.0	5.6	9.6	0.1	2.6	7.5	0.16	<0.01	0.02	<0.01	0.01		0.0
Warm Brook, u/s Chapel-en-le-Frith	10.0	7.85	10.23	1.83	143.0	118.0	25.0	77.5	26.2	1.6	0.1	0.8	18.0	0.08	0.01	0.15	0.04	0.07	7.7	0.01
Roych Brook	12.0	7.4	10.1	3.7	143.0	120.0	23.0	85.0	53.2	1.4	0.1	7.4	17.0	0.04	0.055	0.25	0.04	0.1	6.2	0.025
Sett, Hayfield	12.0	6.55	10.3	1.6	70.0	52.0	18.0	22.5	13.5	0.6	0.2	0.7	15.0	0.05	0.005	0.4	0.03	0.05	4.0	0.02
Sett, New Mills	12.5	6.7	9.98	2.43	79.0	55.0	24.0	30.0	13.5	1.0	0.2	1.0	12.0	0.05	0.01	0.1	0.03	0.07	6.95	0.01
Bollin, Langley	10.5	7.5	11.4	1.6				57.6	20.0	0.92	0.18	0.13	7.0	0.04	0.025	0.0	0.02	0.05	0.4	0.01
Bollin, Jarmin	16.5	7.4	8.75	2.05	135.0	100.0	35.0	85.0	22.5	1.2	0.2	0.2	12.5	0.04	0.01	0.1	0.02	0.07	1.6	0.005
Mobberley Brook	13.0	7.0	4.85	4.4	209.0	143.0	66.0	220.0	45.5	1.2	9.2	2.9	17.0	0.14	0.02	0.25	0.02	0.1	7.15	0.02

SITES OF OCCURRENCE OF *Hydropsyche spp.* IN DISTRIBUTION SURVEY

PARAMETERS annual means from Water Authority Data (1979-80).

RIVER : SITE	Temp. °C.	p.H.	D.O. mgl^{-1}	5 Day BOD mgl^{-1}	Total Hardness $CaCO_3$ mgl^{-1}	Total Alk. mgl^{-1}	Cl^- mgl^{-1}	NO_3 mgl^{-1}	NO_2 mgl^{-1}	Total N mgl^{-1}	$N\text{-}NH_3$ mgl^{-1}	$P\text{-}PO_4$ mgl^{-1}	S.S. mgl^{-1}	Cu ppm	Cd ppm	Pb ppm	Cr ppm	Ni ppm	Fe ppm	Zn ppm
Hebden Bridge	8.9	6.5	11.5	1.2	34.0	10.3	21.2	0.93	0.01		0.14	0.04	7.5							
Dove, Darfield	10.7	7.8	10.5				169.5	5.5	0.21		0.98		126.4	0.03	0.01	0.03	0.12	0.04	3.4	0.04
Dearne, Broomhill	11.5	7.6	8.8		424.1	158.1	287.3	8.1	0.54		2.2	1.1	85.2	0.02	0.76	0.01	0.01	0.03	3.6	0.05
Ewden Beck	9.0	7.0	10.4				20.3	1.7	0.1		0.9		9.0	0.05	0.01	0.07	0.05	0.04	1.1	0.11
Seven	8.4	7.5	9.8		88.9	49.8	16.5	1.2	0.03		0.09	0.04	15.5							
Wharfe Tadcaster	10.9	7.8	10.8		169.6	111.4	18.0	2.09	0.03		0.09	0.08	13.5	0.01	0.72	0.01	0.002	0.005	0.48	0.02
Wharfe Boston Spa	10.0	7.9	11.6		158.8	117.5	15.4		0.03		0.18	0.08	19.2							
Wharfe Harewood	9.5	7.8	11.2		141.7	106.1	14.6		0.03	1.55	0.15	0.08	14.9							
Wharfe Bolton Bridge	9.3	7.9	11.5		129.5	110.4	11.3	1.0	0.05		0.05	0.02	8.6							
Wharfe Burnsall	8.6	7.9	11.6	0.5	144.9	129.3	11.5	1.03	0.05		0.07	0.03	5.6							
Dowles Brook	6.4	7.6	11.8	2.4	204.0	101.0	37.8			1.57	0.06		8.8							
Rainworth, Water Ollerton	9.5	7.0	10.3	7.8	365.5	99.5	401.3			1.43	4.27		57.0							
Bagthorpe Brook	8.3	7.6	11.9	2.6	270.6	142.3	122.0			0.89	0.01		5.0							
Churnet, Upper Hulme	7.7	7.0	11.0	2.2	48.9	22.0	19.9			1.17	0.18		32.38							
Churnet, Bridgend	7.3	7.1	11.5	1.9	70.3	41.6	25.7			1.83	0.50		7.7	0.01	0.01	0.01	0.01	0.01	0.48	0.01
Churnet Abbey Green Road	8.5	7.25	10.49	2.3	81.0	43.7	28.8			1.54	0.55	0.08	24.0	0.01	0.01	0.01	0.01	0.01	0.01	0.03
Churnet d/s Wardles Wks.	10.6	7.0	9.8	6.5	107.3	62.7	71.0			2.73	0.85		41.4							
Churnet, Consall	9.5	6.9	10.0	3.6	114.6	64.6	38.8			3.2	0.05		37.0							
Churnet, Froghall	9.1	7.0	10.4	3.7	121.7	66.5	44.8			3.25	0.48		47.6							
Churnet, Oakamoor	9.4	7.1	10.6	3.5	140.5	64.7	40.4			3.32	0.42		50.6							
Churnet, Alton	9.2	7.1	10.6	3.2	137.5	66.3	38.5			3.28	0.44		54.0							
Blythe, Cheswick Green	9.6	7.7	10.6	3.4	204.6	115.8	41.8			7.93	0.33		10.9							
Blythe, Temple Balsall	10.3	8.0	11.0	3.0	273.8	163.8	46.0			5.12	0.18		9.2							
Blythe, u/s Eastcote Brook	9.9	7.6	9.5	3.5	236.2	140.8	48.2			8.09	1.06		14.4	0.03	0.01	0.01				0.05
Blythe, Stonebridge	10.2	7.7	9.2	3.1	242.7	142.8	50.8			8.17	0.9		12.6							
Langley Brook																				
Bourne, Over Whitacre	9.62	7.7	9.9	1.6	322.1	204.0	67.5			11.43	0.15		18.8	0.02		0.03		0.01	0.03	
Bourne, Daw Mill Bridge																				
Anker, Leathermill Bridge	10.0	7.6	10.4	3.4	346.0	205.3	145.4			7.21	1.05	2.54	17.5	0.01	0.01	0.01	0.02	0.02		0.03
Ouze, Fullwell Bridge	9.7	8.1	10.2	2.4			39.8	9.1			0.11			0.01	0.01	0.01	0.01	0.01		0.01
Tove, Bozenham Mill	11.4	8.2	10.3	2.8			38.6	8.4			0.11			0.01	0.01	0.01	0.01	0.01		0.01
Tove, Coppenham Bridge	11.3	8.1	10.7	3.1	127.0	197.7	40.2	7.8			0.14	0.95								
Clipstone Brook																				
Ouse, Milton Ernest																				
Claydon Brook, Winslow																				
Claydon Brook, Padbury	9.8	8.1	10.3	2.8	143.0	198.8	50.5	9.6			0.2	3.5		0.01	0.01	0.01	0.01	0.01	0.26	0.01
Ouzel, Stanbridge Ford	10.7	8.0	8.3	5.2	144.0	224.3	78.1	11.8			1.63	3.2		0.01	0.01	0.01		0.01	0.14	0.04
Ouse, Olney	12.4	8.2	10.5	2.6	146.0	211.2	49.3	8.8			0.15	1.05					0.01			
Ouse, Ravenstone Mill	12.4	7.1	9.8	3.1			40.8	7.8			0.22									
Ouzel, Caldecote Mill																				
Ouzel, Willen	12.0	8.1	9.5				58.7	9.1			0.32	1.56		0.01	0.01	0.01	0.01	0.01		0.01
Ouzel, Simpson	9.3	8.2	9.0	3.2			50.0				9.93									
Warm Brook u/s Chapel-en-le-Frith																				
Roych Brook																				
Sett, Hayfield	15.0	7.8	9.9	0.9		25.0	24.0	1.1	0.02		0.05	0.10	5.0							
Sett, New Mills	9.7	7.3	10.9	2.1		42.0	20.0	1.41	0.02		0.09	0.20	12.4							
Bollin, Langley	10.5	7.5	11.4	1.6		57.6	20.0	0.92	0.05		0.18	0.13	7.0							
Bollin, Jarmin	10.5	7.5	11.4	1.6		57.6	20.0	0.92	0.05		0.18	0.13	7.0							
Mobberley Brook	10.5	7.3	7.1	3.5		195.0	42.0	2.25	0.18		5.05	1.10	19.0							

The distribution of the other major taxonomic groups in the survey deserves mention. For example, at only nine out of seventy-seven stations were no oligochaetes recorded. Naids may have been present in some of these samples, but having been preserved in formaldehyde these may not have been apparent. Neither oligochaetes or chironomids are favourable groups to be used as indicator organisms as they are too tolerant and ubiquitous. This view is reflected in their lowly position in the Trent Biotic Index and Chandler Score classification systems. Tubificid species are associated with the effects or organic enrichment, for example on the R. Dane at Biddulph, downstream of a water treatment works there were 260 per 0.05 m^2. Similarly on the R. Tean at Beamhurst where organic effluent from a water treatment works, a dairy and a farm enrich the river, the density rises dramatically.

When waters become organically enriched, depositing silt, the growth of heterotrophic aerobes is promoted. This leads to an increased food supply for the worms. Simultaneously the BOD increases, and dissolved oxygen tensions are lowered, thus conditions may become critical for other invertebrate competitors or predators.

Hynes (1971) has shown tubificids to be far more tolerant of phenols and cyanides than many other macroinvertebrates.

The blanketing effect of inert matter has been suggested to suppress most other macroscopic invertebrates, leaving the habitat inhospitable for everything but tubificids. If a stream is contaminated by oil pollution then this may inhibit oviposition by insects. Any combination of these factors may lead to an invertebrate assemblage being dominated by tubificids.

Often Chironomids are associated with the tubificids in very polluted situations. This was true at the heavily polluted sites on the River Tame u/s Blue Billy Tip and in Ford Brook.

Chironomids are the most abundant and widely distributed representatives of the Diptera in the survey, and were found at some sites in numbers exceeding 1000 per m^2. For the purpose of this survey, they were classified as green and red chironomids (reds being C. riparius). This red "blood worm" was most frequently recorded at stations on the River Calder, namely Mirfield and Huddersfield. Due to their high tolerance of environmental conditions such as those encountered immediately below a sewage works, C. riparius is often the most abundant member of the chironomid assemblage.

High incidences of green chironomids (mainly Orthocladiinae, Tanytarsini and Microspectra sp.) are usually associated with either organic enrichment, or slow flowing stretches of river with a silty substratum. The latter situation was true at Rainworth Water, Ollerton. The silty substratum is often a result of man-made conditions, and a canalised section of the R. Erewash at Pinxton and the R. Maun at Edwinstowe are examples of the effect on the fauna in such a situation. The impoverished community at these stations is reflected by low scores on the biotic indexes, the full assemblage consisting of Oligochaetes, Chironomids and Asellus aquaticus.

In less polluted instances, e.g. R. Churnet: Oakamoor, R. Blythe: Temple Balsall, the high densities of green chironomids are associated with the Cladophora growth over gravels of the substratum, the blanket weed providing an hospitable habitat for the larvae. Chironomid distribution generally seems to be influenced more by the changes in substratum rather than current velocity or small changes in water quality. A large increase in the numbers of chironomids reflects a gross deterioration in water quality, but their use as indicators is limited. Organic enrichment will often cause high population densities of a single species. These often become "nuisance organisms" when "midge swarms" rise from the river.

The Hirudinea in the survey were represented by Glossiphonia complanata, Erpobdella octoculata, Erpobdella testacea, Hellobdella stagnalis and Piscicola geometrica. Leeches were recorded at 36 of the stations, but numbers per square metre never exceeded 20 except at R. Churnet, Cheddleton Station where a density of 500 m^2 was recorded. Their distribution appears to be closely associated with that of their food organisms. G. complanata feeds mainly by sucking body fluids of molluscs, whilst Erpobdellae feed on a variety of insect larvae and oligochaete worms. P. geometrica as its name implies is found at stations where there are suitable fish as hosts.

Bryce et al. (1978) reports that G. complanata was recorded from chemical Class 1 and 2 water qualities but in this survey they were found throughout the whole range, e.g. Class 1 = Clipstone Brook; Class 2 = R. Ouzel, Willen; Class 3 = R. Dearne, Broomhill and Class 4 = R. Churnet d/s Wardles Chemical Works. Erpobdellids appear to be consistently more closely correlated with higher water qualities, hardly surprising in view of their dietary requirements. P. geometrica has been recorded in the R. Ouzel at Stanbridge Ford and R. Tean at Checkleybank, both of which sites are known to be supporting good fish populations.

Throughout the survey a varied molluscan fauna was encountered including Ancylus fluviatilis, Potamopyrgus jenkinsi, Limnaea pereger, Planorbis sp., Valvata sp., Physa sp., Sphaerium sp., Pisidium sp. and one find of Anodonta sp. Distributed throughout all the classes of water quality was L. pereger(L.p) probably one of the most tolerant species of freshwater snail. Other very abundant species were A. fluviatilis (A.fl.), P. jenkinsi (P.j) and Sphaerium sp. (Sph.). Particularly high densities of snails were recorded at the following stations:

Claydon Brook, Padbury - P. jenkinsi

R. Ouse, Olney - P. jenkinsi, L. pereger, Valvata and Sphaerium

R. Ouse, Simpson - P. jenkinsi, L. pereger and Sphaerium

R. Devon, Hawton - P. jenkinsi, Planorbis, Valvata and Pisidium

R. Blythe, Henwood Mill - A. fluviatilis, P. jenkinsi, Sphaerium and Pisidium

R. Poulter - P. jenkinsi

The one find of Anodonta on the R. Ouse at Ravenstone Mill was rather out of character as this species is usually associated with slow flow conditions. The sampling station here was man made stretch of river with high banking and relatively deep slow flowing water. Other species common in lowland situations were absent in this survey as would be expected, thus species such as Bythinia, L. stagnalis and Unio are not recorded.

The next major group to be considered are the Crustacea. Three species were recorded in this survey, Asellus aquaticus, Gammarus pulex and Astacus pallipes. A. pallipes was found in very high water quality at Langley Brook, Middleton. This was not a common species in the survey, but from personal experience it has been sampled on other occasions in the R. Severn and in the stream in Bradgate Park, Leicester, both of water quality Class 1. Generally it is difficult to sample due to a very rapid and effective escape reaction. The isopod A. aquaticus is more widespread than the amphipod G. pulex, being present at stations of all four water qualities, e.g. R. Blythe, u/s Eastcote Brook, R. Ouzel, Willen, Mobberley Brook and R. Dove, Darfield representing Class 1 - 4 respectively.

The increase in abundance is generally associated with a decrease in water quality and often a decrease in water velocity may also be well tolerated. In contrast G. pulex favour cleaner waters in true riffle situations where gravel and stony substratum offers suitable

niches for concealment from predators with fast flowing and well oxygenated waters.

High population densities of G. pulex were recorded at Dowles Brook, R. Blythe, Stonebridge, R. Ouse, Milton Ernest, R. Bourne, Fillongley and R. Tean, Checkleybank, all sites of Class 1 or 2 water quality.

Rhyacophila sp. are often associated with communities comprising relatively large numbers of G. pulex together with species of mayfly and stonefly, for example R. Churnet, Upper Hulme and R. Calder, Hebden Bridge. These are generally classic riffle habitats with high water quality.

When proportionally more A. aquaticus than G. pulex are present in the invertebrate assemblage Hydropsyche spp. are more commonly encountered. These are usually sites with decreased water quality, characterised by only Baetis representing the mayfly group. Examples of such sites include R. Tove, Bozenham Mill and R. Goyt, Otterspool Bridge.

Further analyses of caseless caddis associations are undertaken in the following section.

4.2.2. PROCESSING OF RESULTS

Initially manual processing of data was carried out to calculate biological indexes and scores in an attempt to extract information on specific variables which affected Trichopteran distribution. To further the work (on the data collected in the field survey) the data were re-written into a matrix format suitable for computer analysis. Data were punched and stored on the I.C.L. 1904S computer. Major matrices comprised raw field data and corresponding data from the Water Authorities from the sites visited in the survey. Ecological data of this type are usually non-normally distributed, so for the biological figures a log $x + 1$ transformation was performed. The raw and transformed data could then be compared if required (Snedecor and Cochran 1967, p.193).

Once in matrix form the data may be manipulated but the original size of the matrix was too large for the computer to handle. Deletion of variation (sites) having less than five entries was performed leaving a 61 x 61 matrix which could be edited and altered as required.

Correlation coefficients were calculated and significant values extracted for all chemical parameters and biological taxa. Co-efficients of determination were calculated for each data matrix, but upon examination of the two sets of data, comparing raw and transformed matrices the conclusions were not dramatically different, i.e. the same parameters were still important.

The survey data were processed to examine how the distribution of Trichoptera were related to:

a. Chemical Classification of Rivers

b. Relationship with specific chemical analytic data

c. Community structure

d. Indicator value of water quality

4.2.3. RELATIONSHIP WITH CHEMICAL CLASSIFICATION OF RIVER (D of E)

To compare the presence/absence of Hydropsyche spp. and Rhyacophila sp. with the D.of E. Chemical Class, x^2 (chi. square) tests were applied. Tables 4.7 and 4.8 show Water Authority and Aston Data for Hydropsyche spp. followed by the results for Rhyacophila sp. in Table 4.9.

Table 4.7 x^2 test for Hydropsyche spp. (Water Authority Data)

Chemical Class	1	2	3	4	Total
Hydropsyche spp. present	28	14	5	2	49
Hydropsyche spp. absent	3	13	19	12	47
Total No. of sites	31	27	24	14	96
$x^2 = 35.51$ df = 3		$p < 0.001$ * * *			

Table 4.8. x^2 test for Hydropsyche spp. (Aston Data)

Chemical Class	1	2	3	4	Total
Hydropsyche spp. present	14	12	4	0	30
Hydropsyche spp. absent	7	13	17	10	47
Total No. of sites	21	25	21	10	77
$x^2 = 17.51$ df = 3		$p < 0.001$ * * *			

From both sets of data the relationship between Hydropsyche spp. distribution and chemical class is highly correlated.

Table 4.9 x^2 test for Rhyacophila sp. (Aston Data)

Chemical Class	1	2	3	4	Total
Rhyachophila present	9	2	0	0	11
Rhyacophila absent	12	23	21	10	66
Total No. of sites	21	25	21	10	77
$x^2 = 19.96$ df = 3		$p < 0.001$ * * *			

It was concluded that the distribution of Rhyacophila was also related to river water quality as assessed by D. of E. classification

4.2.4. RELATIONSHIP WITH SPECIFIC CHEMICAL

Firstly the data were processed in the way first used by Dr. R. Abel (Private Communication) for processing field data on mayfly distributions.

Essentially, for each site where a species was recorded, the raw chemical data were extracted and converted into annual mean values. From these values the median value was calculated, together with the 95% confidence limits and the 5% and 95% percentiles. Percentiles were calculated by ranking the variates and then calculating the probability plotting point using the Hazen factor ($f = \frac{100}{n + 1}$). Graphs are plotted on log. probability paper, and the percentiles at the 5% and 95% read off. The values are calculated for temperature, dissolved oxygen, BOD and hardness and are shown on Tables 4.10, 4.11, 4.12 and 4.13. Calculations showed the mean and median values to be so close that it is valid to assume that the data are normally distributed. Using an F-test of Equality of Variance (Snedecor & Cochran, 1978) it can be assessed if there is any significant difference between values. The results of these statistical tests are given in Table 4.14.

Individual Parameters:

a. Temperature

From Table 4.10 there appears to be little difference in distribution when pairs of values are tested in relation to temperature for all Hydropsychids and for individual species using this particular test, thus demonstrating the group are distributed over a wide range of temperature.

b. Dissolved Oxygen (Table 4.11)

There is a significant difference between the overall values for dissolved oxygen in all the sample sites and those at which H. angustipennis, H. pellucidula and R. dorsalis are present.

TABLE 4.10 TEMPERATURE °C

Stations	n	Range	Mean	Median	S.D.	95% C.L.	5% + 95% Percentiles
All Stations	80	6.4 - 16.7	10.02	9.9	1.848	9.9 ± 0.41	7.6 13.0
Hydropsyche spp.	41	6.4 - 15.0	9.92	9.7	1.50	9.7 ± 0.47	7.8 13.0
H. angustipennis	14	6.4 - 12.4	10.13	10.1	1.61	10.1 ± 0.85	7.4 14.5
H. pellucidula	9	6.4 - 11.3	9.48	9.5	1.34	9.5 ± 1.03	8.2 11.5
H. instabilis	2	9.5 - 9.6	9.55	9.55	0.07	9.55 ± 1.98	7.3 17.0
H. siltalai	7	8.3 - 15.0	10.13	9.54	2.29	9.45 ± 2.13	7.5 12.0
R. dorsalis	31	6.4 - 15.0	9.51	9.4	1.495	9.4 ± 0.55	7.6 11.4
P. flavomaculatus	19	6.4 - 15.0	9.66	9.7	2.027	9.7 ± 0.97	6.7 13.0
P. conspersa	2	8.3 - 9.9	9.25	9.25	0.919	9.25 ± 8.25	6.6 12.5
Psychomyiidae	6	8.3 -15.0	9.92	9.9	1.491	9.9 ± 1.56	8.7 11.2

TABLE 4.11 DISSOLVED OXYGEN mgl^{-1}

Stations	n	Range	Mean	Median	S.D.	95% C.L.	5% + 95% Percentiles
All Stations	77	3.3 - 11.8	9.93	10.30	1.49	10.3 ± 0.33	8.4 12.0
Hydropsyche spp.	41	7.1 - 11.9	10.39	10.50	0.992	10.5 ± 0.31	8.8 12.0
H. angustipennis	14	9.2 - 11.8	10.50	10.45	0.717	10.45 ± 0.41	9.4 12.3
H. pellucidula	9	9.2 - 11.8	10.34	10.40	0.803	10.4 ± 0.61	8.8 12.0
H. instabilis	2	10.0 - 10.6	10.3	10.30	0.424	10.3 ± 3.79	8.8 12.0
H. siltalai	7	9.6 - 11.8	10.29	10.04	0.735	10.04 ± 0.68	9.0 11.3
R. dorsalis	30	9.24- 11.7	10.65	10.75	0.738	10.75 ± 0.27	9.4 12.0
P. flavomaculatus	19	7.9 - 11.8	10.75	10.9	0.975	10.9 ± 0.46	9.4 12.2
P. conspersa	2	9.5 - 11.8	10.65	10.65	1.626	10.65 ± 14.6	9.4 12.2
Psychomyiidae	6	9.2 - 11.7	10.76	10.9	0.954	10.9 ± 0.99	9.2 13.0

c. Biochemical Oxygen Demand (Table 4.12)

As one would expect this reflects dissolved oxygen results to a certain extent. Significant values were calculated for Hydropsychids in total, H. angustipennis, P. flavomaculatus and Psychomyiidae.

d. Total Hardness (Table 4.13)

This parameter was the final one to be calculated. Significantly different values were recorded for Hydropsyche spp., H. pellucidula and Psychomyiidae.

This processing method was finished at this point, as it was felt to be of only limited use in showing pointers to the key factors influencing caddis distribution. As success depended upon the number of sample sites at which a species was recovered, this may lead to a limited data base, and a rather spurious interpretation.

4.2.5. GRAPHICAL PRESENTATION

An alternative representation of the same data came from attempting to plot the parameters graphically. Dissolved oxygen and BOD were plotted, grading the survey sites from best water quality (according to parameter) to the worst. The mean annual value at that site was recorded and plotted together with the range (Fig.4.1 and 4.2).

Linearly above, distribution of species were entered to display the range over which the species occurred.

Both methods of processing highlighted the broad range over which these trichopteran species were found, one species often overlapping another in the tolerance range.

With the computing facility available, using the large data matrix previously described, a series of correlation coefficients (r) were calculated. A total of 77 variables were compared, thus resulting in 77 - 1 = 76 degrees of freedom.

From tables, values at 80 df (the closest value for r) positive values were as follows:

TABLE 4.12 BOD mgl^{-1}

Stations	n	Range	Mean	Median	S.D.	95% C.L.	5% + 95% Percentiles
All Stations	64	0.5 -	4.05	3.45	2.25	3.45 ± 0.56	1.6 7.8
Hydropsyche spp.	32	0.5 - 7.8	2.9	2.9	1.46	2.90 ± 0.53	1.3 6.0
H. angustipennis	14	2.4 - 7.5	3.3	3.1	1.25	3.10 ± 0.72	2.4 3.85
H. pellucidula	9	2.4 - 7.4	3.71	3.5	1.44	3.50 ± 1.08	2.7 4.1
H. instablis	2	3.4 - 3.6	3.5	3.5	0.14	3.50 ± 1.25	- -
H. siltalai	6	0.9 - 7.6	3.89	3.0	2.60	3.0 ± 2.72	1.3 6.6
R. dorsalis	24	0.5 - 6.47	3.01	2.4	1.848	2.4 ± 0.77	1.0 7.2
P. flavomaculatus	15	0.5 - 3.1	2.24	2.4	0.816	2.4 ± 0.45	2.2 4.3
P. conspersa	2	2.5 - 3.5	3.0	3.0	0.707	3.0 ± 6.33	- -
Psychomyiidae	3	2.6 - 3.1	2.83	2.8	0.251	2.8 ± 0.62	1.2 3.6

TABLE 4.13 TOTAL HARDNESS mgl^{-1} $CaCO_3$

Stations	n	Range	Mean	Median	S.D.	95% C.L.	5% + 95% Percentiles
All Stations	57	30 - 779	248.0	210.0	155.02	210 ± 41.0	66 670
Hydropsyche spp.	28	30 - 420	177.2	140.0	97.11	140 ± 36.8	60 350
H. angustipennis	8	114 - 340	215.4	223.0	79.8	223 ± 66.5	115 360
H. pellucidula	8	114 - 273	183.8	170.0	62.6	170 ± 52.2	98 325
H. instabilis	2	114 - 204	159.6	159.6	63.6	159 ±571.4	- -
H. siltalai	4	81 - 270	143.2	110.0	85.7	110 ±136.3	- -
R. dorsalis	23	34 - 779	172.2	140.0	150.0	140 ± 64.7	48 370
P. flavomaculatus	15	34 - 779	190.7	140.0	179.1	140 ± 99.3	40 500
P. conspersa	2	236 - 270	253.0	253.0	24.0	253 ±215.7	- -
Psychomyiidae	6	76 - 242	149.5	145.5	53.9	145.5 ± 56.5	105 185

TABLE 4.14

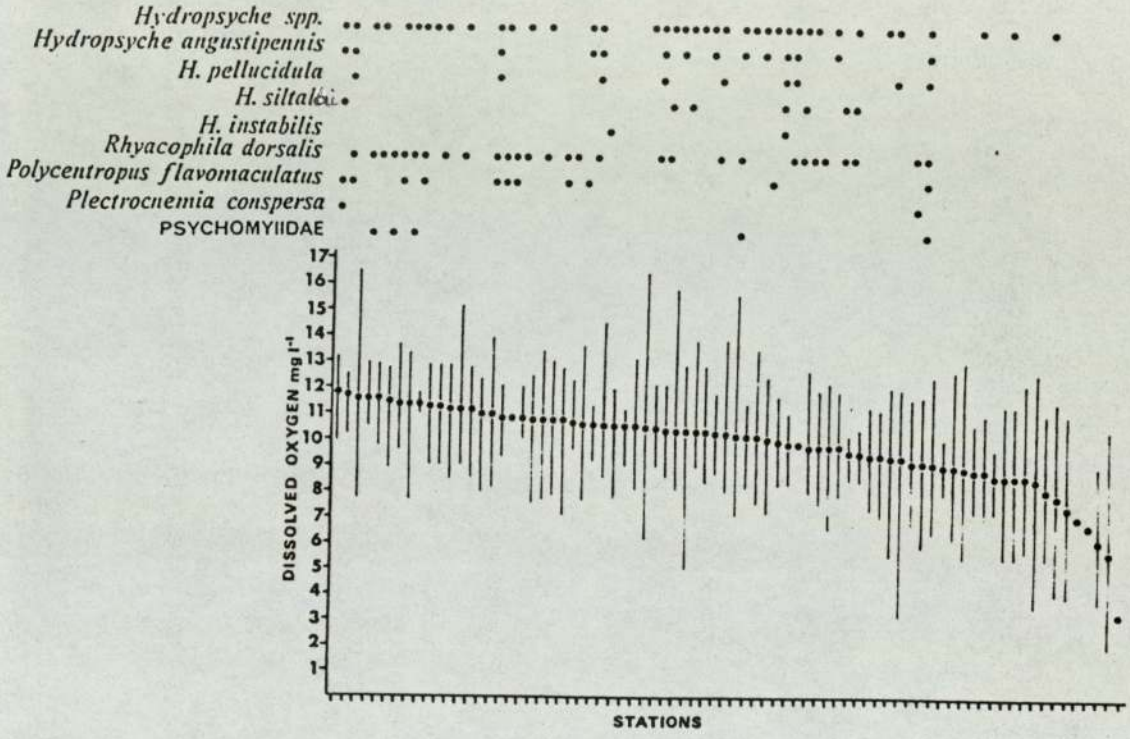
RESULTS OF TESTS OF EQUALITY OF TWO VARIANCES

PAIRS OF VALUES TESTED	PARAMETER			
	Temp.	D.O	BOD	Hardness
All Stations : *Hydropsyche spp.*	S	S	S	S
All Stations : *H. angustipennis*	N.S	S	S	N.S
All Stations : *H. pellucidula*	N.S	S	N.S	S
All Stations : *H. instabilis*	N/A	N/A	N/A	N/A
All Stations : *H. siltalai*	N.S	N.S	N.S	N.S
All Stations : *R. dorsalis*	N.S	S	N.S	N.S
All Stations : *P. flavomaculatus*	N.S	N.S.	S	N.S
All Stations : *P. conspersa*	N/A	N/A	N/A	N/A
All Stations : Psychomyiidae	N.S	N.S	S	S

S = SIGNIFICANT. N.S = NOT SIGNIFICANT

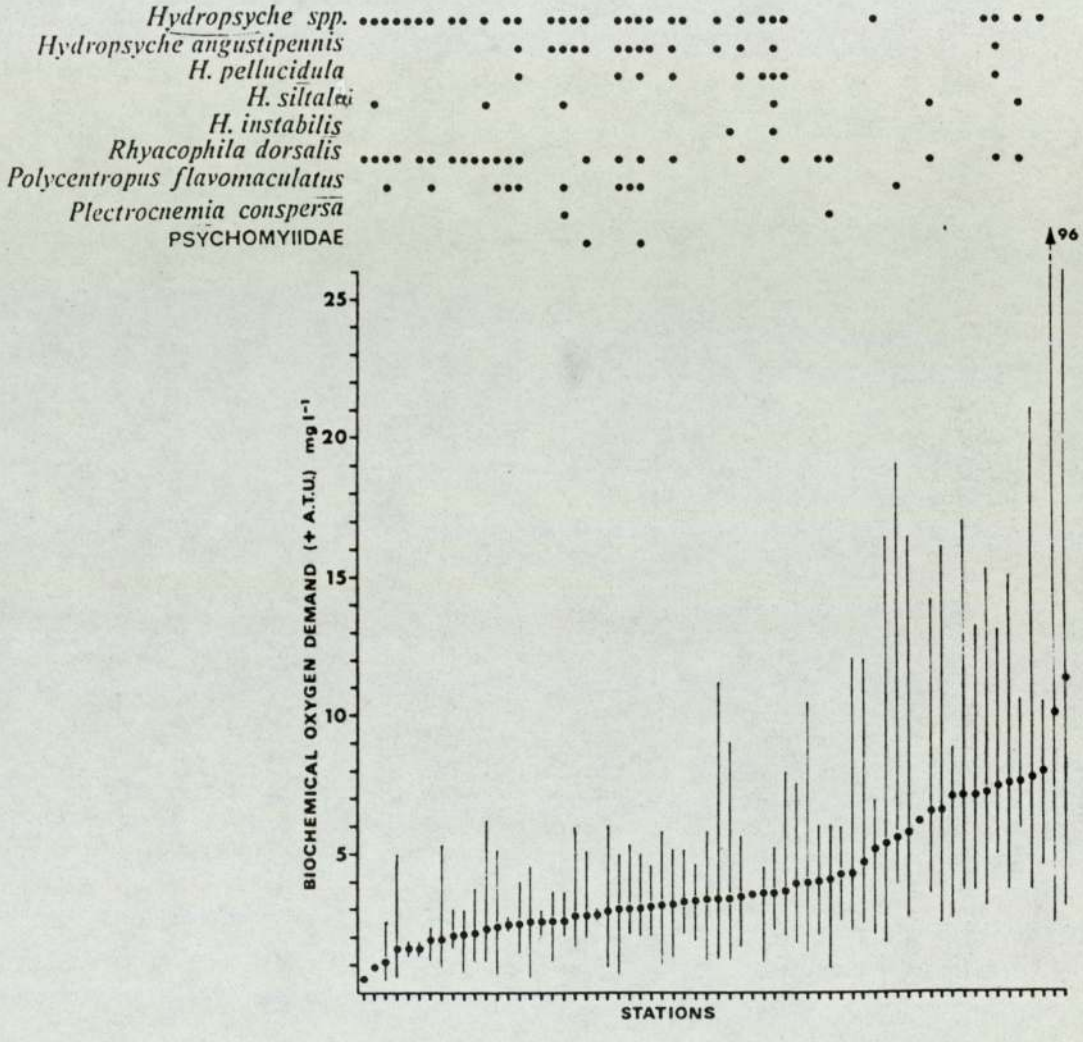
N/A = TEST NOT APPLICABLE

Fig.4.1. Graph displaying the range of dissolved oxygen levels over which species of Trichoptera were distributed.



Stations are arranged from left to right showing highest to lowest water quality, i.e. highest to lowest D.O. Central point gives mean D.O. whilst vertical line indicates range for that station.

Fig.4.2. Graph displaying the range of BOD levels over which species of Trichoptera were distributed



Stations are arranged from left to right showing highest to lowest water quality, i.e. lowest to highest B,O.D. Central point gives mean BOD whilst vertical line indicates range for that station.

0.05 = 0.232

0.01 = 0.302

0.001 = 0.380

Extracting values greater than 0.232 produced a large network of both positive and negative associations between species and chemical factors. A table of the strong association with Hydropsyche spp. is presented in Table 4.15.

Coefficients of determination (r^2) were calculated for each data matrix, i.e. how much of the variation in the y variable is attributable to the linear regression on the x value.

Finally, a series of t tests were performed to compare sites at which H. angustipennis was present v sites where it was absent (Table 4.16).

4.2.6. RESULTS

Examination of the significant positive correlations showed strong links with pH, dissolved oxygen, BOD and copper. Biological associations were strongest with the plecoptera Leuctra spp., ephemeroptera Baetis sp., E. ignita, R. semicolorata, also Ancylus fluviatilis, Hydroptilidae and Oligochaeta.

4.2.7. t-TESTS

The results from these tests substantiated the correlations. Chemical parameters of significance were once again pH, dissolved oxygen, BOD, and temperature. These factors theoretically should be the most important factors dividing sites with presence or absence of Hydropsyche sp. Again, some metals had significant values suggesting that these may warrant further investigation.

4.2.8. ASSOCIATIONS WITHIN COMMUNITIES

Using only biological data from the survey one approach to community structure is using Cluster Analysis as discussed in Chapter 2. Fig.4.3 depicts the data with chemical class and presence/absence of Trichoptera.

Group average data were used but no distinct clusters of sites cor-

TABLE 4.15. Parameters and species found to be correlated with *Hydropsyche* spp.

Temperature

pH

Dissolved Oxygen

Copper

Leuctra sp.

Baetis sp.

E. ignita

R. semicolorata

A. fluviatilis

Hydroptilidae

Oligochaeta

TABLE 4.16

t - tests on *H. angustipennis:* chemical parameters

Parameter	t	dif.	5%sig.= 1.989
1. Temperature	2.52251	75	sig.
2. pH	2.92881	75	sig.
3. D.O.	2.69052	73	sig.
4. BOD	3.65734	74	sig.
5. Total Hardness	0.234332	73	N.S
6. Total Alkalinitv	1.34266	74	N.S.
7. Chloride	0.822147	74	N.S.
8. $N\text{-}NO_3$	0.31211	68	N.S.
9. $N\text{-}NH_3$	1.84101	74	N.S.
10. $P\text{-}PO_4$	0.937905	69	N.S.
11. Suspended Solids	0.612283	61	N.S.
12. Copper	1.66912	67	N.S.
13. Cadmium	1.35975	63	N.S.
14. Lead	2.72096	63	Sig.
15. Chromium	2.2314	62	Sig.
16. Nickel	2.28293	63	Sig.
17. Iron	1.38032	53	N.S.
18. Zinc.	1.81658	63	N.S.

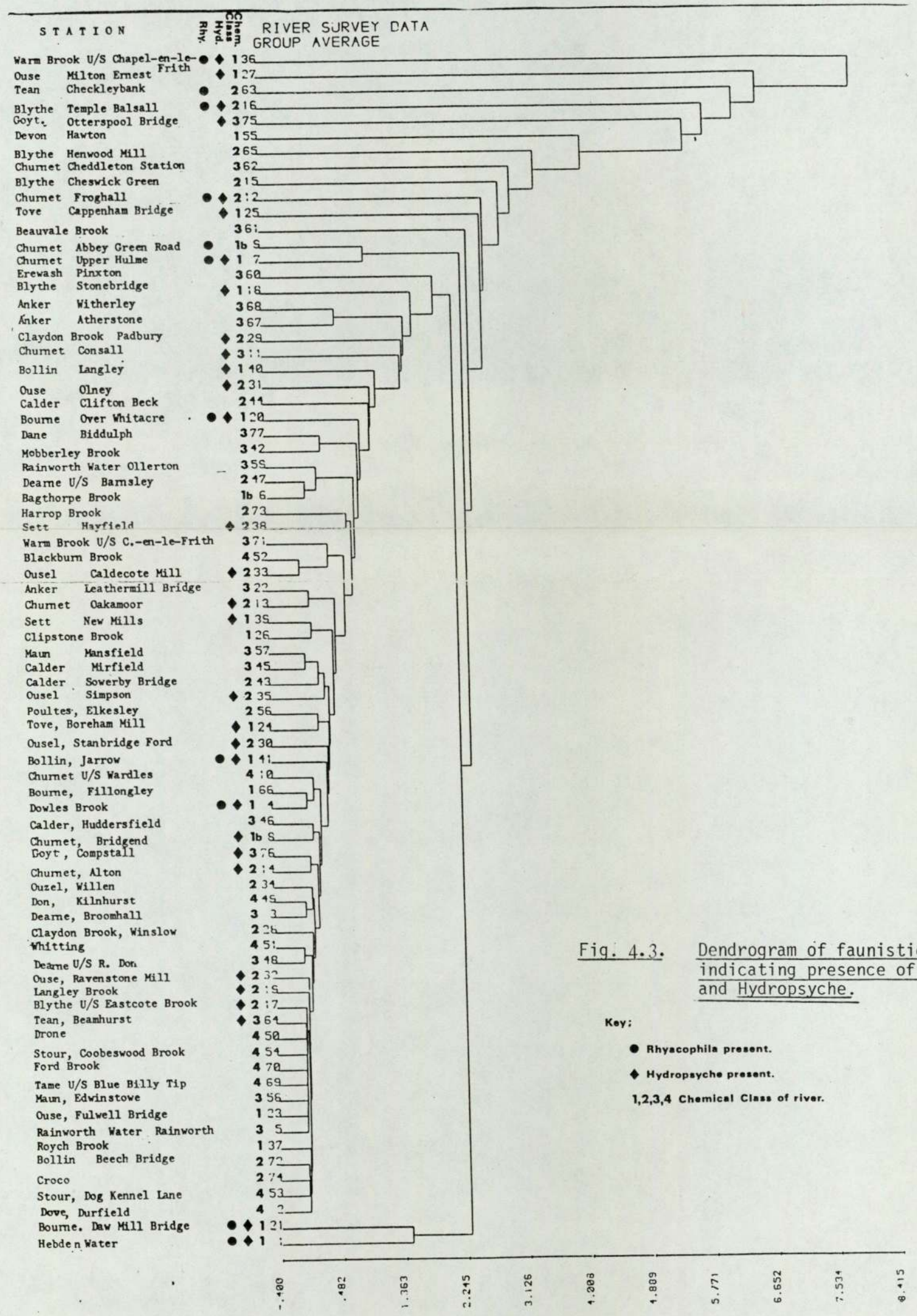


Fig. 4.3. Dendrogram of faunistic similarities indicating presence of Rhyacophila and Hydropsyche.

Key:

● Rhyacophila present.

◆ Hydropsyche present.

1,2,3,4 Chemical Class of river.

relating with water quality and Trichopteran distribution were apparent. The merging and overlapping of sites of different water qualities and faunal assemblages is due to a combination of both biological interactions with physico-chemical parameters. Often, the association of one station with another biologically, will be obscured by some strong environmental parameter. This method is helpful to provide an objective classification of data but cannot express fully the complexities of the relationships between all members of the community when reduced to a two-dimensional format.

To summarise, Rhyacophila appear less tolerant of adverse changes in water quality than Hydropsyche.

There are no Trichoptera recorded at stations with class 4 water quality due to the poor chemical conditions prevailing at these sites and resultant inhospitable environment.

At Chemical Class 3, only Hydropsychids are recorded, the stations on the R. Goyt at Otterspool Bridge and Compstall, R. Tean at Beamhurst and R. Churnet at Consall are similar in that the first three share a BMWP score of 15, TBI of V and a Diversity Index between 1.16 - 2.14. Consall on the Churnet scores higher on all biological assessments, perhaps because it has a higher faunal diversity but very few individuals of each taxon.

All of these stations share associations of Hydropsyche spp. with Asellus, Chironomids and Tubificids. These species are well documented to be rather tolerant of poor water quality. Hydropsyche spp. at each of these stations benefit from rapid water velocity, thus satisfying their oxygen requirements and facilitating net spinning for food capture. However, these chemical and biological conditions would prove too severe for the survival of Rhyacophila sp.

At 12 out of 25 stations of Chemical Class 2 Hydropsyche spp. were present but only at two of these stations, namely Temple Balsall on

the R. Blythe and Froghall on the R. Churnet were Rhyacophila present too.

In brief, the appearance of Rhyacophila in conjunction with Hydropsyche is facilitated by an increase in the quality of water, suitable substratum and plentiful food supply for the predatory Rhyacophila larvae. The occurrence of Rhyacophila dorsalis without Hydropsyche angustipennis at Checkleybank on the R. Tean has been more closely investigated in further studies.

At stations of highest water quality, the frequency of R. dorsalis increases, but Hydropsyche spp. also benefit from improved conditions in a riffle section as illustrated by their presence on the R. Blythe, Stonebridge, R. Bollin, Langley and R. Sett, New Mills.

4.2.9. PRINCIPAL COMPONENT ANALYSIS

Data from 75 sites (variables) were processed to give the correlation co-efficients.

Eigen values for each factor were calculated but it is usual to consider only the first three factors. The percentage of variation and cumulative percentage for the first three factors are summarised below:

	Eigen value	% of Varn.	Cum. %
Factor 1	34.18732	45.6	45.6
Factor 2	11.05759	14.7	60.3
Factor 3	9.42114	12.6	72.9

Figures 4.4, 4.5, 4.5 and 4.7 are scattergrams plotted to display the similarity of the large number of variables in two dimensional form. On the vertical axis, Component 1, the maximum variation is plotted against Component 2 on the horizontal axis.

Factor 1 is often associated with large scale diversity and is often correlated with a general trend rather than a specific environmental parameter. For example, Fig.4.4 has plotted number of species at each

Key to Figures 4.4, 4.5, 4.6 and 4.7

Computer printed numbers = site

Handwritten numbers = value of parameter

No.	Site	No.	Site	No.	Site
1	Hebden Bridge	31	Olney	61	Cheddleton Station
2	Darfield	32	Ravenstone Mill	62	Checkleybank
3	Broomhill	33	Caldecote Mill	63	Beamhurst
4	Dowles Brook	34	Willen	64	Henwood Mill
5	Rainworth	35	Simpson	65	Fillongley
6	Bagthorpe Brook	36	U/S Chapel-en-le Frith	66	Atherstone
7	Upper Hulme	37	Roych Brook	67	Witherley
8	Bridgend	38	Hayfield	68	D/S Chapel-en-le Frith
9	Abbey Green Road	39	New Mills	69	Beech Bridge
10	d/s Wardles	40	Langley	70	Harrop Brook
11	Consall	41	Mobberley Brook	71	Croco
12	Froghall	42	Sowerby Bridge	72	Otterspool Bridge
13	Oakamoor	43	Brighouse	73	Iron Bridge
14	Alton	44	Mirfield	74	Biddulph
15	Cheswick Green	45	Huddersfield		
16	Temple Balsall	46	U/S Barnsley		
17	Eastcote Brook	47	U/S R. Don		
18	Stonebridge	48	Kilnhurst		
19	Middleton	49	Drone		
20	Over Whitacre	50	Whittington		
21	Daw Mill Bridge	51	Blackburn Brook		
22	Leathermill Bridge	52	Dog Kennel Lane		
23	Fulwell Bridge	53	Coombeswood Brk.		
24	Bozenham Mill	54	Hawton		
25	Cappenham Br.	55	Elkesley		
26	Clipstone	56	Mansfield		
27	Milton Ernest	57	Edwinstowe		
28	Winslow	58	Ollerton		
29	Padbury	59	Pinxton		
30	Stanbridge Ford	60	Beauvale Brook		

Fig.4.4. Scattergram to show distribution of sites in relation to numbers of species sampled

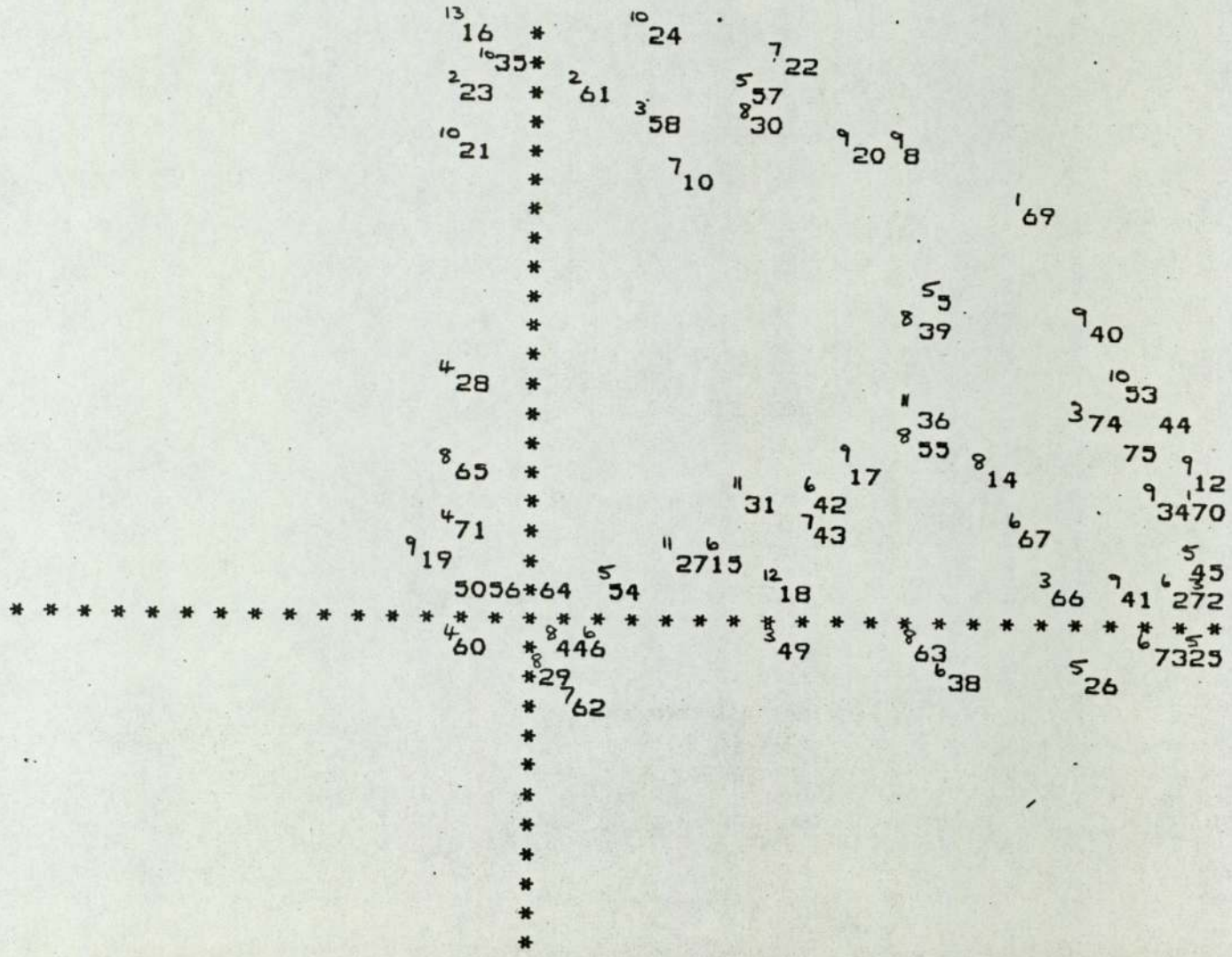


Fig.4.5. Scattergram to show distribution of sites in relation to temperature

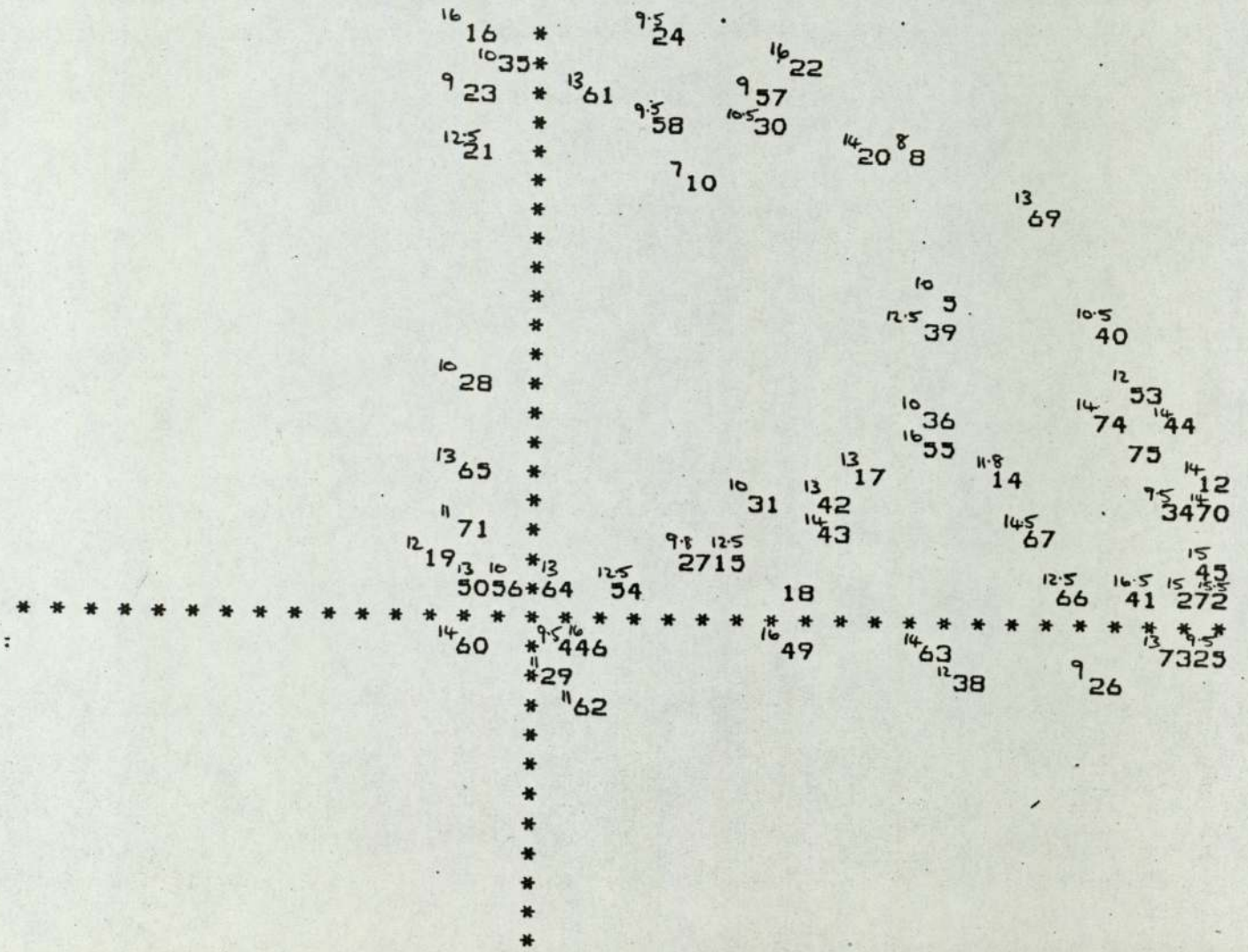


Fig.4.6. Scattergram to show distribution of sites in relation to dissolved oxygen

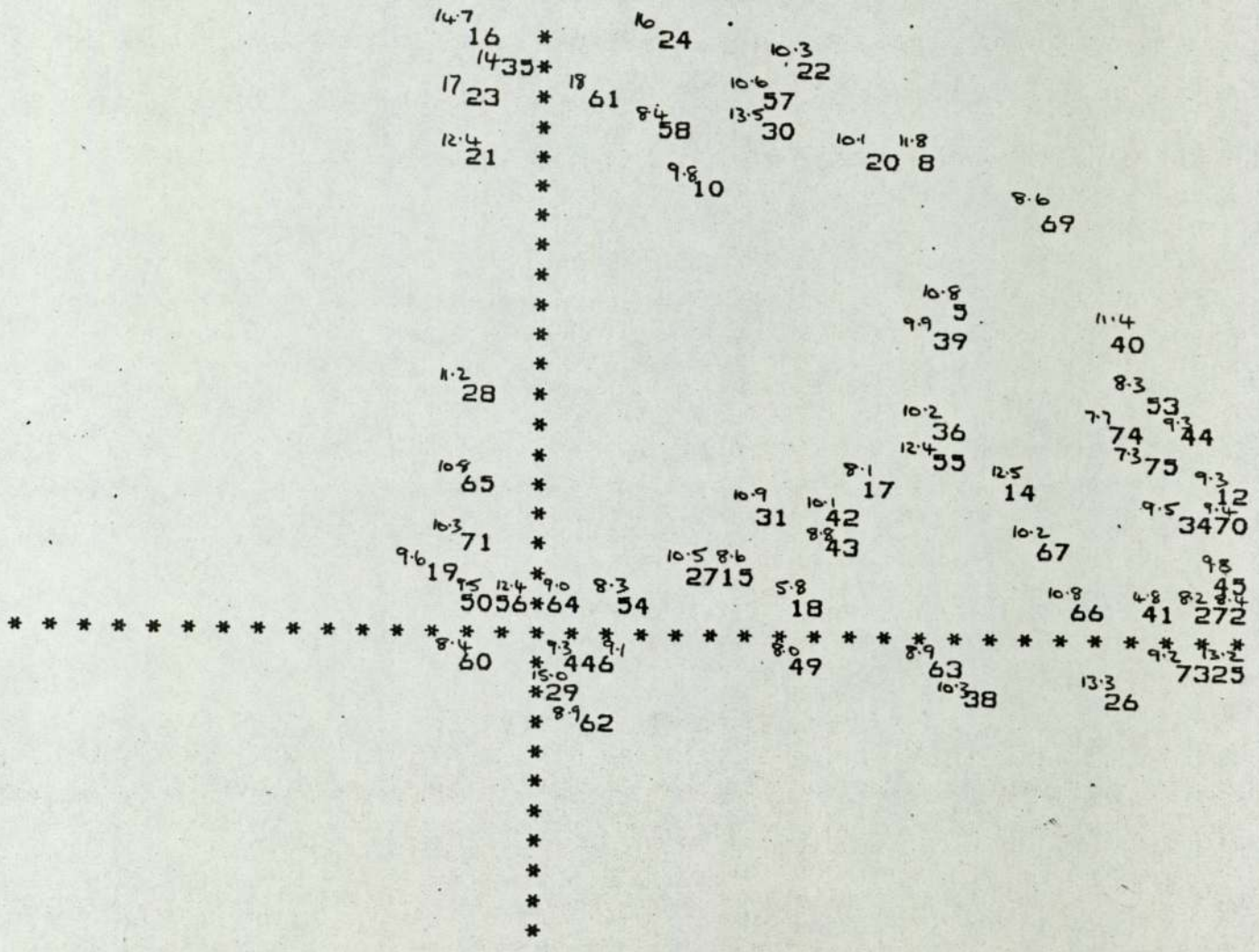
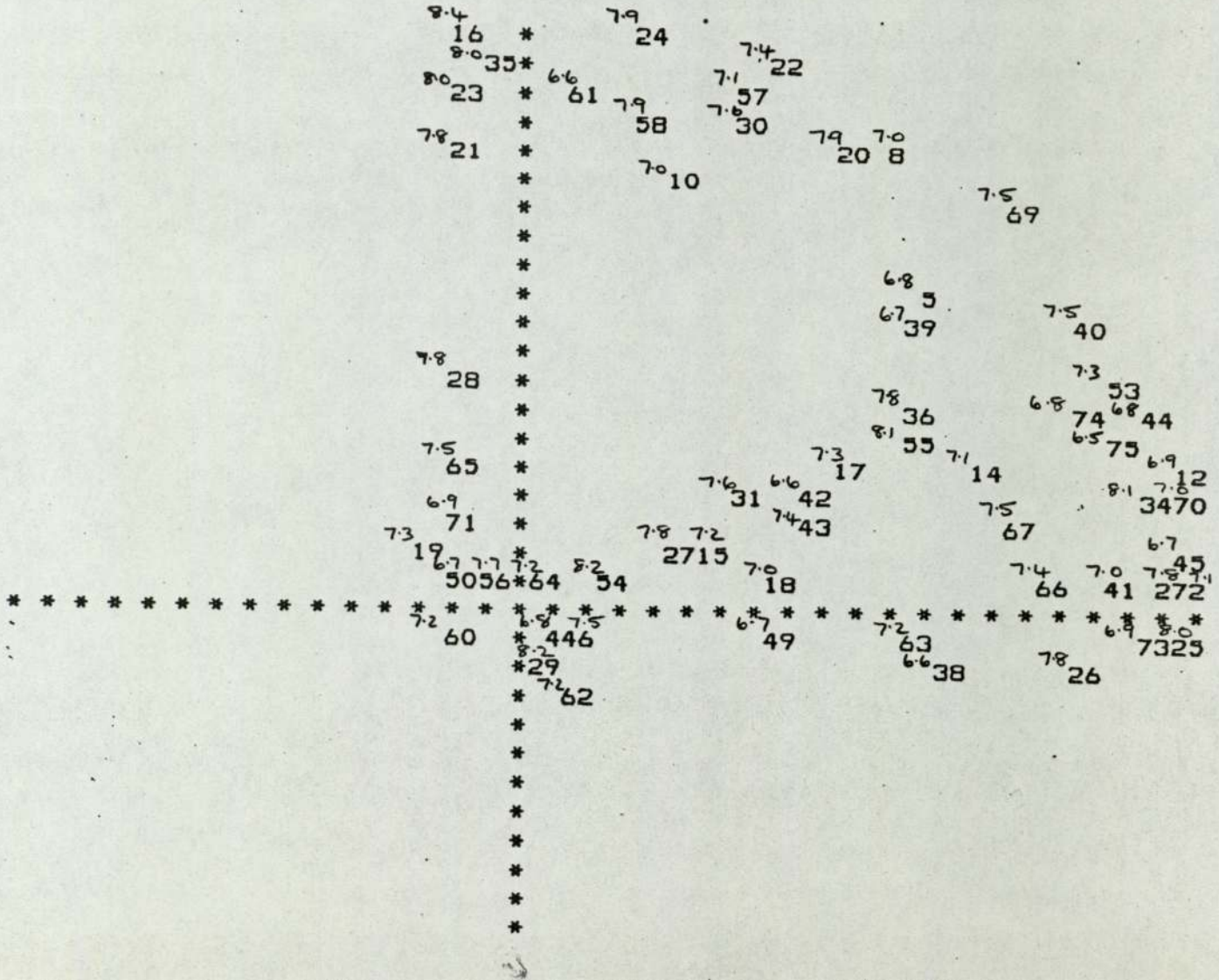


Fig.4.7. Scattergram to show distribution of sites in relation to pH



site to see if there was a general trend overall. As temperature, pH and dissolved oxygen were significant values in t-tests these individual environmental parameters were plotted - but reveal no distinct trend.

Sites do not form a small number of discrete groups with similar values, rather they demonstrate continuous variation. This is substantiated by the gradation of sites already illustrated in the Cluster Analysis work. The general parabola shape is often an indication of a collection of parameters shaping the distribution of species.

Using P.C.A. has not separated streams of high and low water quality very successfully, consequently it is difficult to draw any information from this method on associations of caseless caddis with communities from varying water qualities.

As Hamér and Soulsby (1980) concluded, this method may have more potential if 'classic' sites of clearly documented quality are processed by this method. These data then provide a baseline with which to compare future survey data treated by this method.

4.2.10. DISCUSSION AND CONCLUSIONS

The findings from the computer work on species present in association with Hydropsyche larvae, i.e. Leuctra spp., Baetis spp., E. ignita, R. semicolorata, Ancylus sp., Hydrophilidae and Oligochaeta are logical, as Hydropsyche species are widely found in benthic invertebrate communities, comprising some, if not all of the above species. This data, generated from statistically random sampling of riffle sites in all water qualities reiterates the classical work by Percival and Whitehead (1929, 1930) from their studies on biotopes and benthic communities.

The Percival and Whitehead study defined seven biotopes, five eroding types and two depositing substrata. The Trichopteran species showed greatest abundance in the eroding but relatively stable conditions with stones 9.00 m m - 5.0 cm in diameter with diatomaceous growth (comparable to R. Churnet at Alton).

In the classical torrential headwater biotope they were associated with Ephemeroptera which comprised 33.2% of the fauna, Diptera, 20%, Plecoptera 5% and Trichoptera 31%. The current in this biotope was 4 $msec^{-1}$ with unstable substratum of clean stones (R. Churnet, Upper Hulme). In the next type of biotope with a substratum of cemented stone matrix, the percentage contribution in fauna from Trichoptera was 39.9%, Ephemeroptera 20% and Coleoptera 10% (this could be compared to Dowles Brook). A mixed small stone, unstable substratum was found to be less favourable, Trichoptera comprising 34%, Coleoptera 33% and Ephemeroptera 31% (for example, R. Bollin, Langley). Trichopteran larvae, lastly made a significant contribution in biotopes with flattened stones between 2.5 - 30 cm diameter bearing Cladophora set in a matrix of small particles 0.3 - 0.5 mm. Here Dipterans contributed 40%, Trichoptera 15%, Ephemeroptera 31% and Naididae 12.5%. (This might be compared to R. Ouse at Milton Ernest). In other biotopes, with loose moss on stones, together with potamogeton, Coleoptera, Diptera, Gastropoda and Tubificidae predominate.

Similar observations on the associations of Trichopterans with other species and physical conditions were made throughout this study but H. angustipennis was perhaps more often associated with growths of Cladophora than they reported. This may be due to its provision of suitable habitat and food supply (e.g. R. Tean, Beamhurst).

4.3. THE RIVER CHURNET STUDY

AIM

To investigate the response of benthic invertebrates particularly caseless caddis larvae to the differing water qualities and physical conditions along the length of a river. Furthermore, to study the sequential separation of species down the course of the river and investigate any small scale distribution at individual sites due to variation in microhabitat.

4.3.1. METHODS

The River Churnet, Staffordshire was sampled at 11 stations using cylinder and kick heel sampling methods. Sketch Map 5 shows the sampling sites on the R. Churnet and Table 4.17 gives corresponding Grid References. Samples were taken during the period January 1979 - April 1980 on a three-monthly basis. Biological and chemical samples were all processed and recorded as previously explained. The sampling sites spanned the 38 km length of the river, which rises in the southern Peak District as several small tributaries. The first sampling site at Upper Hulme is at the confluence of two of these, shortly afterwards the river is dammed at Tittesworth Reservoir, this water being used to local domestic supply. Below the reservoir the course continues through Leek where a chemical works producing dyes discharges effluents into it. Further downstream below Leek, the sewage works effluent enters and water quality was also in the past seriously affected by copper works and a sand quarry upstream and downstream of Froghall respectively.

Today, the copper industry has declined but tailings from the quarrying activities still affect the river over a short stretch. Further downstream at Rocester a small sewage works effluent is received by the river before its confluence with the River Dove.

SKETCH MAP 5 Sampling Sites on the R. Churnet

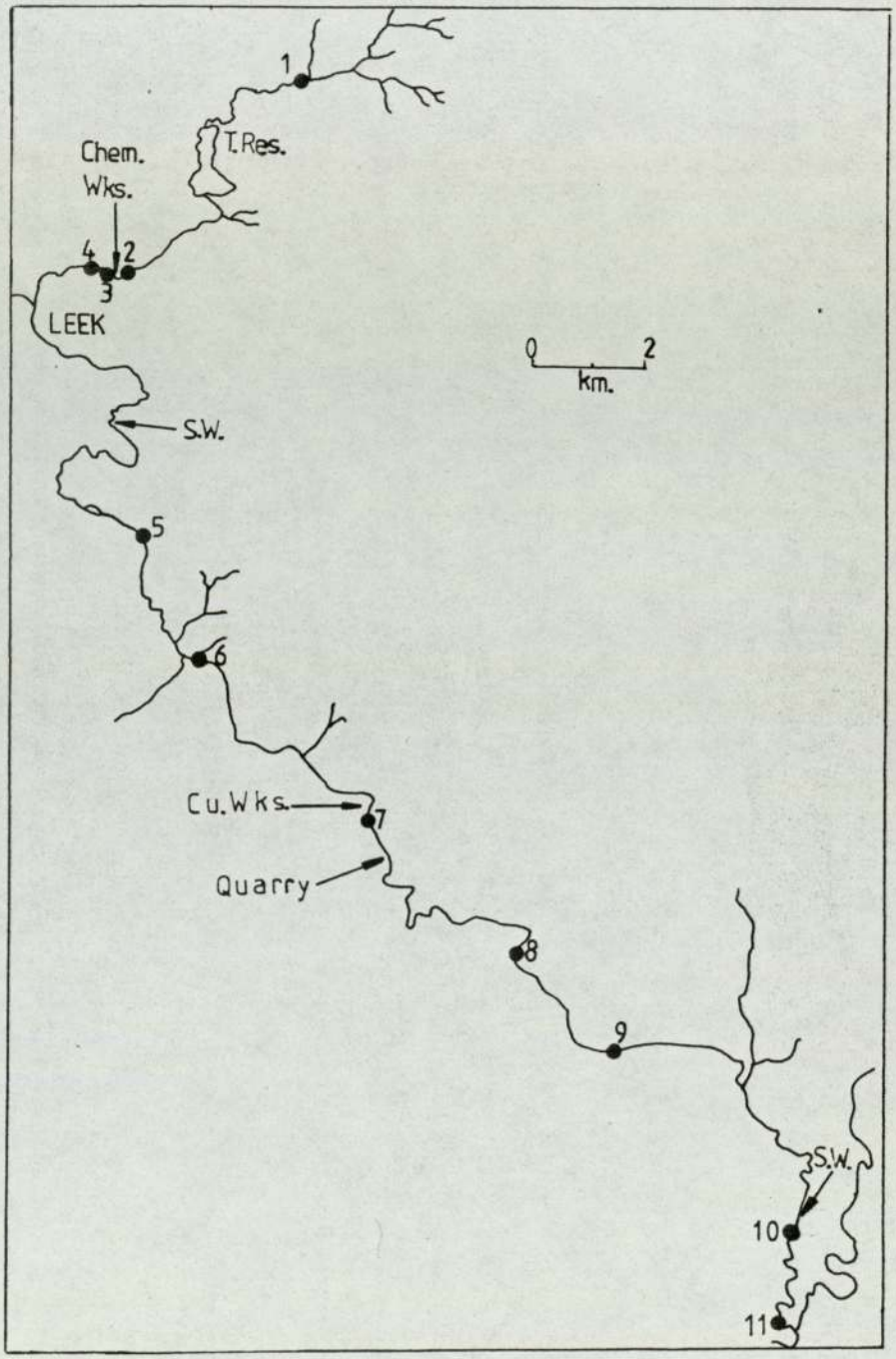


TABLE 4.17

KEY TO SAMPLING SITES ON RIVER CHURNET

No.	STATION	GRID. REFERENCE
1	Upper Hulme	SK 012 610
2	Abbey Green Road	SJ 979 573
3	D/S Chemical Works	SJ 982 578
4	Bridgend	SJ 978 550
5	Cheddleton Station	SJ 975 520
6	Consall	SK 002 487
7	Froghall	SK 025 472
8	Oakamoor	SK 053 448
9	Alton	SK 072 426
10	Rocester	SK 105 395
11	Churnet Mouth	SK 100 380

4.3.2. RESULTS

Table 4.18 summarises the physico-chemical parameters at the eleven stations. During the year, parameters fluctuate as can be seen from the ranges given and some pollutional effects are readily apparent. For example, water temperature usually increases slightly down a river profile if there are no pollutional interferences (Edington, 1965). Along the Churnet, there is a trend of increase in temperature (see Table 4.19). Superimposed upon this pattern, the effects of the chemical works in Leek has a dramatic effect on the temperature immediately below the outfall, similarly there is an increase in temperature recorded at Cheddleton Station due to the addition of organic effluent from Leek sewage works.

As a direct result of effluent discharges the dissolved oxygen and BOD levels at those two stations are markedly altered. The temperature increase caused a decrease in dissolved oxygen, and a corresponding increase in BOD. A slight elevation of BOD is detected at Rocester. This is attributable to the sewage effluent but in comparison to the upstream station it has much less effect on water quality. At Leek the sewage effluent discharges into a shallow, fast flowing river, with a small volume compared to the larger river at Cheddleton Station, and the even bigger river at Rocester where it is a typical lowland reach meandering through gentle flat agricultural and pasture land before the confluence with the R. Dove.

The copper works upstream of Froghall, once a source of metal pollution, no longer discharges large amounts of copper into the river. This can be seen by the data in Table 4.20 and the sand quarry downstream presents only short term turbidity problems.

Biological Data

Each biological sample was processed by sorting, counting and identification. A species list and abundance was compiled. Tables

TABLE 4.18

Physico-chemical parameters for River Churnet 1979-80 expressed as mean and range

	Temp. °C.	D.O mg l^{-1}	BOD mg l^{-1}	Total ox. Nitrogen	Ammonia mgN l^{-1}	S.S mg l^{-1}
Upper Hulme	7.71 0.5-16.0	10.9 9.4-12.2	2.1 0.7-3.0	1.17 0.6-2.2	0.18 0.1-0.5	32.4 1 -131
Abbey Green Road	8.5 1.0-15.0	10.4 8.6-12.2	2.29 1.1-6.2	1.54 1.0-2.1	0.55 0.1-1.8	24.0 3 -207
D/S Chemical Works	10.55 2.5-18.5	9.8 7.9-12.0	6.47 3.5-14.1	2.72 1.7-5.0	0.85 0.1-1.6	41.4 1 -308
Bridgend	7.3 3.0-11.0	11.5 11.0-11.8	1.86 1.1-2.3	1.83 1.6-2.0	0.5 0.1-0.9	7.6 3 - 15
Cheddleton Station	10.4 2.0-21.5	9.3 6.7-11.8	4.1 2.1-6.0	3.2 2.2-5.1	0.73 0.1-2.2	24.8 7 -102
Consall	9.5 2.0-17.2	10.0 8.3-11.8	3.59 2.2-5.2	3.2 2.2-4.7	0.45 0.1-0.9	37.0 10 -106
Froghall	9.1 2.0-16.5	10.4 8.7-11.8	3.66 2.0-7.9	3.25 2.2-4.7	0.48 0.1-1.7	47.6 7 -166
Oakamoor	9.4 2.0-16.0	10.6 9.0-12.2	3.46 1.6-5.6	3.2 2.2-4.5	0.42 0.1-1.4	50.5 8 -157
Alton	9.2 2.0-16.0	10.6 9.2-11.4	3.18 1.2-5.1	3.3 2.4-5.1	0.44 0.1-1.8	54.0 6 -189
Rocester	9.1 0.1-16.0	10.5 7.8-12.8	3.3 1.8-5.8	3.35 2.3-4.4	0.39 0.1-0.8	41.3 6 -239
Churnet Mouth	11.7 3.5-18.0	10.0 8.7-11.4	2.4 2.0-2.7	3.4 3.1-4.0	0.2 0.1-0.3	13.0 9 - 20

TABLE 4.19

TEMPERATURE OBSERVATIONS ON THE RIVER CHURNET

STATION	DATE MAY 1979		DATE AUGUST 1979		DATE JANUARY 1980	
	OWN	W.A.	OWN	W.A.	OWN	W.A.
Upper Hulme	7.2	8.0	13.0	15.5	3.0	1.5
Abbey Green Road	9.5	9.5	13.5	15.0	4.0	3.0
Bridgend				11.0		
Consall	10.0	10.0		17.0		
Cheddleton Station	10.0	10.2	14.5	18.0	5.0	3.5
Froghall	10.3	10.0	15.0	16.5		3.0
Oakamoor		10.5		16.0		3.0
Alton	10.7	10.0	15.2	16.0	5.0	3.0
Denstone						~~5.0~~
Rocester	11.0	11.0	15.5	16.0	5.0	5.0
Churnet Mouth	11.2	12.0	15.7	16.0	5.0	5.0

TABLE 4.20

METAL LEVELS AT STATIONS ON THE R. CHURNET

STATION	Cu $mg\ l^{-1}$	Cd $mg\ l^{-1}$	Pb $mg\ l^{-1}$	Cr $mg\ l^{-1}$	Ni $mg\ l^{-1}$	Zn $mg\ l^{-1}$	Fe $mg\ l^{-1}$
Upper Hulme	0.012	0.002	0.02	-	0.01	0.035	0.80
Abbey Green Rd.	0.022	0.001	0.02	0.002	0.01	0.060	0.76
Cheddleton Station	0.021	0.002	0.03	0.005	0.01	0.050	2.00
Froghall	0.021	0.002	0.03	0.005	0.01	0.050	2.41
Alton	0.008	0.002	0.01	0.005	0.01	0.010	1.29
Rocester	0.010	0.001	0.02	0.005	0.01	0.010	1.54

4.21 and 4.22 show examples of the faunal lists compiled from cylinder samples and kick heel samples. For each station a Trent Biotic Index, Chandler Score and revised BMWP Score was calculated and Department of the Environment (D o E) classification assigned according to 1970 D o E report. These results are tabulated in Table 4.23.

The number of taxa in the invertebrate assemblages was counted and these data were generally found to reflect the changes in water quality. This complements physico-chemical findings. Changes recorded at the eleven stations, by both cylinder and kick heel methods may be seen in Figs.4.8 and 4.9.

At Upper Hulme, fifteen taxa were present and represent a typical unpolluted upland river assemblage, comprising predominantly plecopteran species, ephemeropterans, gammarids and trichopterans. High T.B I and score values show it to be of good water quality as D o E class suggests Often in such conditions the fewer number of taxa are due to oligotrophic conditions of moorland run off.

As may be seen from Table 4.23, at Abbey Green Road, the standard of water quality remains high. Twenty-two taxa were present of great diversity. Here the river is larger, with a well established riffle community. The substratum of mixed boulders and gravel is stable, and not shifting as the gravel and shale flakes at Upper Hulme. The substratum type, together with good water quality provides many microhabitats for a variety of differently adapted invertebrates. Hydropsyche, Rhyacophila and Polycentropus spp. were all present at this station, demonstrating the range of conditions available for the predatory carnivorous Rhyacophila - and of suitable flow and oxygen for Hydropsyche and Polycentropus spp.

The drop in water quality immediately below the chemical works reflects acute pollution from a point source. The invertebrate assemblage is poor, predominantly organisms with a high tolerance such as tubificids,

TABLE 4.21. R. CHURNET 0.05 m^2 CYLINDER SAMPLES

SITE →	CHURNET										
FAUNA ↓	Upper Hulme	Abbey Green Road	D/S Chemical Works	Bridg-end	Cheddle-ton Station	Consall	Froghall	Oakamoor	Alton	Rocester N/A	Churnet Mouth N/A
Platyhelminthes							3				
A. fluviatilis	2	3		3							
P. jenkinsi											
L. pereger											
Planorbis sp.											
Sphaerium sp.						2					
Pisidium sp.											
Tubificidae	1	11	4	11	55	1	600		5		
G. complanata			1	2	7	2					
E. octoculata			3		18				3		
E. testacea											
H. stagnalis	1										
H. sanguisuga											
Hydracarina				3		1					
G. pulex				10	1						
A. aquaticus			7		19	7					
Baetis sp.	13	3		3	10	10	4	8	2		
Caenis sp.											
E. ignita		4									
E. dancia	1										
E. venosus							5		2		
R. semicolorata		1			7	1	26	4	2		
Heptagenia sp.											
Paraleptophlebia sp.	4										
Protonemura sp.							2				
Amphinemura sp.	1										
Nemoura sp.	1			1							
Leuctra sp.				1		1	1				
Perla sp.									5		
I. grammatica	4	3				7		4			
Chloroperla sp.	25										
Gyrinidae											
Haliplidae							1				
Elminthidae	5					1	24				
Dytiscidae		1									
Sialis lutaria											
H. angustipennis	1			1		1			1		
H. pellucidula								1	8		
H. siltalai						1					
H. instabilis						1					
H. fluvipes											
Rhyacophila dorsalis	3						7	7	1		
P. flavomaculatus	1		1								
P. conspersa		1									
Glossosomatidae											
Phryganidae											
Sericostomatidae						1					
Limnephilidae					1			1			
Hydroptilidae											
Molannidae											
Simulium sp.			2	6							
Dicranota sp.	7				3						
Tipula sp.									1		
Atherix sp.									1		
Chironomidae (Green)	5	2	9	12	22	2	32	225	3		
Ceratopogonidae							4				
No. of taxa	16	9	7	11	10	15	12	7	12		
No. of Indv.	75	29	27	53	143	39	709	250	34		
T.B.I.	X	VII	IV	IX	VI	IX	VIII	VII	VIII		
Chandler Score	911	468	232	518	391	651	453	473	419		
B.M.W.P. Score	79	55	24	39	33	63	51	45	47		
Shannon-Weiner Diversity Index	3.1430	2.6810	2.3960	2.9540	2.6080	2.7420	1.0226	0.6958	3.235		

TABLE 4.22. RIVER CHURNET HEEL KICK SAMPLES

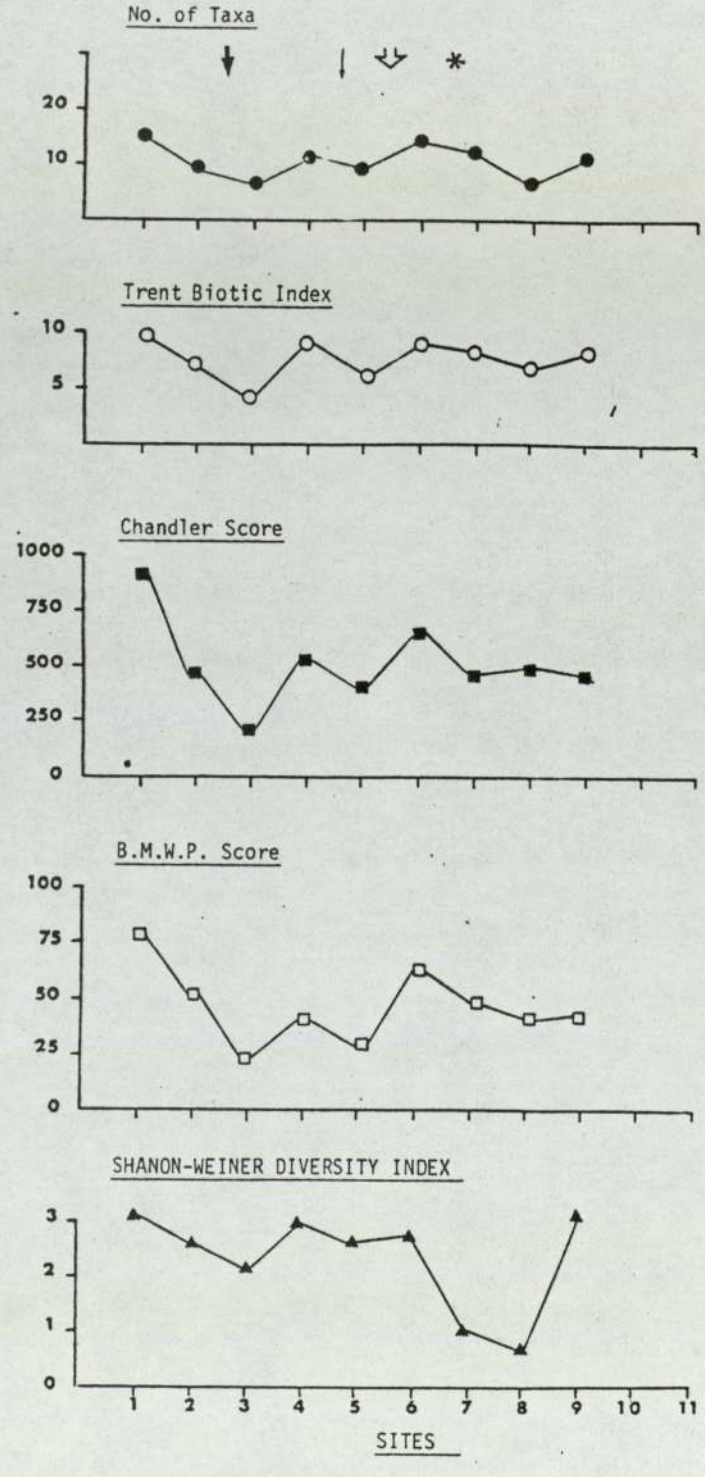
SITE →	CHURNET										
FAUNA ↓	Upper Hulme	Abbey Green Road	D/S Chemical Works	Bridg-end	Cheddle-ton Station	Consall	Froghall	Oakamoor	Alton	Rocester	Churne Mouth
Platyhelminthes								14	1		
A. fluviatilis		6		5	3		1			4	2
P. jenkinsi				57							
L. peregor		22	44							23	
Planorbis sp.			26			1		1			
Sphaerium sp.						1				8	
Pisidium				12		1		5			
Tubificidae	3	29	61	19	64	6		15	10	5	15
G. complanata			4	5	8	1		3		8	1
E. octoculata				3	30	1		9			
E. testacea						12					
H. stagnalis					1	1					
H. sanguisuga			11			1					
Hydracarina	44	27		22				6	2	8	6
G. pulex				26	2	1	4	72	3	5	
A. aquaticus			71		534	30	28	58	22	6	
Baetis sp.	1	14	3	2		10	23	20	42	12	29
Caenis sp.						3					
E. ignita							18	15	36		26
E. dancia						1					
E. venosus	3			3			1				
R. semicolorata						3					3
Heptagenia sp.						4					
Paraleptophlebia sp.											
Protonemura sp.	6					36					
Amphinemura sp	21										
Nemoura sp.											
Leuctra sp.	19					3	1		9		
Perla sp.											
I. grammatica	14					15				11	
Chloroperla sp.	7										
Gyrinidae		2									
Haliplidae		1	2			2		5	5		
Elminthidae					1			3			
Dytiscidae		32		5							
Sialis lutaria		1									
H. angustipennis	14	1				2	1	7	26	22	
H. pellucidula											
H. contubernalis											
H. siltalai						1					
H. instabilis						1					
H. fluvipes											
Rhyacophila dorsalis				2			12		17	3	
B. flavomaculatus	3			4							
P. conspersa											
Glossosomatidae											
Sericostomatidae	4					1					
Limnephilidae	6	6			1	1	2	8			8
Hydroptilidae								1	1		
Molannidae											
Simulium sp.		7								9	
Dicranota sp.	2			7					3		5
Tipula sp.			1	1				2		6	
Atherix sp.										11	7
Chironomidae (Green)	34	34		21	30	32	65	5	17	17	32
Ceratopogonidae		1					1				
No. of taxa	16	14	9	16	10	27	12	18	15	16	11
No. of Ind.	182	183	223	194	674	173	157	249	196	158	134
T.B.I.	X	VIII	VI	VIII	VI	X	VII	VIII	IX	IX	VII
Chandler Score	912	713	253	560	377	692	779	693	948	587	580
B.M.W.P. Score	67	47	30	65	36	109	60	76	64	63	33
Shannon-Weiner Diversity Score	3.2694	3.097	2.349	3.2248	0.9994	3.5235	2.4565	3.2035	3.2166	3.6910	2.8834

TABLE 4.23

SUMMARY OF BIOLOGICAL DATA FROM THE R. CHURNET

STATION	T.B.I.	CHANDLER SCORE	B.M.W.P. SCORE 1980	D.of E. CLASS 1970	NO.of TAXA
Upper Hulme	X	1114	81	A	15
Abbey Green Rd.	X	1198	98	A	22
d/s Chem.Works	V1	253	33	B/C	8
Bridgend	V111	560	58	A	16
Cheddleton Station	V1	377	36	B/C	10
Consall	X	1693	119	A	27
Froghall	V111	779	76	A	13
Oakamoor	1X	994	94	A	21
Alton	1X	948	74	A	17
Rocester	1X	587	67	A	17
C. Mouth	1X	647	69	A	17

Fig.4.8. R. CHURNET 0.05 m^2 CYLINDER SAMPLES



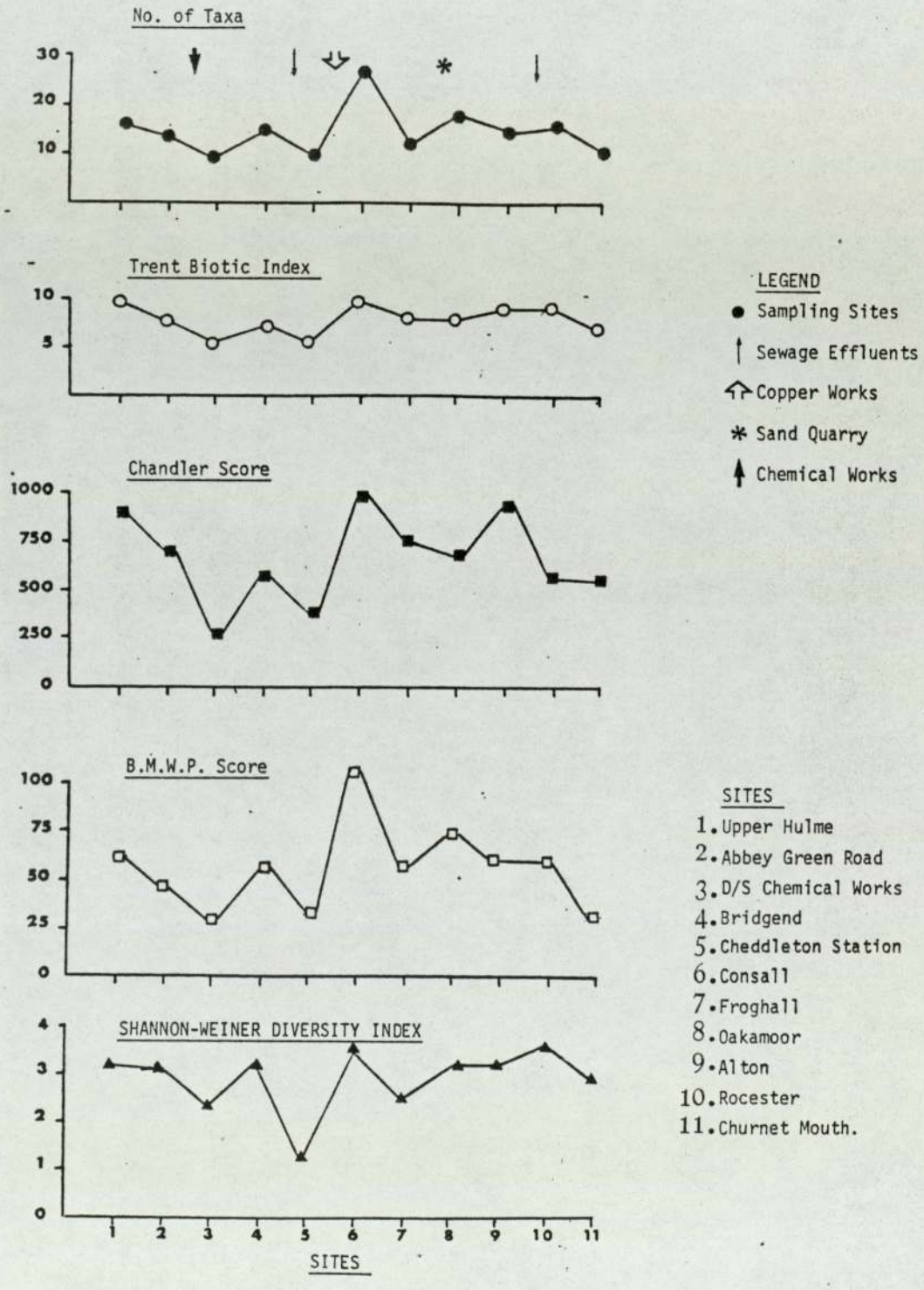
LEGEND

- Sampling Sites
- Sewage Effluents
- Copper Works
- Sand Quarry
- Chemical Works

SITES

1. Upper Hulme
2. Abbey Green Road
3. D/S Chemical Works
4. Bridgend
5. Cheddleton Station
6. Consall
7. Froghall
8. Oakamoor
9. Alton
10. Rocester
11. Churnet Mouth.

Fig.4.9. R. CHURNET HEEL-KICK SAMPLES



leeches, chironomids and snails. Rapidly the water quality recovers at Bridgend with Gammarus sp., mayflies, trichoptera and various diptera in the sample.

The discharge of sewage effluent takes its toll on the fauna again at Cheddleton Station. The diveristy of fauna is decreased and the the water quality is again reflected by oligochaetes, leeches, Asellus aquaticus, chironomids and snails in abundance.

Moving further downstream, the river appears to undergo a self-purification sequence. The apparent rapid increase in quality at Consall may also be attributable to hospitable substratum, a decrease in temperature combined with a higher concentration of nutrients. The increased suspended solids make it particularly suitable for particle feeders such as the Hydropsychids which are abundant here. Similarly, further downstream at Alton the net-spinning species proliferate due to the ideal combination of conditions, substratum, flow rate and nutrient status of the water.

At Rocester, downstream of the effluent again favours Hydropsyche, exhibiting similar conditions, but at the Churnet Mouth slower flow eliminates their presence.

4.3.3. DISTRIBUTION OF CASELESS CADDIS IN THE R. CHURNET

The distribution pattern which emerged is depicted in Table 4.24. In a pollution-free river system, there is a sequential separation of species down the length of the river (Illies and Botosaneanu, 1963) and a discernable small scale distribution of species at any particular site due to variations in mircohabitats (Ulfstrand, 1967).
Edington and Hildrew (1973) found both of these patterns to be evident in their study of net spinning Trichoptera in the R. Usk, South Wales. Although in the R. Churnet two main pollutional effects are evident, which may slightly distort the natural pattern, there is still a definitive distribution of species downstream. Some species have been proven experimentally to be specialised for living in varying water velocities,

Hydropsychids being characteristic of riffle sections and Polycentropids of pools.

Polycentropid larvae in the R. Churnet

Polycentropid larvae were found at the first three sampling stations, i.e. Upper Hulme, Abbey Green Road and Bridgend (Table 4.24), stations of high water quality. At all stations there are microhabitats available suitable for net spinning in water of velocity not exceeding 20 cm^{-1}. These larvae tend to aggregate at suitable positions where prey density is highest - their diet varying according to availability of chironomids, Ephemeroptera nymphs and Plecoptera nymphs (Higler, 1978). This is logical as it is at these sites where the species are not abundant as a food source. Feeding is initiated by prey touching and vibrating the net which the larvae spins. The vibrations stimulate the larvae to then capture the prey.

Hydropsyche larvae in the R. Churnet

Distribution of Hydropsyche spp. is also sequential and influenced by microhabitats, but a third distribution, i.e. geographical, has to be considered. H. angustipennis is a common species in the Midlands, but with restricted distribution elsewhere in Britain. Similarly in the north of England, H. siltalai is the predominant species. A typical sequence of appearance of Hydropsyche spp. in a clean river in Britain appears to be Diplectrona felix in the headwaters, H. siltalai, H. pellucidula followed by H. contubernalis and/or Cheumatopsyche lepida, (Edington and Hildrew, 1973 ; Badcock, 1974 1975; ; Boon ,1976; 1978a and Edington , 1968).

H. angustipennis does not strictly conform to any place in this pattern, and is found widely from small headstreams to the lower reaches of rivers. This species particularly favours sites such as outflows from lakes and ponds, e.g. Sutton Park stream, West Midlands, and it has been noted to thrive in mildly organically polluted conditions, e.g. Langley

TABLE 4.24

DISTRIBUTION OF CERTAIN SPECIES OF CASELESS CADDIS IN THE RIVER CHURNET

	Upper Hulme	Abbey Green Road	Bridgend	Cheddleton Station	Consall	Froghall	akamoor	Alton	Denstone	Rocester	Churnet Mouth
Polycentropidae											
Plectrocnemia conspersa		0									
Polycentropus flavomaculatus	0	0	0								
Hydropsychidae											
H. instabilis		X			X						
H. siltalai		X	X		X		X	X	X	X	
H. angustipennis	X	X		X	X	X	X	X	X	X	X
H. pellucidula							X	X			
H. contubernalis								X		X	
Rhyacophilidae											
R. dorsalis	∇	∇	∇	∇		∇	∇	∇	∇	∇	∇

Mill Sewage Works. It appears extremely tolerant of a wide range of conditions. The data collected appear to conform to this general pattern although D. felix is absent. Edington and Hildrew (1973) found D. felix to have a summer maxima of 15°C and to tolerate only small daily ranges, so the temperature regime at Upper Hulme may be unsuitable. Consulting Tables 3.14 and 3.15 it can be seen that the maximum temperature exceeds 15°C.

H. instabilis is slightly more tolerant to elevated temperatures, and is the next in the series, being present at Abbey Green Road and Consall. Its absence from the stretch of river between these two stations suggests that the conditions imposed by the addition of the effluents make it inhospitable.

Following the established pattern, H. siltalai appears at Abbey Green Road and Consall, where it co-exists with H. instabilis. H. pellucidula appears next in the sequence, if the blanket distribution of H. angustipennis is ignored. H. contubernalis is found at Alton, co-existing with H. siltalai H. angustipennis and H. pellucidula. It again is found at Rocester - a typical site where H. contubernalis can thrive whereas other species would be limited by water velocity.

Rhyacophila larvae in the R. Churnet.

Only one species R. dorsalis is found in the Churnet. All Rhyacophilidae are free living predators, their major food sources being Baetis sp. and Simulium sp. It is reasonable to assume that they are not limited in the Churnet by dissolved oxygen levels or food availability and are consequently found along its length.

In her paper Markinkovic-Gospodnetic (1966) on a study of caddis, especially of Rhyacophila spp. in a stream on the slopes of Mt. Bosnia stated that it appears that zonation and sequential distribution separates 11 species. This is tentatively attributed to changes in water temperature, velocity and volume.

4.4. DISTRIBUTION OF CASELESS CADDIS IN THE R. BLYTHE

A similar distribution survey, using the same sampling methods was undertaken on the river Blythe.

4.4.1. METHODS

This 30 km reasonably high quality river of the West Midlands flows from the south side of Solihull, northwards to Coleshill where it is joined by the R. Cole and both have their confluence with the R. Tame. The R. Cole is of far inferior quality, having a course through industrial Birmingham (see Sketch Map 6).

Only one major effluent is discharged into the R. Blythe, from Barston sewage treatment works via Eastcote Brook, otherwise it is free from pollution, flowing through agricultural land. Table 4.25 gives the grid references of the sampling stations.

TABLE 4.25 SAMPLING STATIONS ON THE R. BLYTHE

No.	Station	Grid Reference
1	Cheswick Green	SP 124 755
2	Henwood Mill	SP 181 793
3	u/s Cuttle Brook	SP 201 755
4	Temple Balsall	SP 209 763
5	u/s Eastcote Brook	SP 213 800
6	Stonebridge	SP 214 831

Sampling the Blythe, it was possible to compare the distribution of caddis flies to that of the R. Churnet.

4.4.2. RESULTS

Table 4.26 summarises the chemical data collected from the six stations. The species distribution of the caseless trichopteran larvae is depicted in Table 4.27 and a summary of the biological status is given in Table 4.28. As may be seen from the chemical data, all stations

SKETCH MAP 6. SAMPLING STATIONS ON R. BLYTHE

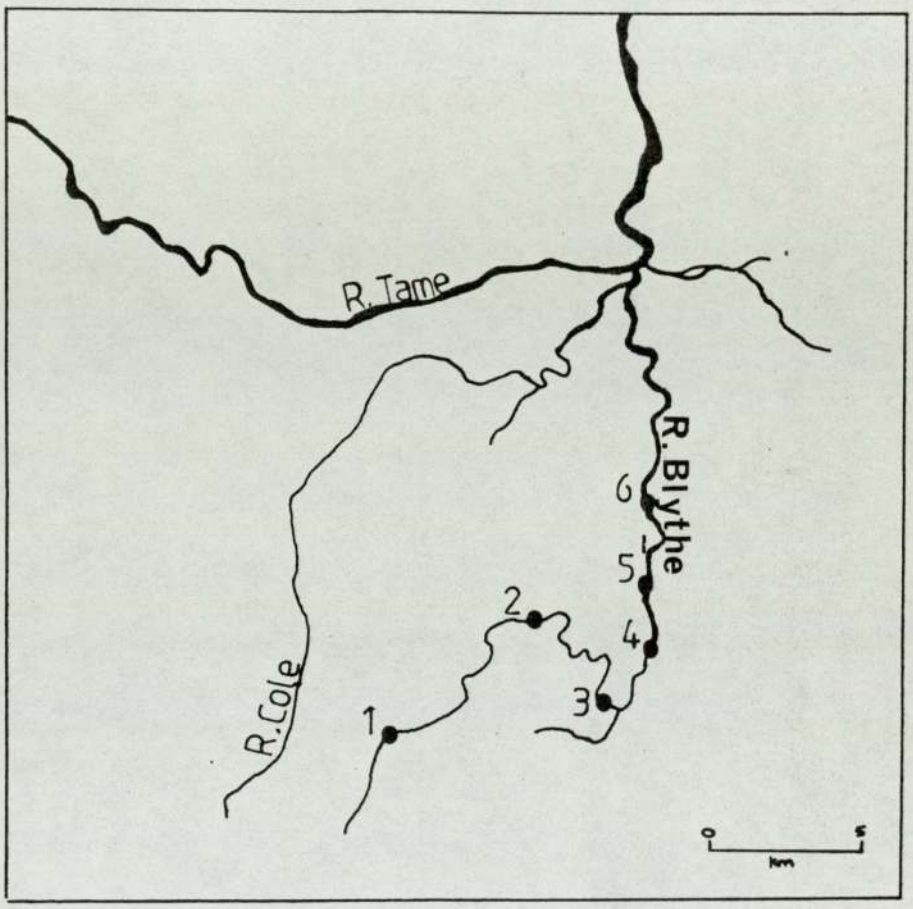


TABLE 4.26. CHEMICAL DATA R. BLYTHE

	Temp. °C.	pH.	D.O. mg l^{-1}	BOD mg l^{-1}	Total Hardness mg l^{-1} ($caCO_2$)	Alkalinity mg l^{-1} ($CaCo_3$)	Total ox. Nitrogen mg $N1^{-1}$	Ammonia mg $N1^{-1}$	S.S. mg l^{-1}	Copper mg l^{-1}	Cadmium mg l^{-1}	Lead mg l^{-1}	Chromium mg l^{-1}	Nickel mg l^{-1}	Zinc mg l^{-1}
Cheswick Green	2.0	7.3	11.4	6.1	260	102	9.7	0.8	18	-	0.002	0.02	-	0.02	0.04
Henwood Mill	2.0	7.45	11.7	4.0	264	116	7.3	-	14	0.01	-	0.07	0.01	0.02	0.06
u/s Cuttle Brook	2.0	7.25	10.6	5.7	294	144	7.8	-	20	-	0.001	0.03	-	0.02	0.03
Temple Balsall	2.0	7.4	11.4	4.0	304	124	7.8	0.6	12	0.01	0.002	0.10	0.05	0.04	0.09
u/s Eastcote Brook	1.5	7.55	11.4	5.1	318	131	8.6	0.6	15	-	0.002	0.05	-	0.03	0.07
Stonebridge	2.0	7.6	11.0	5.0	260	142	9.0	0.6	16	-	0.001	0.03	0.01	0.02	0.03

TABLE 4.27. DISTRIBUTION OF CASELESS CADDIS IN THE R. BLYTHE

STATION / SPECIES	CHESWICK GREEN	HENWOOD MILL	U/S CUTTLE BROOK	TEMPLE BALSALL	U/S EASTCOTE BROOK	STONEBRIDGE
P. flavomaculatus		X			X	X
H. siltalai	X	X		X	X	X
H. angustipennis		X	X	X	X	X
H. pellucidula				X		
R. dorsalis		X	X	X	X	X

X = Present

TABLE 4.28 SUMMARY OF BIOLOGICAL DATA FROM THE R. BLYTHE

STATION	TBI	CHANDLER SCORE	BMWP SCORE 1980	D. of E. CLASS 1970	NO. of TAXA
1	VIII	628	64		16
2	VIII	646	67	A	20
3	VIII	608	60	A	19
4	VIII	921	72	A	21
5	VIII	643	77	A	18
6	VIII	660	68	A	15

are very similar in water quality, which substantiates the findings of the biological sampling. The R. Blythe is a good fishing river, and may be compared to the upper to middle reaches of the R. Churnet.

At each station on the R. Blythe the benthic invertebrate community is diverse, and well equipped to provide food for the Rhyacophila present but also suitable to provide a habitat for Hydropsychids and Polycentropids. In the proposed sequence along a river profile, these sites are most suitable for larvae of the middle portion of that sequence. D. felix and H. instabilis are absent, but they are typically associated with headwater conditions. H. contubernalis, usually at the end of the sequence is also absent, as it is usually found in the lowland zone of large rivers. Absence of P. conspersa, but presence of the more tolerant P. flavomaculatus also fits logically into the theoretical pattern proposed.

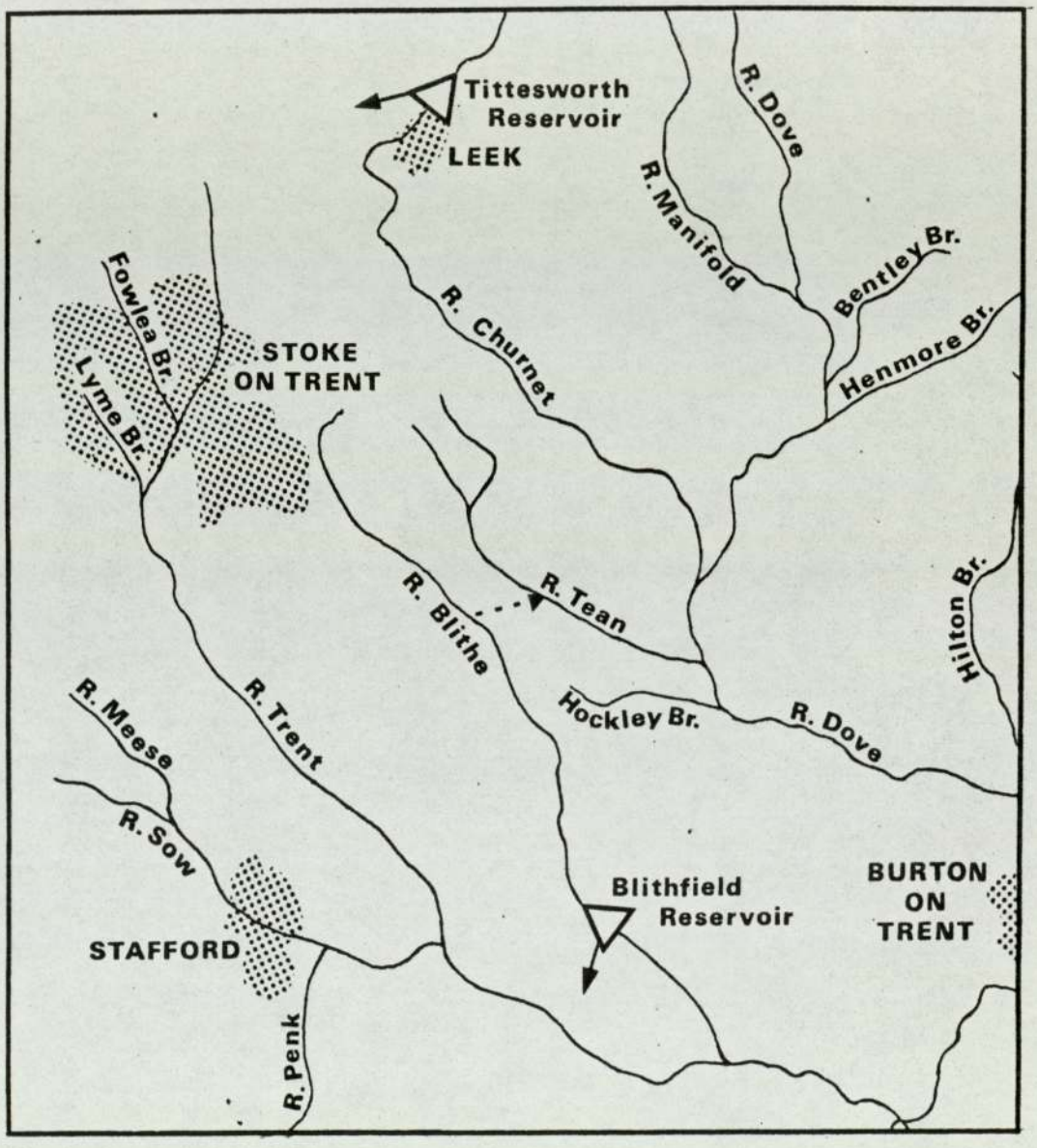
4.5. R. TEAN STUDIES (ORGANIC POLLUTION)

Biological samples, using the cylinder samples were taken on a monthly basis on the R. Tean at Checkleybank and Beamhurst (upstream and downstream of sewage works). This enabled a comparison of the two stations to be made, over all seasons and provided a data base for further experimentation at the Hydrobiology Research Station. Chemical data were compiled in the same way as for other sampling occasions.

4.5.1. METHODS

The R. Tean rises north of Checkley at Huntly where it is a fast flowing stream of small volume. Its overall length is 15 km to its confluence with the R. Dove. Stoke-on-Trent and associated "Potteries" area is the major centre of population and industry affecting the R. Tean. This situation arises because the effluent which should be discharged into the R. Blithe, is diverted into the Tean Valley for processing (see Map 7). The Blithe is maintained as a high quality river as it drains into Blithfield Reservoir which is used

MAP 7. POSITION OF R. TEAN IN RELATION TO MAJOR CENTRES OF POPULATION AND THE R. BLITHE



as a source of drinking water. Consequently, the sewage works at Checkley are known as the Blithe Valley Water Reclamation Works, although located in the Tean Valley. The effluent from the Potteries is a source of heavy metals, particularly cadmium which is a byproduct of the glazing process. As the R. Tean flows through predominantly agricultural land, the run-off from the land is not a great pollutional problem, the other major point sources came from the sewage works and from further downstream from the dairy at Fole.

Description of Sites

The two sampling stations Checkleybank, in the village of Checkley (Grid. Ref. SK 028 377) and Beamhurst (Grid Ref. SK068 357) are both riffle stretches. At Checkleybank the river is approximately 2 - 3 m wide with a current velocity at 64 cm sec^{-1}. The substratum is of small stones and gravel and the site is shaded by overhanging trees. The river at Beamhurst, 2.5 km downstream from Checkleybank, has a current velocity of 80 cm sec^{-1}. The substratum again is of small stones but covered almost totally with Cladophora in the summer months. A small drain, from a dairy farm, also drains into the river at this point, causing an intermittent point source from farmyard run off. The position of these sites is illustrated in Sketch Map 8.

Three cylinder samples were taken at each station, one from the left hand side close to the bank, one from the centre and the third from the right hand side of the river, on a monthly basis.

5.2. RESULTS

Table 4.29 shows the species list for Checkleybank and Beamhurst on the R. Tean. Histograms on (Figs.4.10, 4.11, 4.12, 4.13 and 4.14) show the numbers of individuals sampled at both of the stations over the sampling period, February 1979 - January 1980. Figure 4.15 shows the number of taxa, TBI, Chandler Score and Diversity

MAP 8. R. TEAN SAMPLING SITES AND LOCATION OF THE CHECKLEY CHANNELS AT SEWAGE WORKS

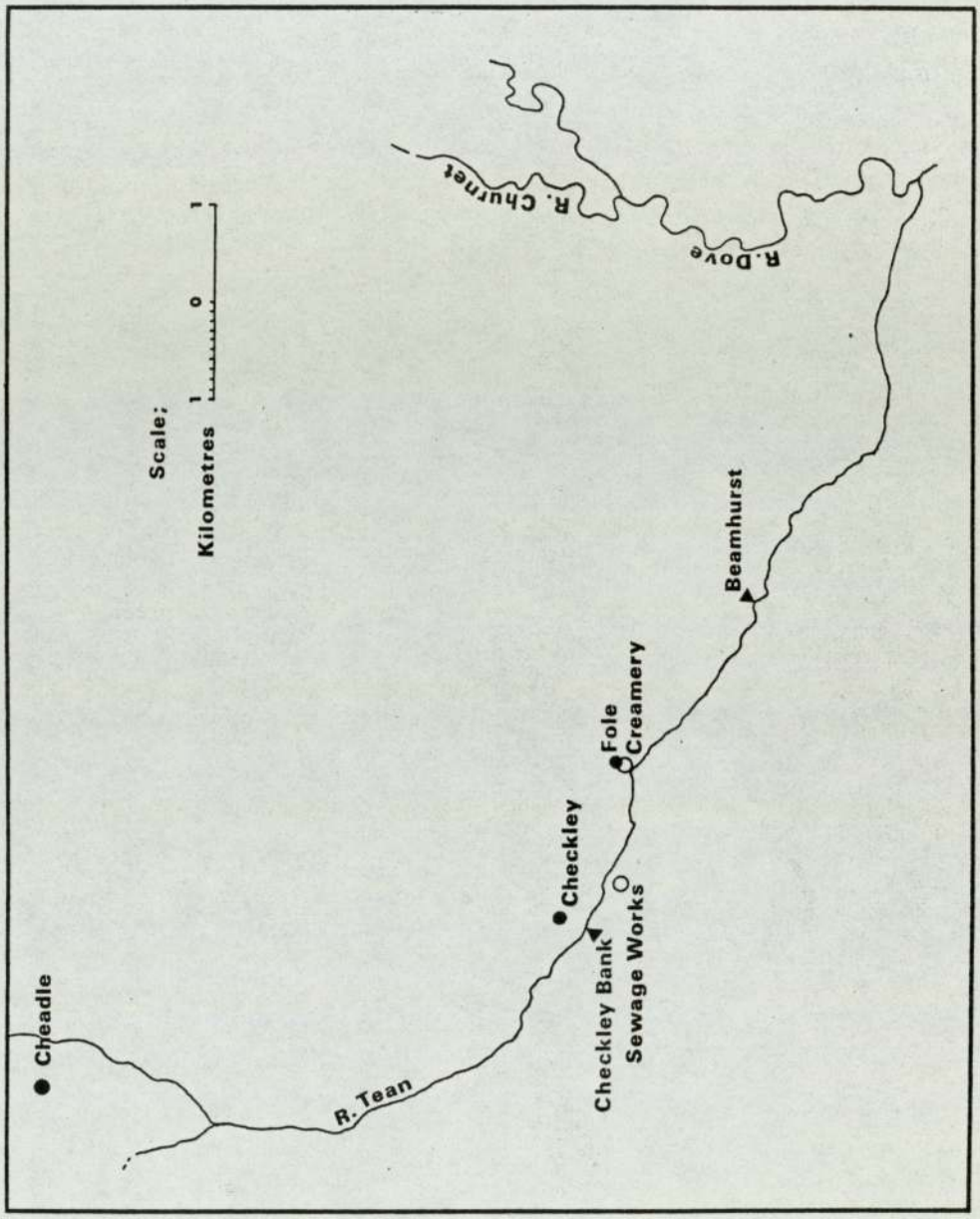


TABLE 4.29 SPECIES LIST FOR R. TEAN AT CHECKLEYBANK AND BEAMHURST

CHECKLEYBANK	BEAMHURST
Naididae	Naididae
Tubificidae	Tubificidae
G. complanata	G. complanata
P. geomatrica	E. octoculata
Hydracarina	H. stagnalis
G. pulex	Hydracarina
A. aquaticus	G. pulex
C. torrentium	A. aquaticus
I. grammatica	B. rhodani
B. rhodani	E. ignita
E. ignita	H. angustipennis
E. venosus	R. dorsalis
E. dispar	Limnephilidae
C. rivulorum	Sialis sp.
R. semicolorata	Haliplus sp.
H. angustipennis	Dicranota sp.
R. dorsalis	Pedicia sp.
Limnephilidae	Chironomidae
Polycentropus sp.	P. jenkinsi
Psychomyid sp.	A. fluviatilis
Hydroptilid sp.	Pisidium sp.
B. elevatus	
Simulium spp.	
Dicranota sp.	
Tipula sp.	
Chironomidae	
Empididae	
P. jenkinsi	
A. fluviatilis	
L. pereger	
Zonitoides sp.	

FIG.4.10. NUMBERS OF OLIGOCHAETA AND HIRUDINEA SAMPLED AT CHECKLEYBANK AND BEAMHURST

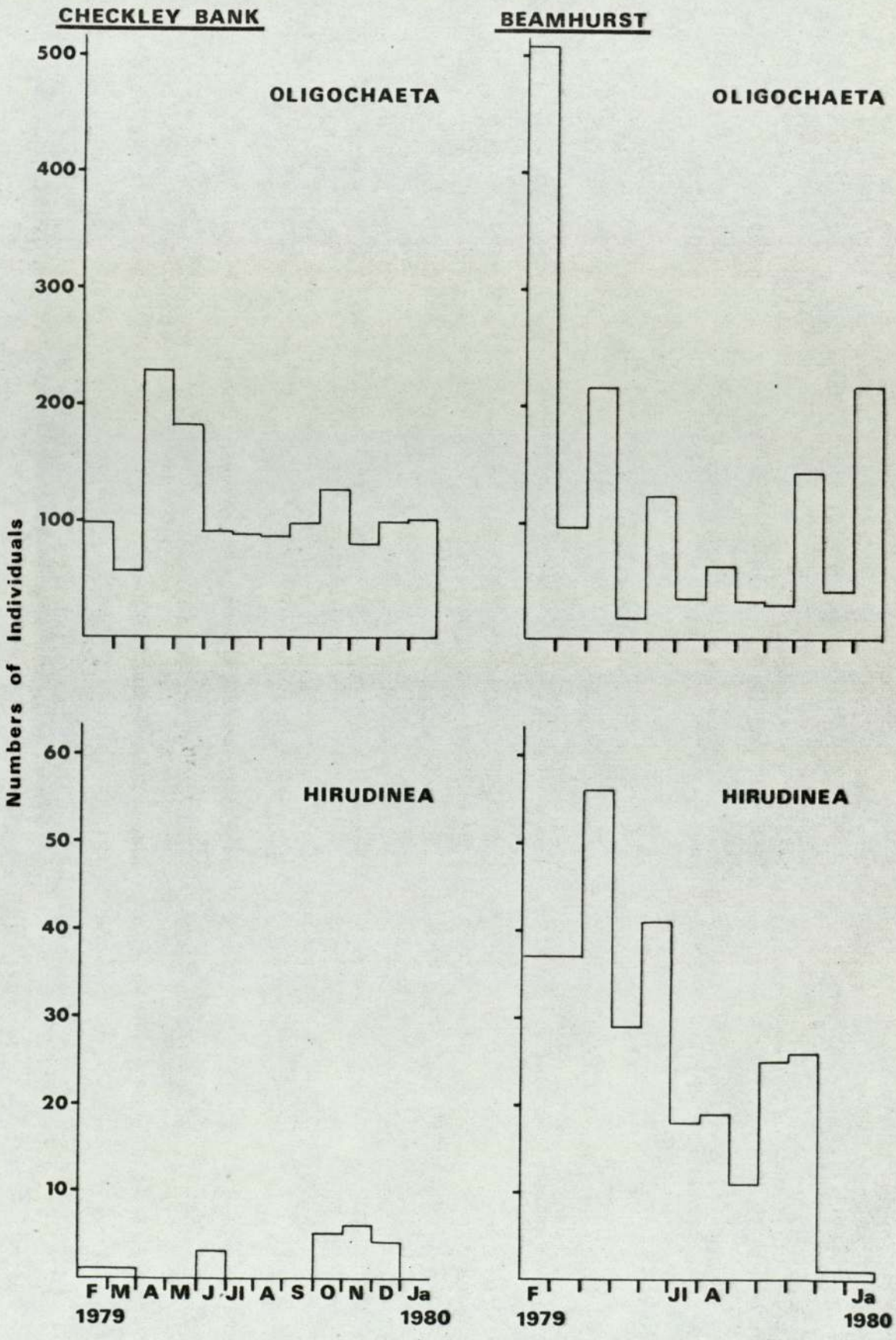


FIG.4.11. NUMBERS OF CRUSTACEA SAMPLED AT CHECKLEYBANK AND BEAMHURST

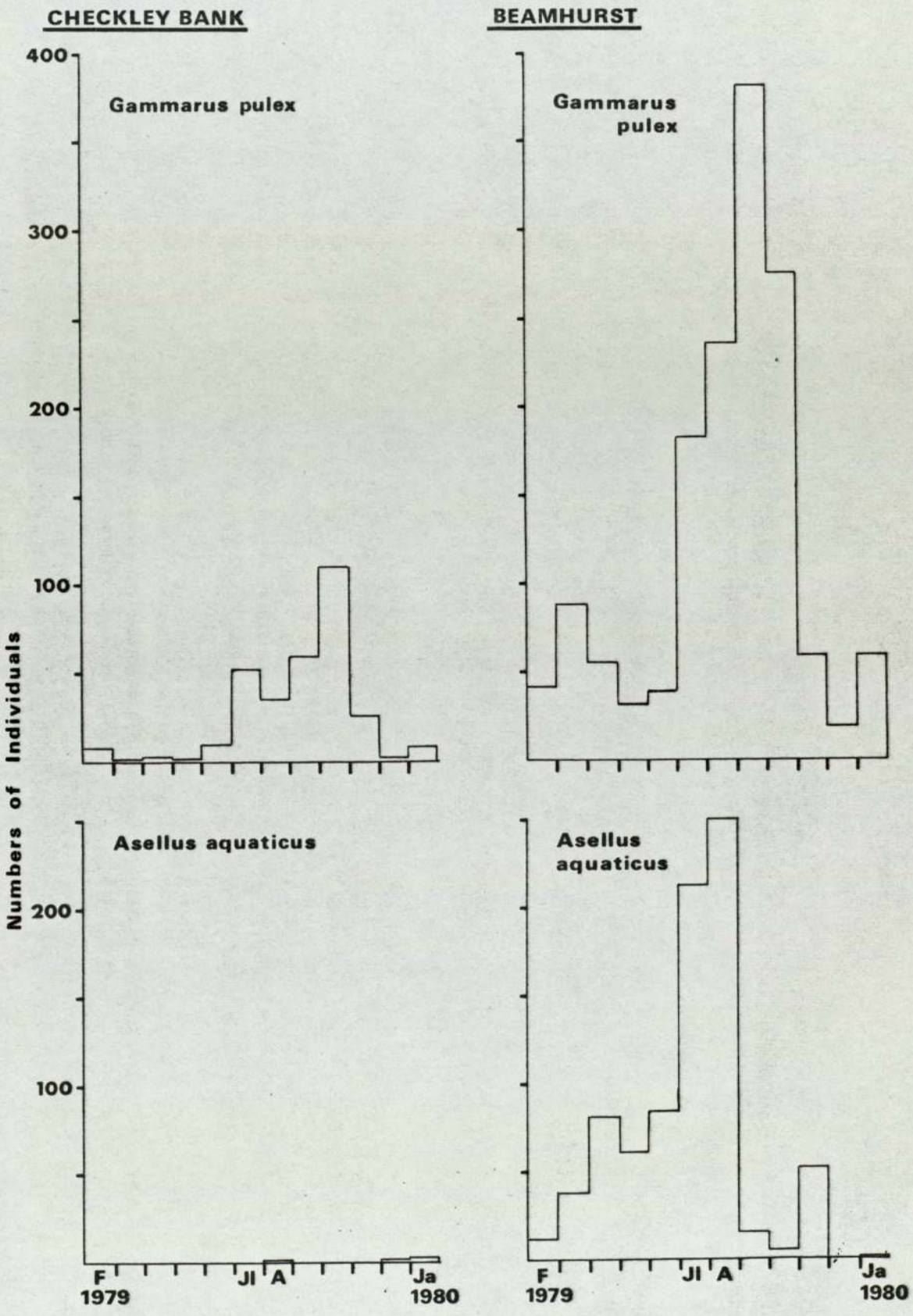


FIG.4.12. NUMBERS OF EPHEMEROPTERA, H. angustipennis and R. dorsalis SAMPLED AT CHECKLEYBANK AND BEAMHURST

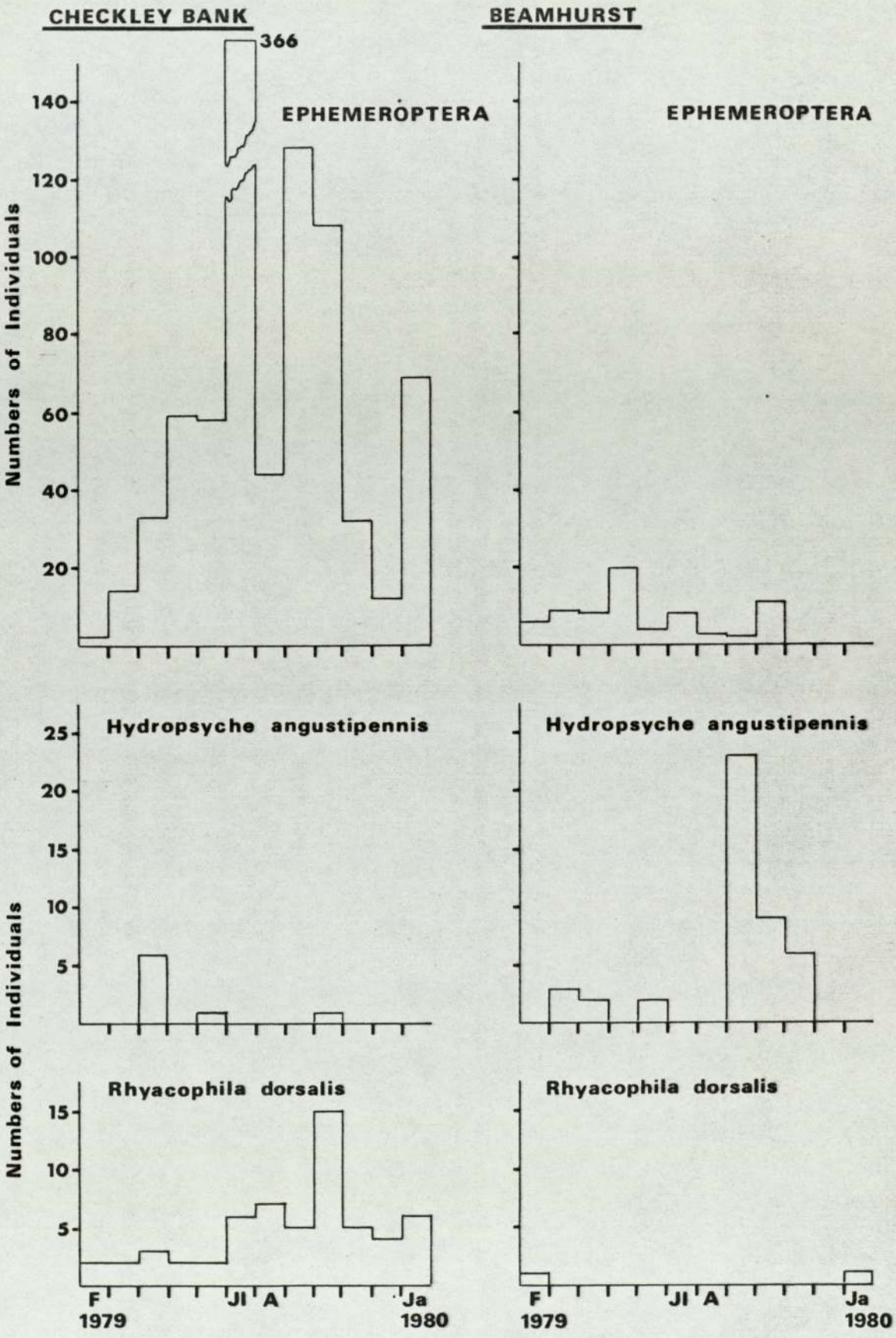


FIG.4.13. NUMBERS OF CHIRONOMIDAE AND OTHER DIPTERA SAMPLED AT CHECKLEYBANK AND BEAMHURST

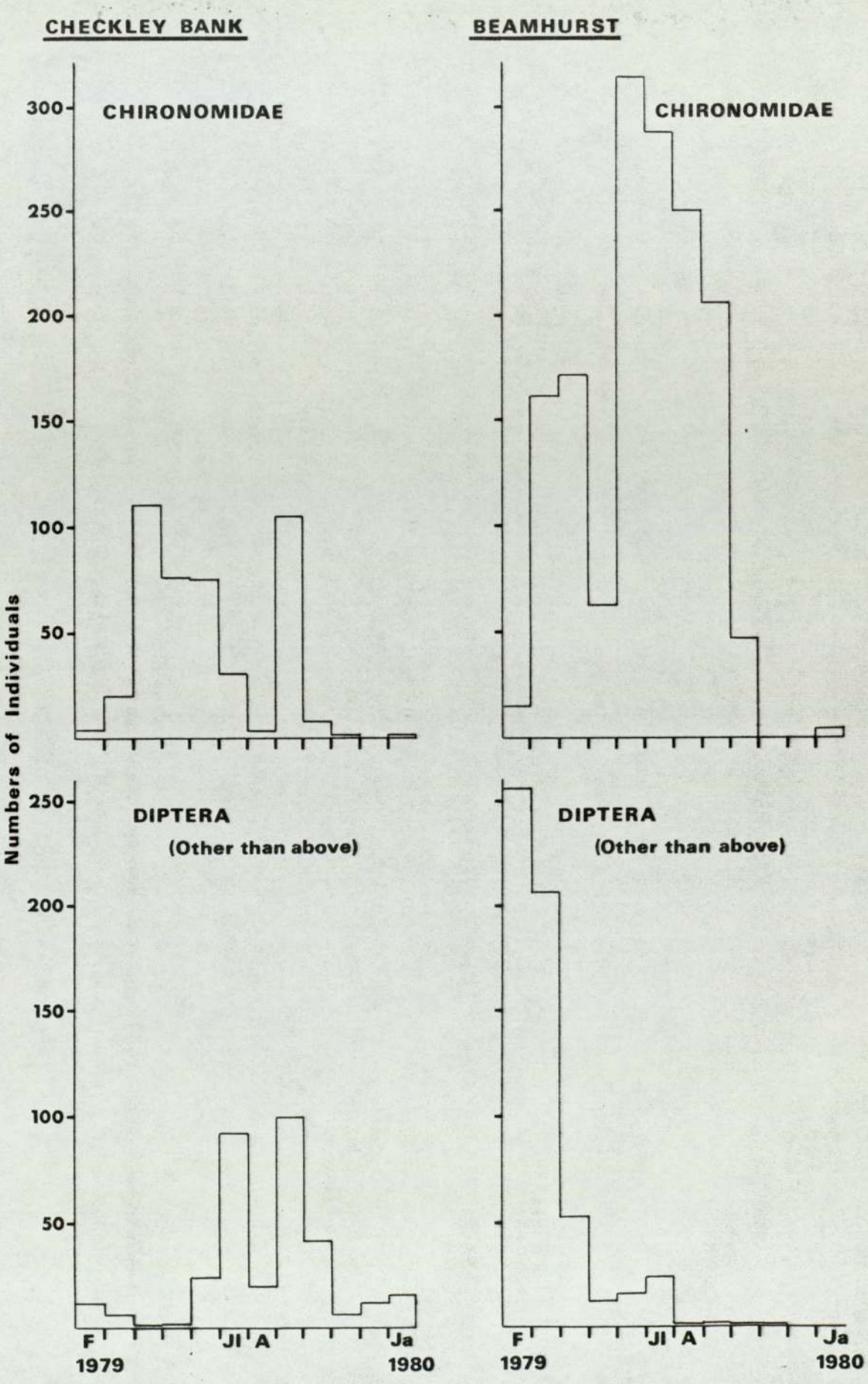


FIG. 4.14. NUMBERS OF MOLLUSCA SAMPLED AT CHECKLEYBANK AND BEAMHURST

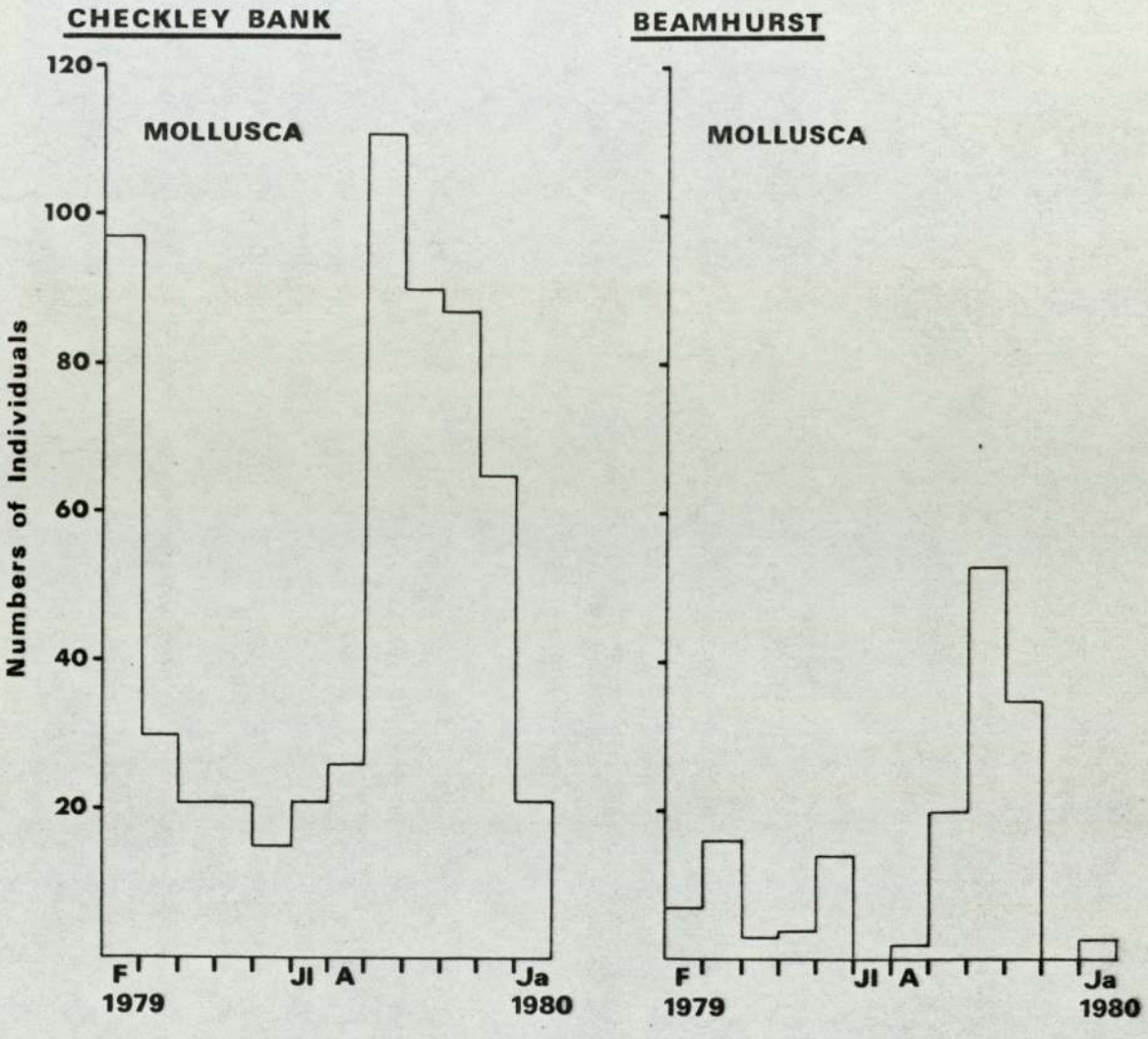
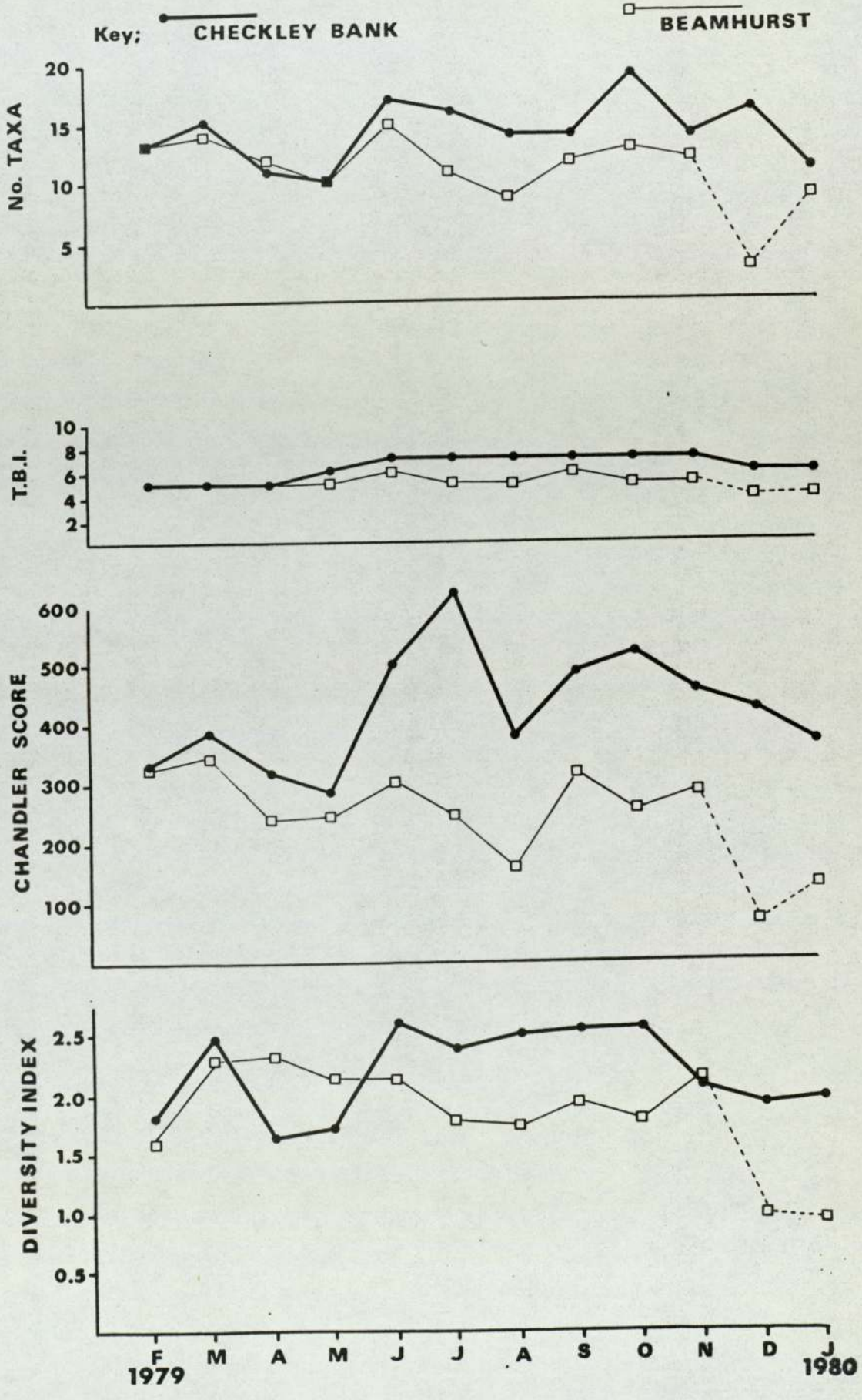


Fig.4.15. Summary of Biological Data from Checkleybank and Beamhurst. R. Tean.



Indexes calculated for each station. As may be seen from the data, the Checkleybank site generally demonstrates a higher water quality, greater species diversity and higher score than the R. Tean at Beamhurst. Fig.4.16 presents the relative abundance of organisms sampled on a monthly basis by bulking the 3 x 0.05 m^2 cylinder sampler results. Table 4.30 records the numbers of R. dorsalis and H. angustipennis taken from each station from Feb. 1979 - September 1980.

4.5.3. DISCUSSION AND CONCLUSIONS

As may be seen from the histograms, R. dorsalis occur each month at the Checkleybank station, but only intermittently are Hydropsyche found. When they do appear, it is in very small numbers, the six recorded in April 1979 at Checkleybank were pupae rather than larvae.

Although the riffle conditions appear ideal for the Hydropsyche, and the water quality should favour their survival it appears that competition from the Rhyacophila and Polycentropids exclude them from this site. They may even be eliminated because the small instars are food organisms for the Rhyacophila.

The invertebrate assemblage at this station is indicative of high water quality, generally comprising Gammarus pulex, large numbers of assorted mayflies, including Baetis spp., Ecdyonurus venosus, Ephemerella ignita, occasionally plecoptera such as Isoperla grammatica and many molluscan species, for example Hydrobia jenkinsi, Sphaerium and Pisidium spp. Oligochaeta also provide a reasonable contribution numerically but only a small proportion of the biomass.

At Beamhurst, although a rich fauna is still present, the organic enrichment to the river alters the species spectrum. The predominant Trichopteran species here is Hydropsyche angustipennis, with R. dorsalis appearing only intermittently, apparently in the early months of the year if at all. These may appear, having been washed downstream by spates. The organic effluent (estimated by conductivity tests to be

FIG.4.16. RELATIVE ABUNDANCE OF ORGANISMS SAMPLED ON A MONTHLY BASIS AT CHECKLEYBANK AND BEAMHURST

(sample = 3 cylinders = 0.15 m^2)

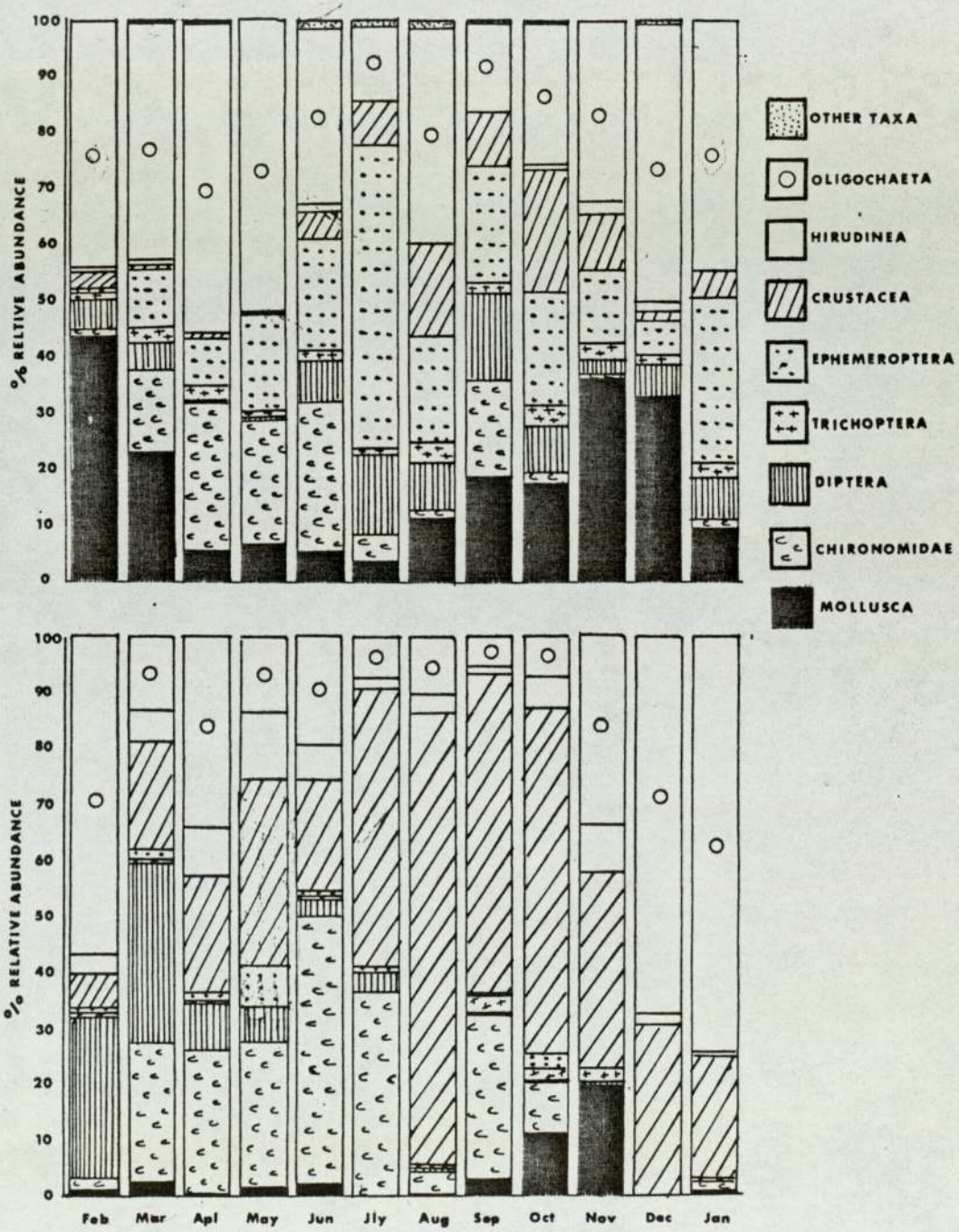


TABLE 4.30 NUMBERS OF R. dorsalis and H. Angustipennis RECORDED AT CHECKLEY BANK AND BEAMHURST, R. TEAN

Date	R. dorsalis		H. angustipennis	
	Checkley Bank	Beamhurst	Checkley Bank	Beamhurst
1979				
Feb.	2	1	-	-
Mar.	2	-	-	3
Apr.	3	-	6(pupa)	2
May	2	-	-	-
June	2	-	1	2
July	6	-	-	-
Aug.	7	-	-	-
Sept.	5	-	-	22
Oct.	15	-	1	9
Nov.	5	-	-	6
Dec.	4	-	-	-
1980				
Jan.	6	1	-	-
Feb.	-	-	-	-
Mar.	2	-	-	2
Apr.	10	-	-	2
May	7	-	-	-
June	2	-	-	4
July	10	-	-	1
Aug.	8	-	-	5
Sept.	7	-	-	1

30%:70% mix of effluent to river water) increases the nutrient status of the river, and encourages Cladophora growth.

Associated with the blanket of algae are large numbers of Asellus aquaticus, a most tolerant crustacean and a largely increased number of G. pulex, presumably encouraged by warmed water and increased food supply.

The more sensitive ephemeropterans present at Checkleybank disappear from this station leaving only Baetis, a tolerant species in the invertebrate community. The conditions favour rapid population increases in the Diptera, particularly the Chironomids, and the Tubificids, and so we see H. angustipennis associated with an assemblage typical of a mildly organically polluted river. The current velocity offers sufficient speed for successful net spinning, and the stones, together with the algae inummerable suitable sites. The increased amount of suspended solids provides ample food, and decreased competition from other insect species provides ideal conditions for their success.

This combination of physico-chemical parameters, causing alterations in community structure interact to exclude R. dorsalis from this downstream station. These results back up findings from the main field distribution survey of the superior tolerance of Hydropsyche to Rhyacophila in organically polluted rivers, and demonstrates the need to rate them differently on biological indexes and scores.

Further work, investigating the comparative tolerances of Hydropsyche and Rhyacophila will be dealt with in Chapter 4.

4.6. R. YSTWYTH AND R. RHEIDOL (Metal Pollution Studies)

As metals had been positively correlated with the distribution of Trichoptera in the large distribution survey, it was decided to investigate this more closely in the field situation.

4.6.1. METHODS

Two rivers in West Wales, known to be seriously affected by zinc pollution were investigated. The Ystwyth and Rheidol are zinc polluted

due to old mine workings in the immediate vicinity in the Cambrian mountains. Some classic field studies have been carried out in this area in the past. Both rivers have been studied over many years by various workers who have reported their chemical and biological status. A summary of these records is given in Tables 4.31 and 4.32 (Brummage et al., 1980).

The sampling area is illustrated on Sketch map 9 overleaf. Sites were sampled using the cylinder method on the Ystwyth upstream at Yr Allt (Grid. Ref. SN 848 755) and downstream of the workings at Cwmystwyth (Grid. Ref. SN 792 738).

The Rheidol, which flows from the slopes of Plynlimon and into the Nant-y-moch Reservoir was sampled some way below the dam at Ponterwyd (Grid. Ref. SN 757 845). It continues its course through the Rheidol Gorge and forms cataracts at Devil's Bridge before completing its course to the sea. The Ystwyth, like the Rheidol meets the sea at Aberystwyth.

A faunal list was compiled for each site and water samples were taken for analysis to accompany the biological data.

The aim of the field study was to observe and record the field conditions of acute zinc pollution and its effect on caseless caddisfly ecology.

Animal material, after identification and counting was dried to constant weight and acid digestion carried out to assess the metal levels.

4.6.2. RESULTS

The results of cylinder samples at each of the three sites are given in Table 4.33. Table 4.34 gives water quality data and metal levels from each station, and Table 4.35 shows the concentration factors calculated for zinc in animal material from stations on the R. Ystwyth.

TABLE 4.31. SUMMARY OF THE PARTIAL RECOVERY OF THE RIVER YSTWYTH 1919 - 1975

Date	Fish	Invertebrates	Flora	Metal Concn. in lower river ($mg\ l^{-1}$)		Reference
				Lead	Zinc	
1919-21	None	9 spp - mostly Insecta	Very poor - limited to algae and bryophytes	0.4-0.5		Carpenter (1924)
1922-23	None	26 spp - mostly Insecta	No change recorded	Nil-0.1		Carpenter (1924)
1939-40	None	63 spp. - restricted to Insecta, Platyhelminthes and Hydracarina	Very poor - still limited to algae and bryophytes	Nil.0.05	0.7-1.2	Jones (1940)
1953-56	Brown trout in lower reaches	No marked change	Very poor - continued absence of angiosperms			Jones (1958)
1975	Brown trout, sea trout, salmon, eel, minnow, stickleback and lampreys	Crustacea, Trichoptera, Oligochaeta and Hirudinea still rare or absent		0.02-0.03	0.32-0.48	SWWRD (1976) UWIST (1976)

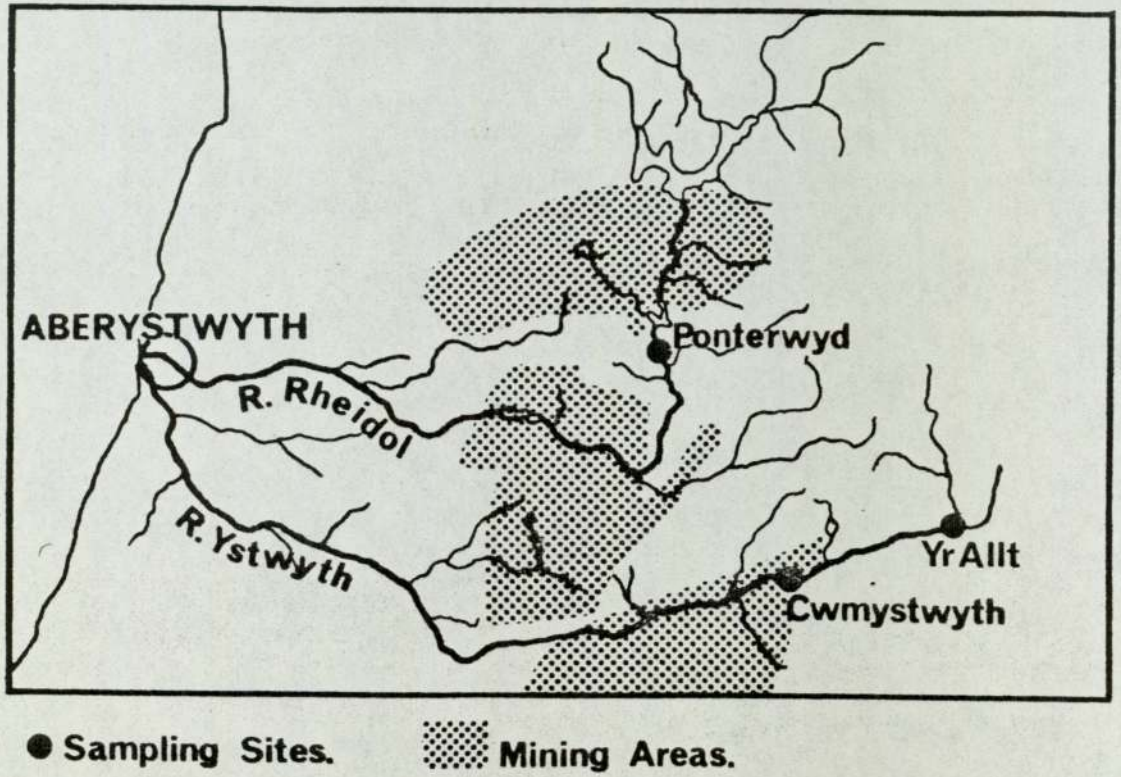
After Brummage *et al.*

TABLE 4.32 SUMMARY OF THE PARTIAL RECOVERY OF THE RIVER RHEIDOL 1919 - 1975

Date	Fish	Invertebrates	Flora	Metal Concn. in lower river (mg l^{-1})		Reference
				Lead	Zinc	
1919-21	None	14 spp - mostly Insecta	Very poor - limited to algae and bryophytes	0.2-0.5		Carpenter (1924)
1922-23	None	29 spp - mostly Insecta	Reappearance of angiosperms in lower reaches	Nil-0.1		Carpenter (1924)
1931-32	Brown trout, eel, stickleback	99 sp - mostly Insecta but with representatives of all the main groups	Increase in abundance of angiosperms	0.02-0.1	0.14-0.30	Laurie & Jones (1932) James *et al.* (1932)
1947-48	Brown trout, sea trout, eel, stickleback	125 spp - mostly Insecta (97 spp), other groups still rare.	Angiosperms mostly confined to lower reaches			Jones (1949)
1971-72	Salmon and sea trout fisheries established	Little change apparent		Nil.0.04	0.20-0.83	Jones & Howells (1975)
1975		Crustacea, Trichoptera, Oligochaeta and Hirudinea still rare or absent				UWIST (1976)

SHOWING SAMPLING SITES ON THE

R. YSTWYTH and R. RHEIDOL.



SKETCH MAP 9. SHOWING SAMPLING SITES ON R. YSTWYTH AND R. RHEIDOL

TABLE 4.33 BIOLOGICAL DATA FROM R. YSTWYTH AND R. RHEIDOL

R. YSTWYTH u/s SPECIES LIST	No. of Individuals 0.05 m^2	R. YSTWYTH d/s SPECIES LIST	No. of Individuals 0.05 m^2	R. RHEIDOL SPECIES LIST	No. of Individuals 0.05 m^2
Hydracarina	1	Naididae	>100	Naididae	7
G. pulex	4	Hydracarina	1	*I.grammatica*	2
Baetis sp.	57	*I. grammatica*	58	Chironomidae (Green)	60
Amphimemura sp.	1	*Chloroperla ap.*	2		
I. grammatica	11	*E. aenea*	1	*Stigeoclonium*	
Elminth (L)	2	*L. volkmari*	3	*Navicula*	
H. angustipennis	11	*R. dorsalis*	8	*Cosmarium*	
R. dorsalis	2	*Simulium sp.*	50	*Surirella*	
P. flavomaculatus	1	Chironomidae	10	*Closterium*	
Chironomidae (Green)	3			*Fragilaria capucini*	
Clinocera sp.	1	No algae or			
Spirogira		diatoms present			
Ulothrix					
Oscillatoria					
Synedra					
Diatoma					
T.B.I. -	9		8		6
CHANDLER SCORE -	452		492		124
B.M.W.P. SCORE -	54		39		12

TABLE 4.34. WATER QUALITY DATA FOR SITES ON THE R. YSTWYTH and R. RHEIDOL

SITE →	YSTWYTH 1 u/s ZINC	YSTWYTH 2 d/s ZINC		RHEIDOL 3	
DETERMINAND					
Temperature ^{o}C	13	15		16	
pH.	6.3	6.3		6.3	
D.O. mgl^{-1}	10.3	10.25		10.25	
B.O.D mgl^{-1}	0.6	0.8		0.9	
Total Hardness mgl^{-1} ($CaCO^{3}$)	7.0	10.0		7.0	
Ca Hardness mgl^{-1} ($CaCO_3$)	5.0	5.0		5.0	
Mg Hardness mgl^{-1} ($CaCO_3$)	2.0	5.0		2.0	
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)	0.0	0.0		0.0	
Total Alkalinity mgl^{-1} ($CaCO_3$)	0.05	0.1		0.05	
Chloride mgl^{-1}	4.2	4.2		4.2	
Nitrate $mgNl^{-1}$	0.1	0.15		0.15	
Ammonia $mgNl^{-1}$	0.3	0.4		0.4	
Phosphate mgl^{-1}	0.1	0.5		0.7	
Suspended solids (105^{o}C) mgl^{-1}	2.0	5.0		2.5	
Copper mgl^{-1}	0.962	0.174		0.357	
Cadmium mgl^{-1}	0.001	0.01		0.005	
Lead mgl^{-1}					
Chromium mgl^{-1}					
Nickel mgl^{-1}					
Iron mgl^{-1}	1.416	2.681		6.55	
Zinc mgl^{-1}	0.918	4.062		0.513	

4.6.3. DISCUSSION AND CONCLUSIONS

The zinc levels at the upstream and downstream sites on the R. Ystwyth were shown to be markedly different. At Yr Allt the level was 0.918 mgl^{-1} Zn compared to 4.062 mgl^{-1} Zn at Cwmystwyth. These levels are both higher than the zinc concentration at Llanfarian Bridge, near the mouth of the Ystwyth where Water Authority Harmonised Monitoring (W.A.H.M) record a zinc level of 0.443 mgl^{-1} over the annual period 1979 - 1980.

Similarly on the R. Rheidol the zinc level at Ponterwyd was found in this study to be 0.513 mgl^{-1} Zn compared to W.A.H.M station levels at Penybont Bridge further down the Rheidol to be only 0.197 mgl^{-1} (1979 - 1980).

On both rivers the decreased concentrations recorded at W.A.H.M stations is due to increased volume of water having a diluting effect, and the fact that these are mean values taken over one year. The individual values found in this study were at summer low flow conditions and thus were more concentrated. Jones (1960) reported increased zinc concentrations on the R. Ystwyth in the drier summer period.

Examining the data collected from the R. Ystwyth, both sites have very similar water quality characteristics with high dissolved oxygen, very soft water and slightly acidic conditions. In a soft water, acidic environment, the metals will generally be in their potentially most toxic form. There is a marked increase in metal concentrations at Cwmstwyth which was one of the most intensively mined sections of the Ystwyth valley in the past. Here rivulets drain tailings which spill to the edge of the river.

Physical conditions at both sites were comparable, both being riffle sections with large stones and shallow fast flowing water, although the upstream station was overshadowed by riverbank trees - whilst the Cwmystwyth site was very exposed.

Biologically, in the river as a whole there was a distinct lack of cased caddis, Mollusca and Hirudinea and few Crustacea. Comparing the upstream and downstream sites, i.e. above and below the diffuse pollutional source the most noticeable change was the reduction in diversity. Absence of G. pulex, Hydropsyche, Polycentropids, algae and diatoms. As expected and recorded by previous workers, the hardy metal-resisting stoneflies were in evidence at all three sites investigated. It appears that the mayfly Baetis, dominant at the upstream Ystwyth site was replaced by I. grammatica, a very tolerant stonefly at the downstream station. R. dorsalis is the only caseless caddis which survives the rigors of conditions at station 2, H. angustipennis and P. flavomaculatus being present upstream only.

A number of factors may restrict the ability of Hydropsyche to survive at the downstream site although the dissolved oxygen and flow rate should be sufficient to maintain them. Firstly, there is a lack of algae at the downstream site, an important factor in the habitat and diet of Hydropsyche. It has been noted in previous field work accounts that H. angustipennis is closely associated with abundant growths of filamentous algae (e.g. R. Tean, Staffs.) whereas R. dorsalis prefers the bare stones of an exposed riffle section - presumably so that predation is easier. In the R. Rheidol, the chemical quality of the water is virtually identical to that of the R. Ystwyth except that metal levels are lower.

The poor diversity of fauna is probably due to the physical environmental conditions and consequent lack of suitable habitats for benthic invertebrates. Large boulders encrusted with a thick layer of diatomaceous residue appear to be the factor restricting the fauna to predominantly worms and midge larvae. Although theoretically this abundance should be advantageous as a food supply to the Hydropsychids, their frequency is restricted due to physical conditions limiting the spaces available for successful attachment and net spinning. The

limitations for the predatory Rhyacophila at this station would be lack of suitable food organisms. Differential sensitivity to toxicants and the ability to bioconcentrate metals seem to be two important factors in survival of the Trichoptera.

On the R. Ystwyth Jones (1940) found no mollusca, crustacea, worms or leeches present at zinc levels of 0.7 - 1.2 mgl^{-1} Zn, but in a tributary of the Ystwyth at 57 mgl^{-1} Zn insect larvae were still present. In further work (Jones, 1958) when levels of zinc had fallen to 0.2 - 0.7 mgl^{-1} Zn the fauna still comprised only the more resistant R. semicolorata, H. lateralis, B. rhodani, C. tripunctata and Esolus parallelopipedus.

This differential tolerance by various invertebrate groups is also shown in work by Weatherley et al. (1975), where zinc levels of 0.1 - 0.4 mgl^{-1} Zn in Lake Burley restricted the fauna to a few mollusca, crustacea, odonata and ephemeroptera. Sprague et al. (1965) documents work on the North West Miranichi River where zinc concentrations over 1.5 times the 7 day LC50 for salmon, still had populations of caddis-flies and midges unaffected by the zinc and copper pollution. However, no hatch of the caddisfly Hydropsyche or mayflies occurred in this river, suggesting a differential sensitivity to life stages, as with copper toxicity.

In the Molonglo River (Australia) it was reported that in the zinc polluted parts of the river the fauna was reduced to bugs, beetles, caddisflies, diptera and stoneflies. Herbst (1967) records that in the heavily zinc polluted R. Sultz (80 ppm $CaCO_3$) that G. pulex and L. orata were present only when the zinc concentration was less than 0.25 mgl^{-1} Zn. A larger variety of insecta were present between concentrations of 0.8 - 6.5 mgl^{-1} whilst Baetis withstood 25 mgl^{-1} and Rhyacophila, 29 mgl^{-1} Zn.

More recently work by Abel and Green (1981) records data from two similar tributaries East and West Allen in N.E.England. The West Allen is heavily zinc polluted 0.45 - 3.65 mgl^{-1} Zn whereas East Allen is not so severely affected <0.001 - 0.36 mgl^{-1} Zn. Surber sampling and chemical analyses over 18 months monitoring took place. Only 18 species were re-

corded in W. Allen compared with 30 sp. in E. Allen. The most zinc tolerant species were again the stoneflies Leuctra sp., Chloroperla torrentium, mayflies B. rhodani and R. semicolorata, chironomids and the cased caddis Limnophilus sp., together with R. dorsalis, the caseless caddis. Under experimental conditions, the Limnophilus survived for two weeks in a solution containing 100 mgl^{-1} Zn.

A further study by Abel and Green investigates decrease in food consumption by Limnophilus in a zinc polluted environment. Essentially consumption was decreased by 10 - 30% which may be of great ecological significance to the survival of trichoptera larvae in the field situation.

The Ystwyth

The data from the Ystwyth sites (Table 4. 35) show that metal levels in caseless Trichoptera show a positive correlation with total metal levels in the river water. Brown (1977) working on invertebrates in the zinc and copper polluted R. Hayle reports zinc levels at a maximum of 2.5 mg l^{-1}. This compares with 4.062 mgl^{-1} in the Ystwyth. She reports an almost proportional increase in copper and zinc concentration in the caseless caddisfly larvae in these conditions. In his paper (1940a) Jones noted that campodeiform Trichoptera were far more common in the Ystwyth than cased forms. This still appears to be true, but in the R. Hayle Brown found several species of cased caddis. The case-dwelling types bioconcentrate less metal than the 'free-living' forms at the same site, this probably being due to their case affording a measure of protection. In the R. Hayle, Brown (1977) reports significant correlation of metal levels in invertebrates with increase in zinc at all but one site although there are consistently high zinc concentrations (0.42 ± 0.15 mgl^{-1}) throughout the year at that site. She suggests that animals exposed to high concentrations of metals may adapt to these conditions. From laboratory investigations, where concentrations were far in excess of any environmental level, the H. angustipennis continued

Table 4.35

Concentration Factors of zinc for species at Stations on the R. Ystwyth.

Station	Species	Concentration Factor
1		
Zn = 0.918 mg l^{-1}	Stoneflies	481.5
in the river	*Hydropsyche sp.*	1409.6
water	*Baetis sp.*	1402.2
	Rhyacophila sp.	191.2
	Gammarus sp.	163.4
2		
Zn = 4.062 mg l^{-1}	*Rhyacophila sp.*	125.5
in the river	*Simulium sp.*	131.4
water	Stoneflies	145.9

to bioconcentrate the metal.

On the Ystwyth the metal uptake at site 1 was greatest in Hydropsyche>Baetis>Stoneflies>Rhyacophila>Gammarus (Table 4. 5). At site 2 Stoneflies>Simulium>Rhyacophila although the increase in uptake of the Rhyacophila has increased approximately four-fold, the same approximate increase in metal levels as in the river water. Complementary evidence on the ability of caseless caddis and other insecta to bioconcentrate zinc comes from a recent paper by Harding et al. (1981).

In a study of heavy metals in the Derwent Reservoir Catchment, N. England elevated levels of zinc, lead and cadmium were found in Bolts Burn, a tributary of the Derwent, due to mining activity.

Maximum concentration of zinc was 0.175 mgl^{-1} although not such extreme concentrations as may be found in West Wales, the data demonstrate that caseless caddis, stoneflies and mayflies are the most resilient species to zinc pollution. Levels of zinc in these species are recorded below.

R. dorsalis	u/s Zn source	561 ppm Zn
	d/s Zn source	687 ppm Zn
Leuctra spp.	u/s	414±62 ppm Zn
	d/s	1293±153 ppm Zn
E. venosus	u/s	2750 ppm Zn
	d/s	15050 ppm Zn

Harding et al. compare the long term use of invertebrates for monitoring with algae or plants, e.g. Lemanea - the use of this alga would be limited because of the relatively short growing periods, therefore invertebrates may be far more useful. The concentrations of metals in invertebrates is probably a reflection of the permeability (Beament, 1961). Caseless Trichoptera had the highest level of metals as seen by this study, the work by Brown, suggesting high permeability of Hydropsyche and Rhyacophila sp. Work on pesticide toxicity to egg masses

of the caddis Triaenodes tardus, Milne (Belluck & Felsot, 1981) reports that they are sensitive to agrochemicals in the ppt-ppb range. The gelatinous covering has to be penetrated before bioconcentration can occur in the eggs.

Kenega (1973) has described bioconcentration as a two-stage phenomenon: (1) adsorption (or pesticide on to a biological surface) and (2) absorption into the tissue matrix. It is not known if this is true in the case of larvae subjected to metals, but it is a possibility.

The extremely high values of zinc in Hydropsyche may in part be due to contamination by particles containing zinc, and also zinc in their gut contents. As their ecology and feeding habits differ from those of Rhyacophila this may be the reason that there is approximately 74 times as much zinc in Hydropsyche at site 1.

The concentration factors for animals at both sites are considerable (see Table 4.45) illustrating that they are very efficient monitors. Caseless caddis may be as useful as stoneflies for indicating the presence of metals in a river, and their differing tolerance to organic pollution may be of use in indicator systems. Using traditional indexes, designed to assess organically polluted waters, the suppression of species due to metal toxicity may not be immediately apparent. Upon examination of a biological sample, the restricted community will provide evidence suggesting toxic pollution.

As indicators of pesticide contamination they may also prove a valuable guide or indicator, this is suggested in work by Marking and Chandler (1981). They tested non-target aquatic organisms for toxicity to bird control pesticides, for example Hydropsyche, mayfly, mollusc and frog. Compared to other species tested Hydropsyche was extremely sensitive to methiocarb, a repellent.

Work by Nehring et al. (1979) compared the reliability and sensitivity of detection of lead pollution using Hydropsyche and other aquatic invert-

ebrates by atomic absorption spectrophotometry and the concentration factor method. It was found that both methods provided equally reliable and accurate results, but that co-incidentally he found an advantage using the concentration factor of metals by invertebrates. At one stage of Nehring's experiments, a flood scoured away all the lead-containing sediments of the river bed. The lead levels were then below the detectable limits of 0.1 mgl^{-1} with A.A.S. Analysis of invertebrate material by the concentration factor method revealed estimated lead levels to now be 0.04 - 0.07 mgl^{-1} at the previously heavily polluted sites on the river. Advantages of the concentration factor method are firstly easier detection of trace levels of pollutants at levels not detectable by A.A.S., secondly dried insect samples do not deteriorate with age, thereby eliminating the necessity of immediate analysis for reliable results and finally aquatic insects may provide a more realistic analysis of the total metal in the stream including the metal laden substratum not accounted for by water samples.

In conclusion, in the field situation caseless caddis larvae may prove valuable "in situ" biomonitors of heavy metal pollution.

5. EXPERIMENTAL STUDIES

5. EXPERIMENTAL STUDIES

5.1. Objectives

The aims and objectives of this section of the study were broadly four-fold. Firstly to carry out intermediate field-laboratory studies at the Checkley Hydrobiology Research Station to establish the respective tolerances of Hydropsyche angustipennis and Rhyacophila dorsalis larvae to three different water qualities. This was a natural progression from the field work, to investigate the effect of organic pollution upon these two species.

Following the evolution of a satisfactory method of maintaining large numbers of caseless caddis larvae under laboratory conditions, the remaining three aspects of study were performed. Acute toxicity studies on H. angustipennis were completed with specific toxicants, namely copper, zinc, ammonia and two herbicides diquat and terbutryne. The next experiments investigated the bioconcentration of metals by H. angustipennis, incorporating animals used in the acute toxicity tests above. This work was extended to investigate the accumulation of zinc using radioisotope techniques.

Finally, experiments were undertaken using respirometric techniques to elucidate the effects of single and combined parameters such as temperature, dissolved oxygen, pH and ammonia in an effort to establish tolerance limits with regard to the autoecology of this species.

5.2. Chronic Laboratory-Field Studies of H. angustipennis and R. dorsalis.

An investigation into the tolerance of these two species of caseless caddis larvae to three different water qualities was carried out at the Aston Applied Hydrobiology Field Station at Checkley, Staffordshire. This field station is situated on, and belongs to Severn-Trent Water Authority on the Blithe Valley Water Reclamation Works site.

For ecological experimentation on fish and aquatic invertebrates, three simulated channels had been constructed in parallel to the R.

Tean. Each channel is 300 m in length, with alternating riffle and pool sections (see Fig.5.1). Water from the R. Tean and sewage effluent from the Blithe Valley works are mixed and used to provide three different water qualities.

Channel A.	River water only	
Channel B.	75% river water	25% effluent
Channel C.	50% river water	50% effluent

Intensive monitoring of water in each channel is carried out continuously by probes and recorders (and by automatic sampling) housed in a hut at the top of the channels. pH is monitored using an E.I.L. Model 2836 Industrial pH Meter, whilst dissolved oxygen is monitored by a SIMAC Model 505 D.O. meter.

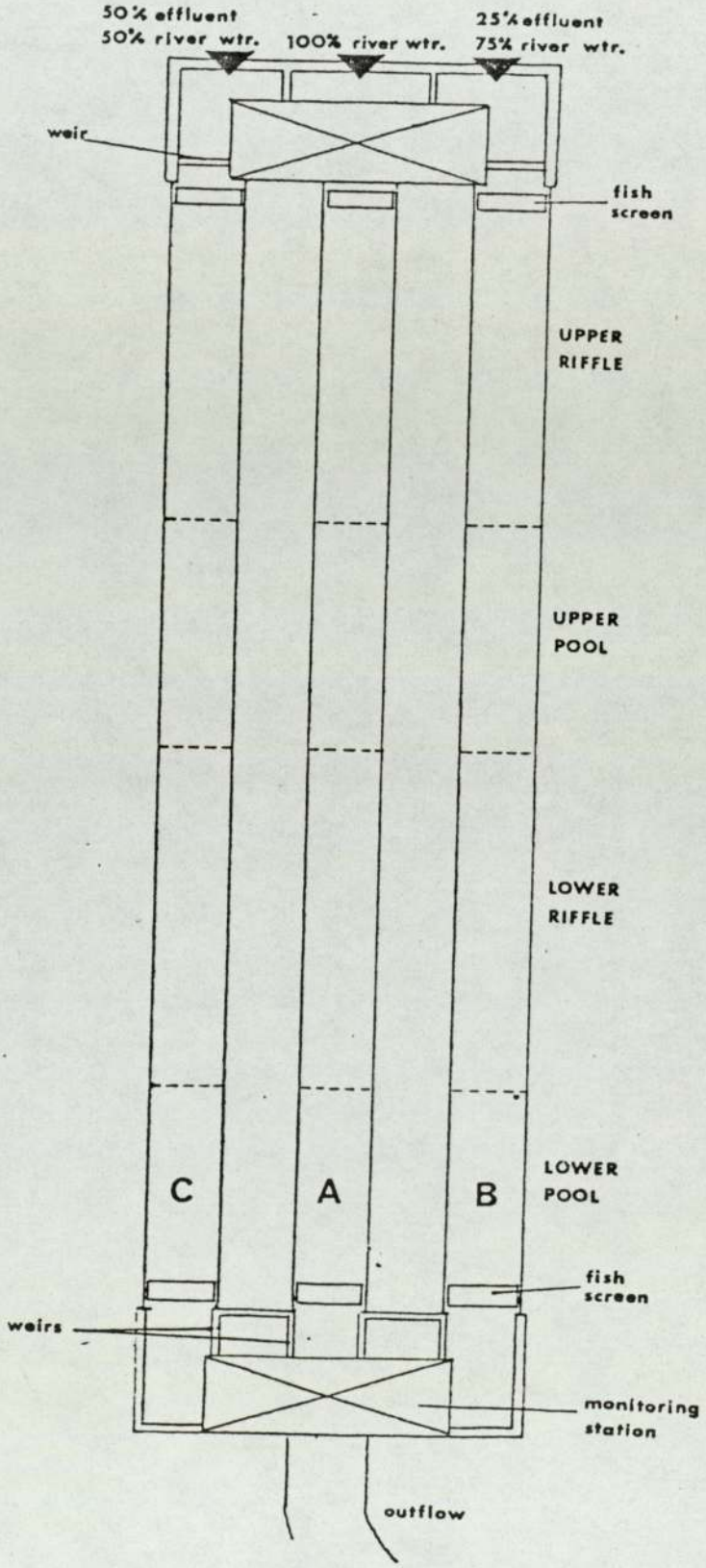
Advantage was taken of the opportunity to study under intermediate field-laboratory conditions, the survival/mortality of H. angustipennis and R. dorsalis in different mixtures of river water and sewage effluent.

5.2.1. Methods

Three 10 litre plastic tanks were set up inside the monitoring hut at the top end of the channel system (Fig.5.1). A system of monopumps and overflows was constructed so that water was pumped up from the respective channel at approximately 4 mins/litre into three tanks marked A, B and C. Tank A received river water only, tank B, 25% effluent and tank C, 50% effluent.

Containers in which to keep captive the fifth instar H. angustipennis larvae and final instar R. dorsalis were constructed. For the omnivorous H. angustipennis, bags made of fine mesh nylon curtain netting, weighed down with stones and secured with a plastic tag sufficed to contain the larvae. The animals were consequently afforded a suitable environment in which to spin their nets; they were not fed during the experiments as sufficient detritus was present in the water being

Fig.5.1. Plan of Checkley Channels



supplied to the tanks on which they could feed.

The R. dorsalis larvae being predatory and free ranging, required a different type of container. Individual pockets were sewn in nylon curtain netting, the open tops of the pockets being secured by a plastic strip such as that used to bind together folders. This was suspended over the tank using dowel rods, so that the pockets containing individual larvae hung in the water within the tanks. The larvae were maintained on small Simulium larvae pumped into the tanks with the water.

Twenty-five H. angustipennis were placed in each bag and were replicated for the three tanks, and ten R. dorsalis in separate pockets were suspended in each tank. Initially, daily counts, and later twice weekly counts were made of the numbers of larvae surviving in each water quality. The temperature, dissolved oxygen and pH readings were also recorded. From the recordings of mortalities, the data were transferred on to Log-Probability scale paper and Median Lethal Times calculated.

The experimental period for H. angustipennis ran from Day 1 to Day 45, immediately followed by the experimental period for R. dorsalis, Day 46 to Day 70.

5.2.2. Results.

Figs.5.2, 5.3 and 5.4 summarise the temperature, dissolved oxygen, and pH readings taken during the full 70 days of experimentation on both species of caseless caddis. The increase in the effluent content from A (river water) to B (25% effluent) to C (50% effluent) is reflected in these readings. Tank C, with 50% effluent is consistently warmer, with lower D.O and pH values than tank B containing only 25% effluent. This in turn is consistently higher for all three parameters than tank A which contained river water only.

Table 5.1 records the maxima and minima for temperature, D.O. and pH in each of the tanks.

Fig.5.2 Temperature, Dissolved oxygen and pH recorded in tank receiving 100% river water

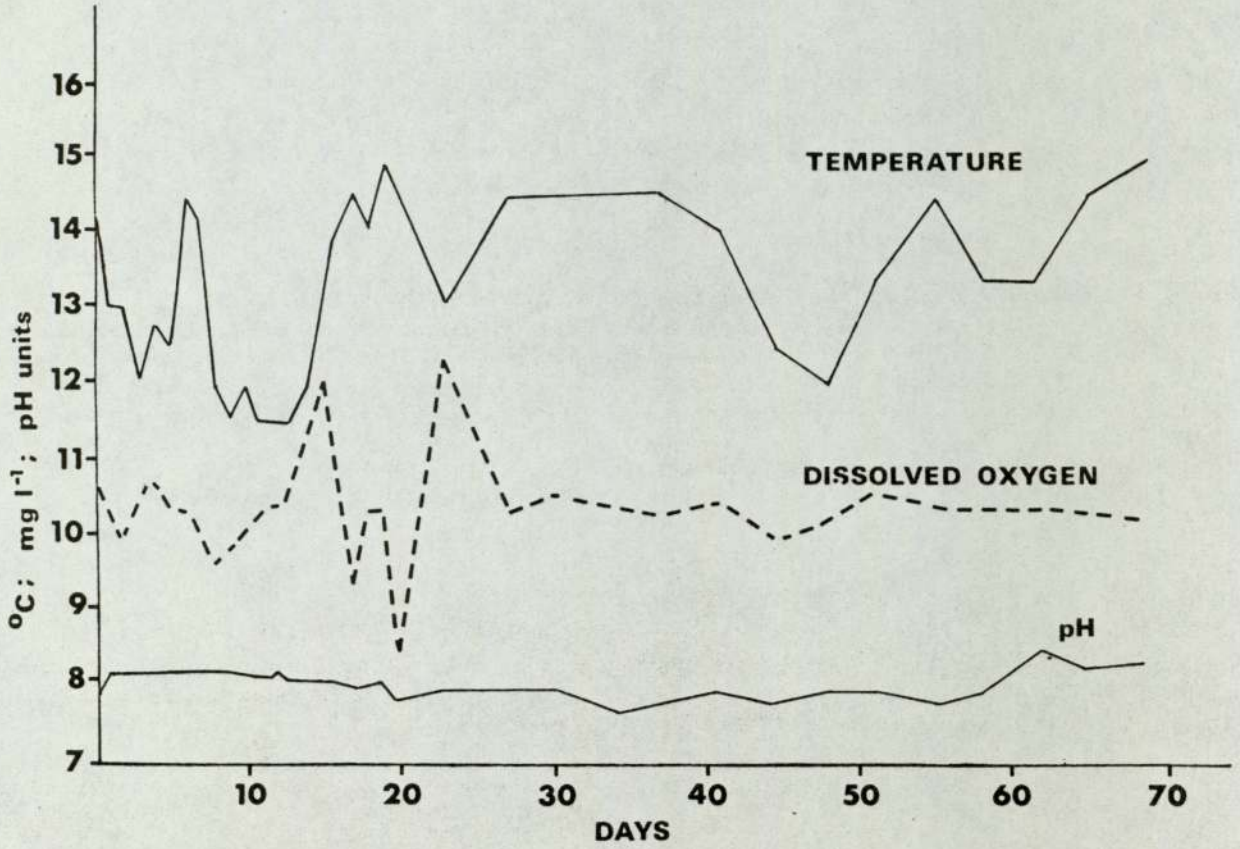


Fig.5.3. Temperature, Dissolved oxygen and pH recorded in tank receiving 25% effluent, 75% river water

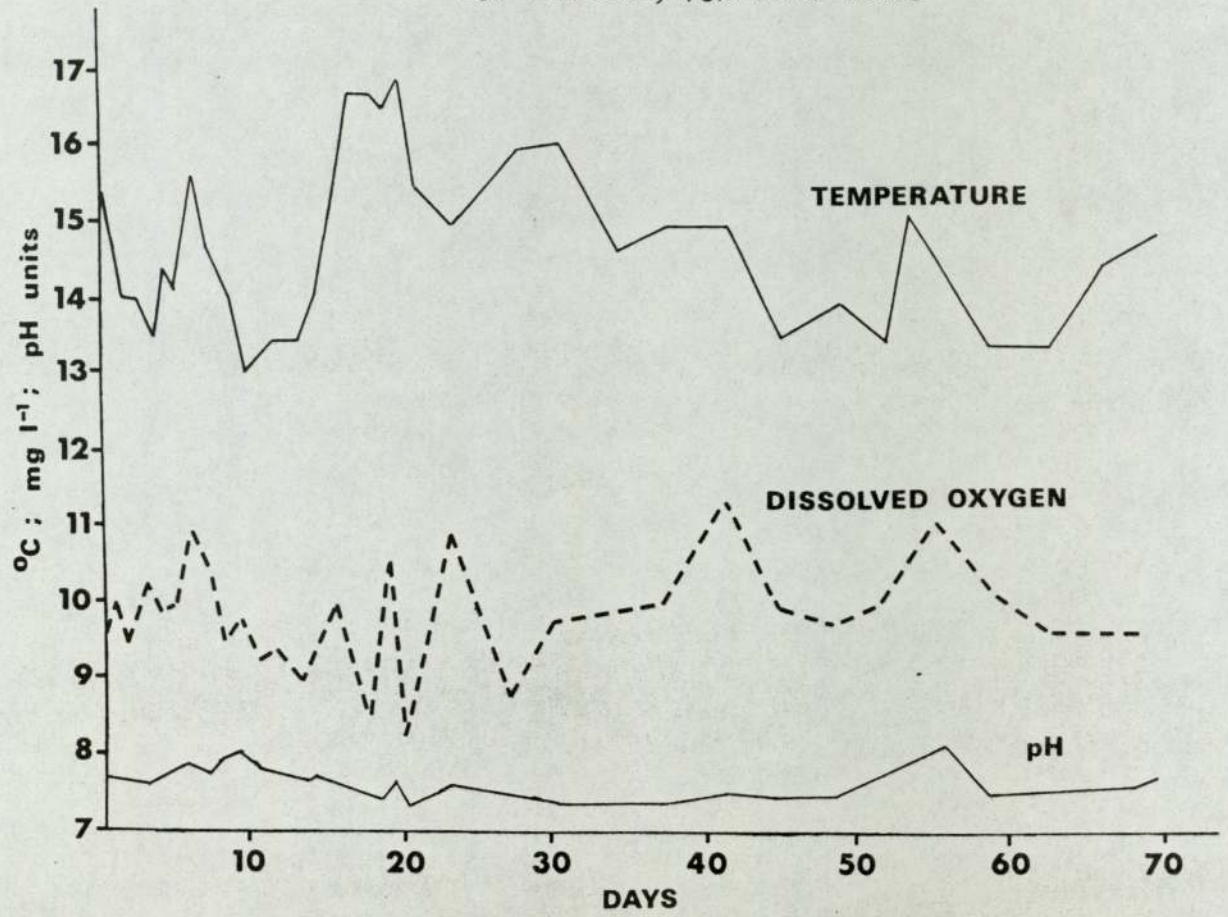


Fig.5.4. Temperature, dissolved oxygen and pH recorded in tank receiving 50% effluent, 50% river water

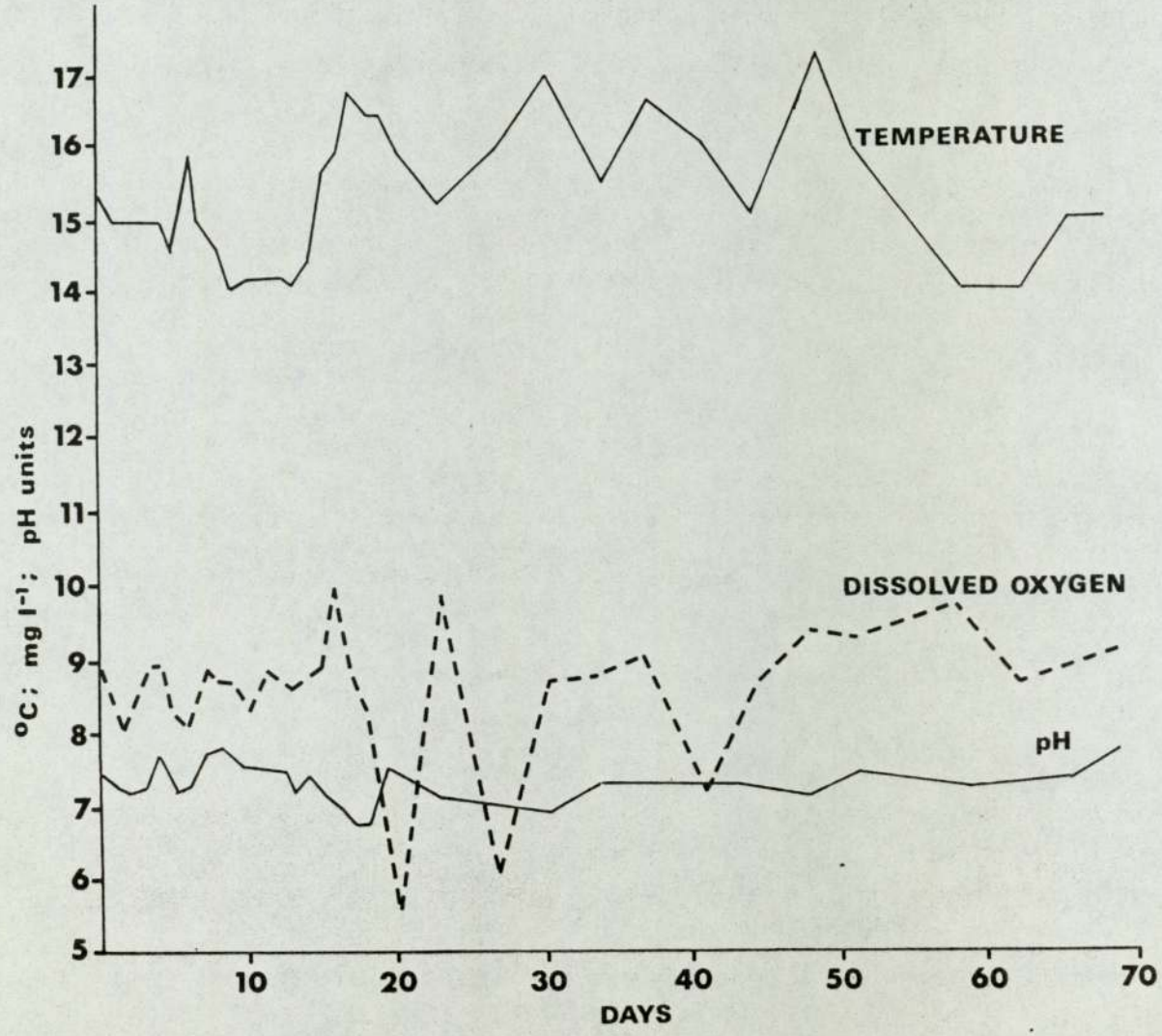


TABLE 5.1 Maxima and Minima for Temperature, Dissolved Oxygen and pH in tanks containing River Water, 25% and 50% sewage effluent.

		River Water		25% Effluent		50% Effluent	
		Min.	Max.	Min.	Max.	Min.	Max.
H. angustipennis							
Days 1-45	Temp.	11.5	15.1	13.0	16.8	14.1	17.2
	D.O	8.5	12.6	8.3	11.5	5.5	10.1
	pH	7.6	8.2	7.3	8.2	6.8	7.8
R. dorsalis							
Days 46-70	Temp.	12.0	15.1	13.5	15.4	14.1	17.5
	D.O	10.0	10.8	9.8	11.2	8.8	10.0
	pH	7.7	8.5	7.5	8.2	7.2	7.8

LT.50 values calculated for H. angustipennis and R. dorsalis in the three water qualities are presented in Figs. 5.5 and 5.6 respectively. A summary of these results may be found in Table 5.2.

5.2.2. Discussion and Conclusions

It may be seen from the data summarised in Table 5.2 that in every water quality the Hydropsyche larvae are more tolerant than those of R. dorsalis. For both species the tolerance increases as water quality improves, thus LT50 values are extended from 50% effluent to 25% effluent to river water only. In experimental conditions, larvae have to be disturbed frequently for counting the numbers alive and dead. This presumably stresses the larvae and certainly disturbs H. angustipennis in net spinning activities. It may be that their LT.50 values would have proved to be even longer in a true field situation.

However, the differential tolerances apparent from this study may explain the distribution of the two species at two sites, upstream and downstream of the sewage works. This work appears in Section 4.5 and is summarised in Table 4.30

For R. dorsalis, the gradients of decreasing tolerance with increasing concentrations of effluent, substantiates field observations from the R. Tean and Checkley Channels. R. dorsalis is commonly found in the Checkleybank riffle upstream of the sewage works, but is only recorded twice at Beamhurst during a 19 month sampling period. At Beamhurst, the river is downstream of the sewage works, and a dairy outfall, and by conductivity readings the water quality is estimated to be equivalent to 33% sewage effluent. Presumably the fall in water quality and changes in physico-chemical parameters precludes its survival.

The explanation of the distribution of H. angustipennis in the experimental, versus the field situation, is a little more complex. From the LT.50 values, theoretically these larvae should be more successful in river water only than in effluent solutions, hence there should

Fig.5.5 LC50 values for H. angustipennis in three different water qualities

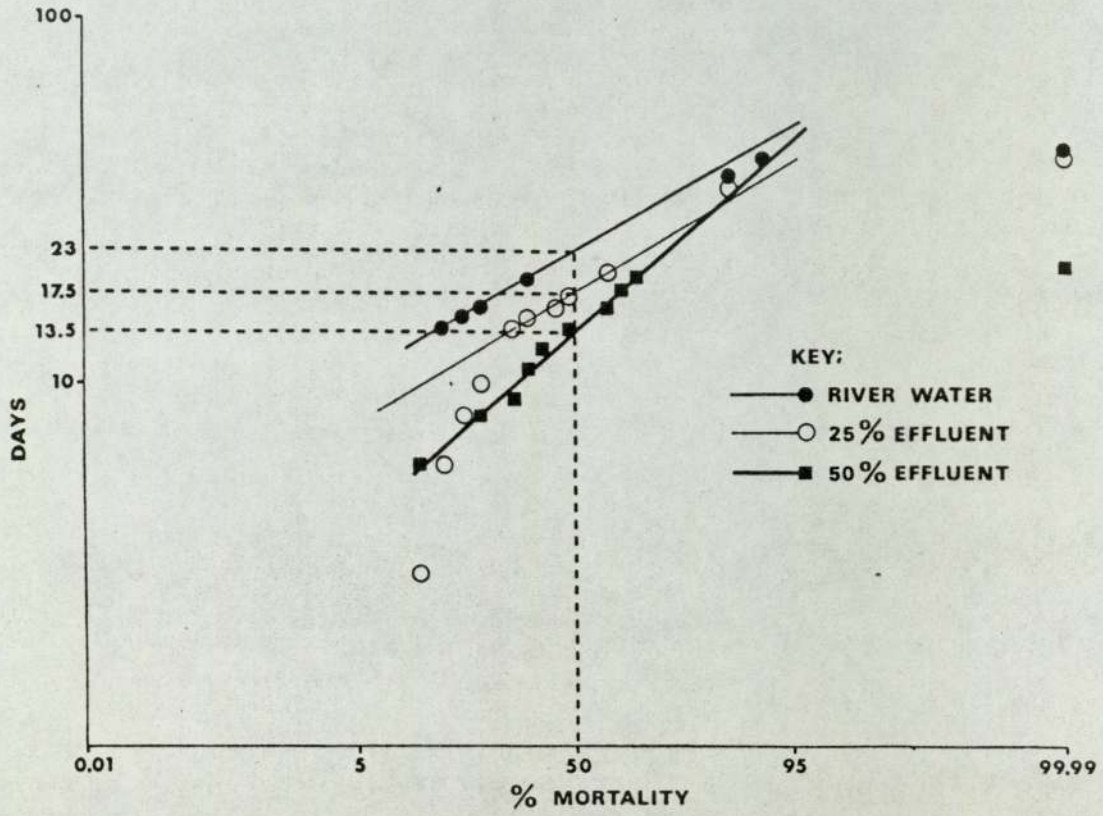


Fig.5.6. LC50 values for R. dorsalis in three different water qualities

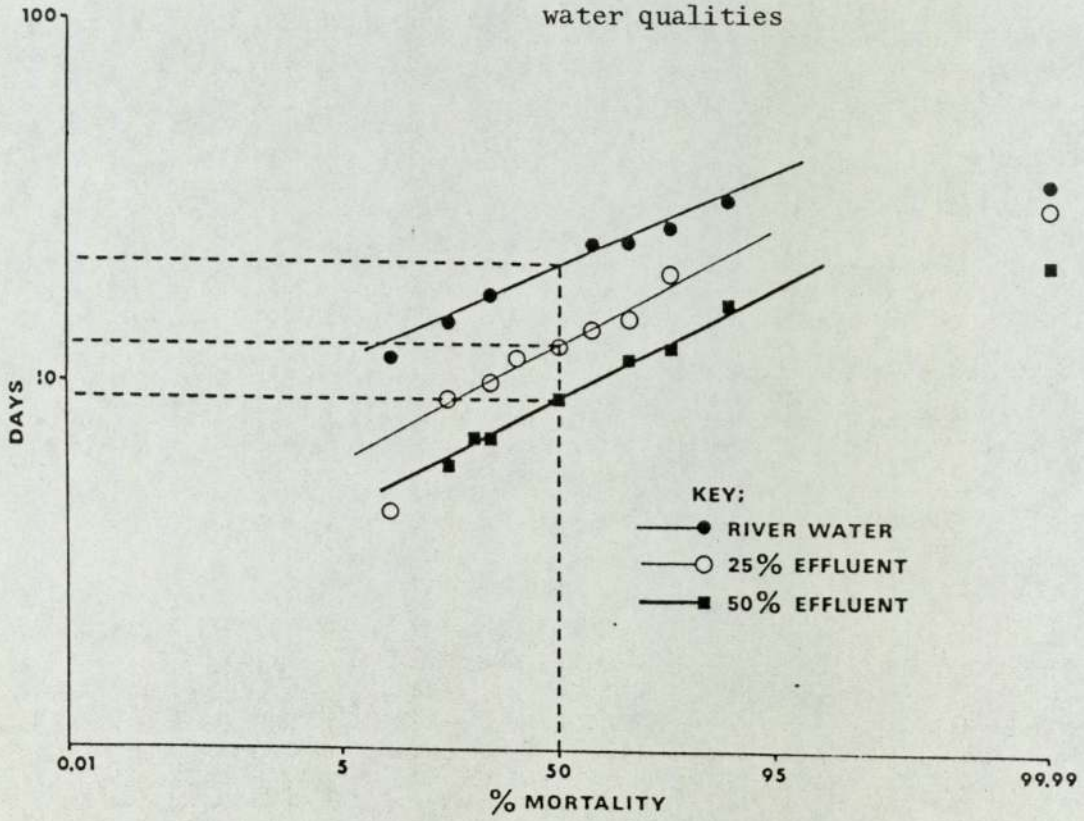


Table 5.2. Median Lethal Times for H. angustipennis and R. dorsalis in three different water qualities

Species	Water	LT50
H. angustipennis	River water	LT50 = 23.0 days
	25% effluent	LT50 = 17.5 days
	50% effluent	LT50 = 13.5 days
R. dorsalis	River water	LT50 = 20.0 days
	25% effluent	LT50 = 12.5 days
	50% effluent	LT50 = 8.0 days

be frequent occurrences in the riffle at Checkleybank. However, only on three occasions are H. angustipennis recorded at this station in 19 months.

Observations by Edington (1981) in the field have noted that R. dorsalis will take small Hydropsyche larvae. This factor may explain the absence of Hydropsyche at Checkleybank, where all other parameters favour its survival. In contrast to this, at Beamhurst, H. angustipennis are frequently sampled (12 months out of 19) and were found in relatively large numbers (e.g. 22 individuals per 0.05 m^2 cylinder, Sept.1980). Their success may be attributable to their higher tolerance than R. dorsalis to organic effluents, causing R. dorsalis to be eliminated and thus removing a predator of the Hydropsyche larvae. Another reason may be that an organic effluent increases the nutritional load and that this is an advantageous factor for larval growth and survival. Certainly, from personal observations, large densities of H. angustipennis are present at stations below sewage outfalls and below lake outflows, both sites being rich in detritus on which the Hydropsyche larvae may feed.

If R. dorsalis, the predator, were absent from Checkleybank, then H. angustipennis should be capable of surviving in the higher quality water, as demonstrated in the LT.50 tests.

5.3. ACUTE TOXICITY STUDIES

5.3.1. Introductory Review

Increasingly in recent years there has been interest in the environmental effects of heavy metals. Specifically in the freshwater ecosystem, ideally each trophic level should be tested in order that potential risk may be calculated. Effects of the discharges of toxic substances into the aquatic ecosystem need to be monitored, quantified and toxicity tests carried out in order that safe levels to the biota may be established and advised.

The term 'Heavy metals', although not rigidly defined, is generally held to refer to those metals having a density greater than five. They are serious toxicants, even if present in only trace amounts and Neiboer & Richardson (1980) also point out that the term is used where there are connotations of toxicity and because of this, data on some lighter elements are sometimes included in general accounts of heavy metals. Neiboer & Richardson proposed that the term should be abandoned entirely in favour of a classification separating metal ions into those which are oxygen seeking, those which are nitrogen/sulphur seeking and those which are intermediate. The authors demonstrate the biological relevance of their classification, and their concern for scientific accuracy is commendable, but the term "heavy metal" still arouses interest from many disciplines which would be lost if the term were discarded.

From the field work carried out in this research programme, some heavy metals are of particular interest with regard to Trichopteran species and these will be investigated further after clarification of some further terminology. Heavy metals in the aquatic environment may be dangerous to organisms which bioconcentrate, bioaccumulate or biomagnify them and increase their effects. These three concepts are different, and have been used in this work as by Kneip & Lauer (1973).

Bioconcentration: the ability of an organism to concentrate a substance from the aquatic system (expressed by the concentration factor).

Bioaccumulation: the ability of an organism to concentrate throughout its lifetime, so that the concentration factor is continually increasing (this assumes net accumulation over excretion. N.B. it has been rarely demonstrated for copper).

Biomagnification: the occurrence of a substance at successively higher concentrations with increasing trophic levels in food chains. These workers point out that while biomagnification occurs with chlorinated hydrocarbons, the evidence for its occurrence with

metals in aquatic ecosystems appears to be weak or non-existent.

Toxicity testing data enables one to establish information on individual species and put together a theoretical picture of consequences from heavy metal pollution in the field situation. In practice it is impossible to test every organism from alga to invertebrate to fish at every trophic level for every single or multiple toxicant. Consequently certain systems have to be evolved for toxicological testing.

D. magna was initially the most popular invertebrate test organism as it is easy to culture and maintain in laboratory conditions and tests may be carried out during the whole life cycle.

There are severe drawbacks to utilising one species from the lower taxa, and the large numbers of aquatic insects present in the immature stage in the aquatic environment warrants that some insect larval/nymphal stages should be tested. Also, one species will not usually be present in both the lotic and lentic situation. It is known that differential tolerances are exhibited by a variety of orders of insects, for example, stoneflies extremely tolerant of heavy metals, whilst Asellus and chironomids are able to withstand severe organic pollution. The more information on tolerances of all species present in the aquatic community will assist in conserving and protecting the environment, and in their use as indicators.

Trichoptera are represented frequently in the communities of British rivers. The larval stages of caddis are almost totally aquatic, then after metamorphosis they become ariel. Very little literature exists on the resistance of Trichoptera species to toxicants. The Environmental Protection Agency 1978 produced a report on the Environmental Requirements of Trichoptera, but admits it is seriously lacking in information.

Most toxicological studies which have been carried out are lacking in that:-

i. the instar is not recorded

ii. the conditions are not stated, e.g. temperature, pH, hardness,

dissolved oxygen levels.

iii. it is not noted if the test was carried out under static or flow through conditions.

Generally, the conditions are not standardised and consequently results are difficult to compare. With this in mind, the Environmental Protection Agency (E.P.A.1978) produced a report in the U.S.A. to advise on standard procedure. Pellier (1978) also outlines the considerations to be made when measuring acute toxicity of effluents to aquatic animals.

Alabaster and Lloyd (1980) summarise standard fish toxicity testing procedures in the final chapter of their book. These techniques were established by a working party in preparation for an E.I.F.A.C. (European Inland Fisheries Advisory Commission) paper on the subject.

5.3.2.1. Methods of Rearing Laboratory Animals

Two rearing methods were initially tested and compared, for rearing Hydropsyche angustipennis larvae collected by kick heel sampling from a local stream, Langley Brook.

i. Firstly Philipson's Method (1953) involved 10 H. angustipennis final instar larvae being placed in a 2 litre chemically clean glass jar. Each jar was two-thirds filled with tap water and a substratum of clean stones placed in the base of the jar.

A stirrer was constructed using the outer case of a plastic syringe, the paddles formed by softening and shaping the rounded lobes at the end of the syringe cylinder. This was then fitted to a motor to drive the stirrer. Mesh was placed over the opening of the jar to prevent the escape of any adult flies emerging. For replication of these jars a cog and band system was built to drive four paddles at any one time. The jars were kept in a temperature-controlled room at 18°C - 20°C , and the larvae were fed on a diet of flaked fish food. Twigs were suitably placed in the jars, so that newly emerged adults could raise themselves above the

level of the water.

Water which had been conditioned to the same temperature was used to clean and replenish the jars each week. The larvae were counted on alternate days to measure mortality or emergence of adults.

ii. Wiggins Method (1959)

In the same temperature controlled room, four artificial streams, using a recirculating system originally designed by Hawkes for invertebrate experiments were available. These are very similar to the system documented by Kapoor (1972) in which he reared Plecoptera.

Water is circulated from a 400 litre header tank, through a downpipe with adjustable tap for controlling flow rate, into a 6' long P.V.C. trough. The gradient of the trough is adjustable by a hinge system, and water flows down the trough and into a return tank. It is then pumped back to the header tank to complete the circuit. The water temperature in these channels may be controlled by a refrigeration system in the lower tank (see Photograph 3). Containers, specially for use within these artificial streams were constructed in which to keep Hydropsyche larvae.

Cylinders 10 cm diameter, 14 cm tall were made in 1 cm plastic covered wire meshing, the joint being secured by plastic ties. Over the base and outside of this cylinder fine nylon mesh was stitched and the top of the container covered with a close fitting plastic lid. Wiggins originally made metal cages, on similar lines, in which to rear insect larvae.

Both metal or plastic/nylon containers may be used in either the field or laboratory. In the artificial channels the light weight plastic containers were weighted on the bottom using large clean stones. Water velocity was increased down the channel to a velocity of 50 cm sec^{-1}, water could easily flow through the mesh of the container. This maintained a current suitable for the larvae to respire and feed.

Photograph 3. Recirculating Stream System

10 larvae of H. angustipennis (from Langley Brook) and H. pellucidula (from Temple Balsall, R. Blythe) were placed in each container.

The temperature was maintained at $20^{\circ}C \pm 1^{\circ}C$ at 9.8 mgl^{-1} O_2 and larvae were fed flaked fish food. Counts of animals were made at regular intervals.

5.3.2.2. Results

Fig.5.7 summarises the results obtained.

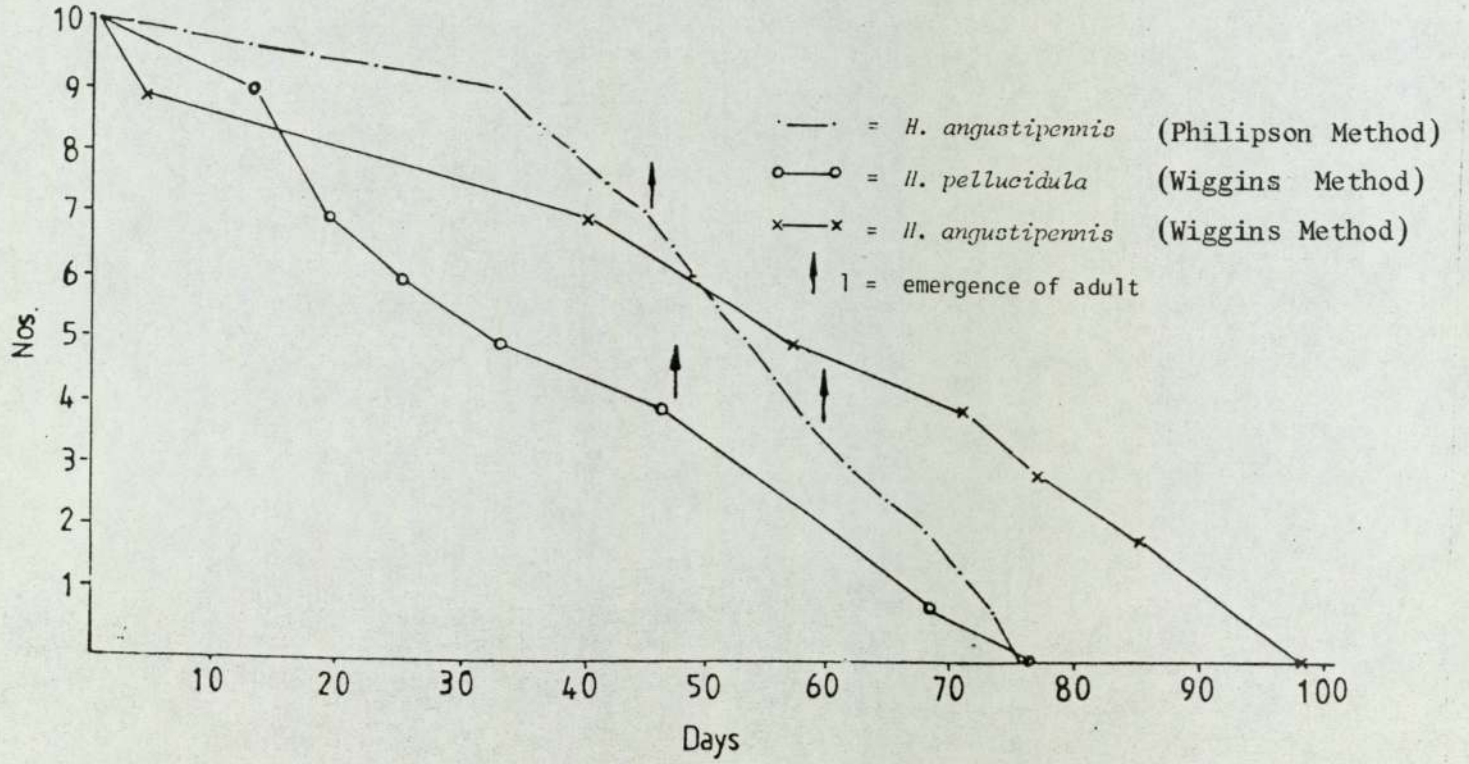
Using the modified Wiggins Method, H. angustipennis were maintained for 98 days in the larval stage. H. pellucidula survived for 76 days during which time one larva had pupated and emerged.

With the Phillipson "stirred" method, to provide a constant circulation of water over the larvae H. angustipennis were maintained for 75 days, two adults emerging during this time. As both methods of rearing, using tap water and artificial diet proved satisfactory for maintaining large numbers of larvae for toxicity testing, it remained to use the easiest method.

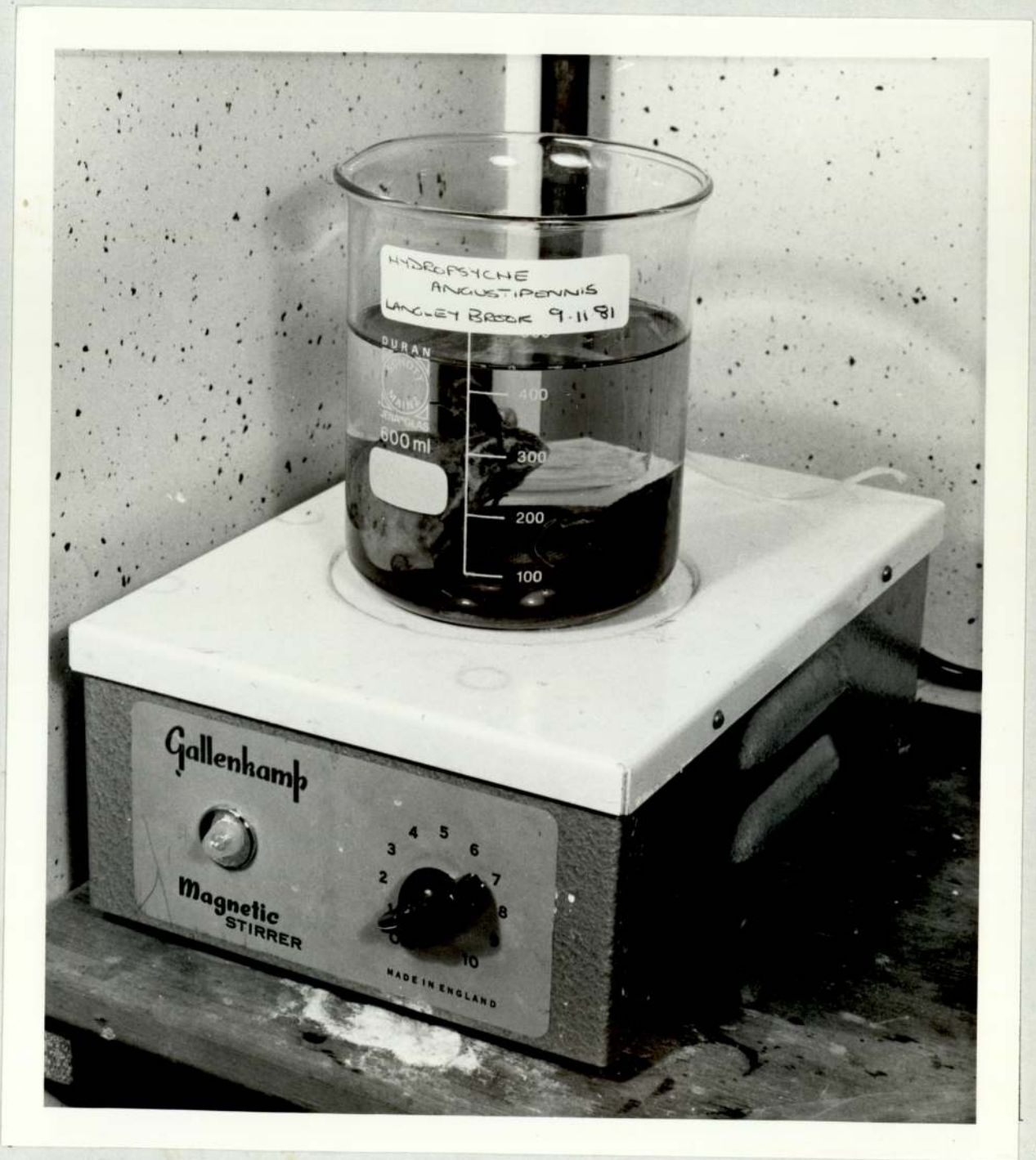
Making containers for the streams was time consuming and they had to be cleaned regularly to avoid the netting becoming clogged with fish food particles. Consequently, the glass container became the most popular method. At a later date, an even simpler and more successful method was discovered of maintaining the larvae in the laboratory.

A large Pyrex beaker, containing the larvae is placed on a magnetic stirrer, the magnetic flea at the bottom being enclosed by a rigid dome of nylon meshing to protect the larvae from damage. This is secured by large stones around the edge. Thus, the water is constantly stirred and the larvae orientate themselves on the stones and sides of the beaker, according to direction of water flow. The containers are easy to clean and the larvae easy to feed, count and maintain at constant temperature in this way (see Photograph 4). They also may easily be acclimated for experimentation .

Fig.5.7 Survival of *Hydropsyche spp.* under different rearing conditions.



Photograph 4. Apparatus for maintenance of H. angustipennis larvae



5.3.2.3. General Toxicity Testing Methods

a. Test Animals

The species used was the final instar of Hydropsyche angustipennis with a head capsule width between 1.28 - 1.56 mm. (Hildrew and Morgan, 1974).

Animals were collected from Langley Brook(Grid Ref.SP.180 980) by kick heel sampling, and held in the laboratory in Langley Brook water prior to testing in aerated 2 l glass jars at 10°C. Individuals from two different collections were never mixed in order to avoid any contamination. Any animals with overt signs of damage, stress or disease were discarded. Larvae were fed, whilst in holding chambers on Phillips Maxiflakes Tropical Fish Food, but were starved for two days before testing.

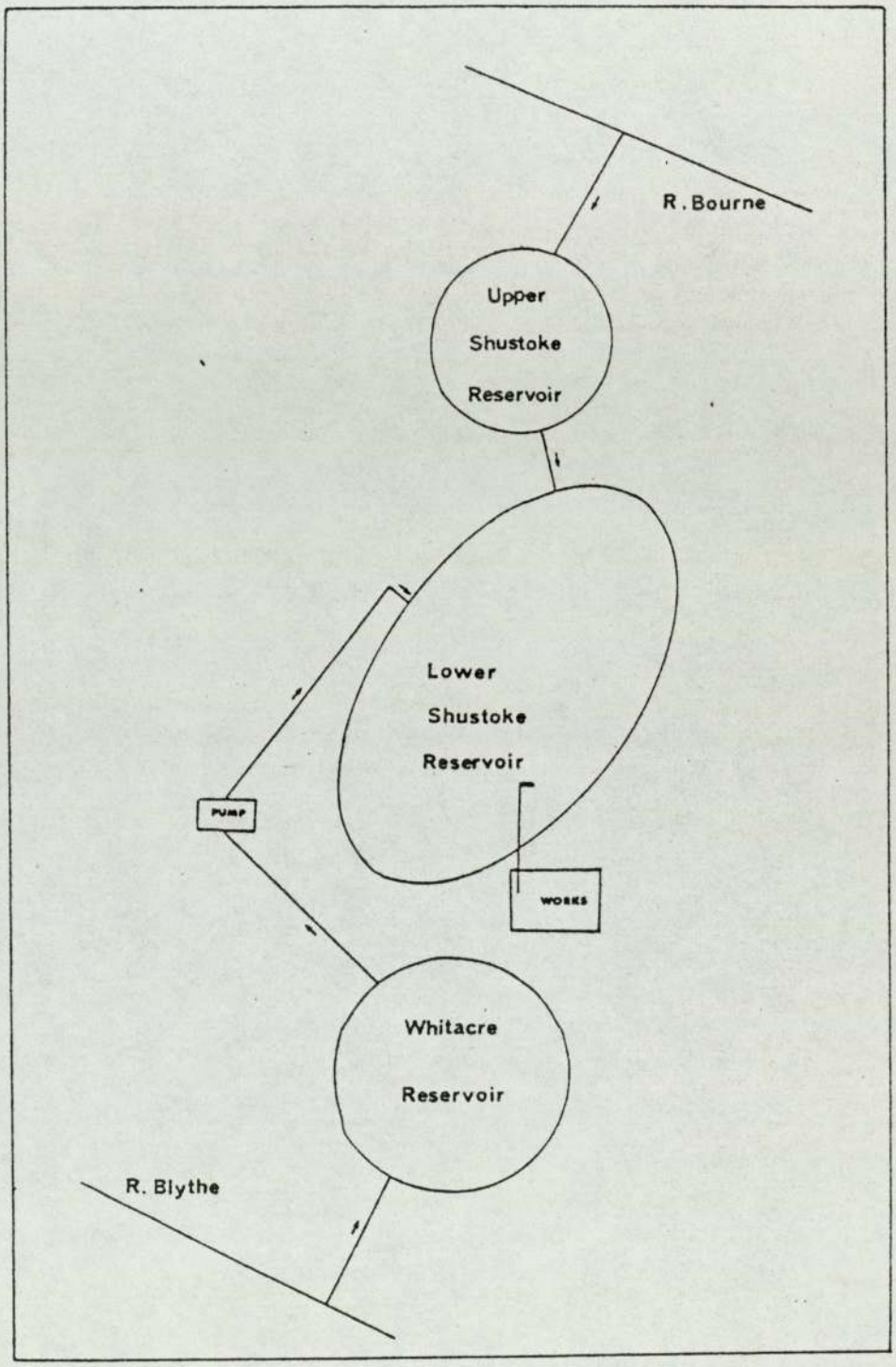
b. Apparatus

250 ml chemically clean glass beakers were used as test containers. These, and all other glassware utilised in the preparation of test solutions etc. had been treated in the following manner:

1. Washed with synthetic detergent and water at least 50°C
2. Rinsed
3. Rinsed and soaked for at least a minute with 5% hydrochloric acid to remove metals and bases
4. Rinsed with copious amounts of distilled water.

Each test chamber was supplied with a standard clean diffuser block and covered with perspex to prevent evaporation or contamination from the air. As glass is known to adsorb metals, levels were checked and test chambers acclimated. 200 ml. of test solution was used in each chamber, containing 5 or 10 animals. Each test solution was renewed daily in all replicate chambers. The temperature was maintained at 10°C ± 2°C using a temperature controlled cabinet. Hardness, pH and dissolved oxygen levels were checked at regular intervals.

Fig. 5.8 Abstraction of Water from R. Blythe and R. Bourne to Shustoke Reservoir



c. Preparation of Solutions

The copper toxicity work was carried out at three different water hardnesses, 19 ppm $CaCO_3$, 100 ppm $CaCO_3$ and 200 ppm $CaCO_3$. The different water hardnesses were achieved by mixing different proportions of Shustoke Reservoir Water (Hardness = 313 ppm $CaCO_3$) and Birmingham Tap water (Hardness = 19 ppm $CaCO_3$). This was stored in large plastic tanks and aerated to drive off any residual chlorine. Shustoke Reservoir water was obtained from Whitacre Water Works (Grid. Ref.SP.235 915). This water is abstracted from the rivers Bourne and Blythe and stored in the reservoir (as in Figure 5.8). Water was collected from a tap inside the works direct from the reservoir, thus ensuring that no copper had been added. The mean, maximum and minimum values for certain water quality determinands are given for Shustoke Reservoir in Table 5.3 (Courtesy S.T.W.A., Whitacre Works). Table 5.4 lists results of water analysis for Shustoke Reservoir and Birmingham tap water.

d. Experimental Toxicant Solutions

For copper, zinc and ammonia studies analar copper sulphate ($CuSO_4.5H_2O$), zinc sulphate and ammonium sulphate $(NH_4)_2SO_4$ were used in preparing the stock solutions with distilled water.
Two basic stocks were made to give concentrations of $1gl^{-1}$ and $10gl^{-1}$ of each toxicant. Pipettes and volumetric flasks were utilised when making up solutions with the appropriate experimental water. Batches of 2 litres of solution were made up at any one time, and for the metals, were stored in nonadsorbative plastic containers.

The herbicides Diquat and Terbutryne were prepared as stock solutions in tap water and calculated on the concentration of active ingredient required.

To summarise:

Copper, Zinc and Ammonia, were made up on a geometric scale of concen-

TABLE 5.3. ANNUAL DATA WATER QUALITY FOR RAW SHUSTOKE RESERVOIR WATER (mgl^{-1})

DETERMINAND	MEAN	MAX.	MIN.
Ammonia mg N l^{-1}	0.09	0.46	0.01
Nitrite mg N l^{-1}	0.095	0.68	0.002
Nitrate mg N l^{-1}	6.9	11.0	1.0
p.H.	8.5	9.1	7.9
Total Hardness	315.0	336.0	276.0
Ca Hardness	210.0	240.0	136.0
Mg Hardness	105.0	176.0	48.0
Chlorides as Cl	57.0	163.0	45.0
Suspended Solids 105^{o}C.	6.8	24.8	1.4
Fluorides	0.23	0.55	0.14

TABLE 5.4. WATER CHEMISTRY RELATING TO TOXICITY TESTING

SITE	Shustoke Reservoir		B'Ham Tap Water	
DETERMINAND				
Temperature ^{0}C	10^{o}		10^{o}	
pH.	7.8		6.4	
D.O mg l^{-1}	10.4			
B.O.D. mg l^{-1}	3.0			
Total Hardness mg l^{-1} ($CaCO_3$)	313.0		22.0	
Ca Hardness mg l^{-1} ($CaCO_3$)	146.0		14.0	
Mg Hardness mg l^{-1} ($CaCO_3$)	167.0		18.0	
Phenolthalein Alkalinity mg l^{-1} ($CaCO_3$)	10.0		0.0	
Total Alkalinity mg l^{-1} ($CaCO_3$)	165.0		10.0	
Chloride mg l^{-1}	45.4		8.5	
Nitrate Mg Nl^{-1}	6.4		0.25	
Ammonia Mg Nl^{-1}	0.4		0.1	
Phosphate mg l^{-1}	0.7		1.0	
Suspended solids (105^{o}C) mg l^{-1}				
Copper mg l^{-1}	<0.01		0.09	
Cadmium mg l^{-1}	0.004		0.05	
Chromium mg l^{-1}	0.003		0.01	
Nickel mg l^{-1}	0.005		0.05	
Iron mg l^{-1}	0.03		1.6	
Zinc mg l^{-1}	0.03		0.4	
Lead mg l^{-1}	0.027		0.005	

tration to give 10, 18, 32, 56, 100, 320, 560 and 1000 ppm (nominally).

Nominal concentrations are quoted because at varying water hardnesses and pH the amount of Cu^{2+} and NH_3 varies. This will be discussed further upon discussion of these individual toxicants.

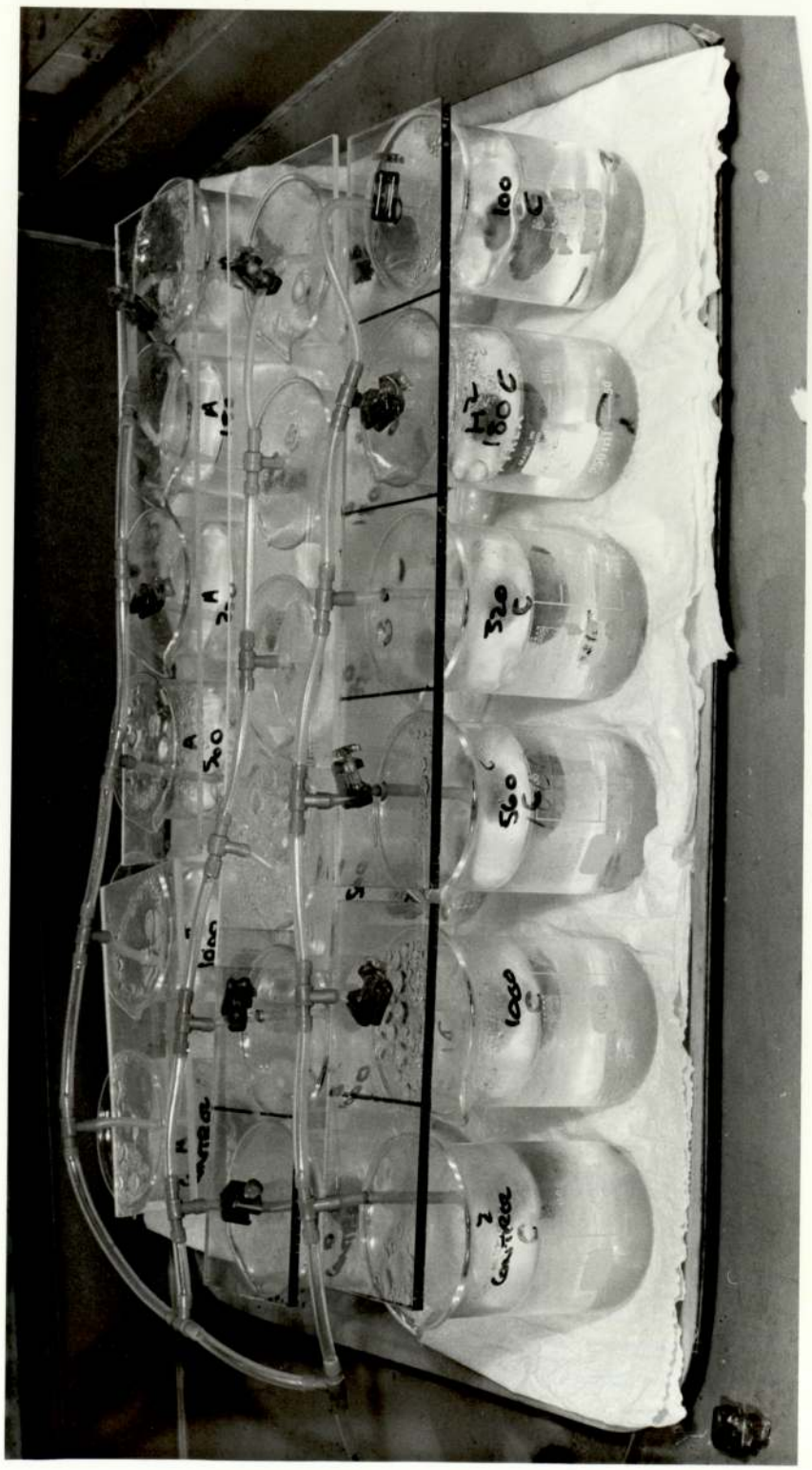
200 ml of test solution was placed in each beaker, and three replicates at each dilution prepared (see photograph 5). Constant aeration was achieved with a standard diffuser block. Either 5 or 10 animals (depending upon availability) were placed in test chambers at random. Cumulative mortalities were recorded at time intervals on a geometric scale throughout the 96 hr test period. If 10% or more of animals in the control chambers died, then the rest of the test was invalidated. Temperature, dissolved oxygen, and pH were monitored throughout the toxicity test, and chemical analyses of metal levels and ammonia were carried out daily. Metal samples underwent acid digestion, and the total concentration was measured by atomic absorption spectrophotometry (A.A.S). The toxic effect of Cu^{++} ions was measured daily using an Orion Model 94 - 29 copper ion selective electrode hooked up to a Corning E.E.L digital 110 expanded scale pH meter. NH_3-N was measured daily by taking a sample of test solution from each test chamber and acidifying with 6 drops con. HCl. for preservation. This was then measured on the auto-analyser.

Herbicide concentrations were maintained by daily renewal of toxicant solutions in test chambers.

e. Observations

Times of observations were based on a logarithmic scale (Bliss 1935 a & b). Animal mortality observations were made at the following times from the start of the test: $\frac{3}{4}$ hr., $1\frac{1}{2}$, 3, 6, 12, 24, 48, 72 and 96 hours and each day thereafter if a long-term test was performed.

Photograph 5. Replicate chambers used for acute toxicity testing of H. angustipennis larvae



5.3.2.4. Checking the Metal Content of Solutions

The Perkin-Elmer Model 306 was used for Atomic Absorption Spectrophotometry using an air acetylene flame.

A calibration was made using standard metal solution as recommended, and readings were taken on a flat bed recorder (Perkin-Elmer 56). Every effort was made to ensure samples were not contaminated during preparation. All glassware was washed with detergent concentrated nitric acid and double washed in distilled water before use. Water samples and metal solutions were prepared in the following manner: 100 or 250 ml. aliquots of sample were transferred to 250 ml pyrex flasks or beakers, depending upon amount of test solution available. 5 ml. of concentrated nitric acid (Fisons A+.Ab Grade HNO_3 Sp.gr. 1.42, 70% HNO_3) was added to the solution by pipette. The sample was then reduced to dryness on a hotplate or mantle in a fume cupboard (approx. 4 - 6 hours). Resultant residue was dissolved into 2 ml. 50% hydrochloric acid (A.A.S grade, BDH HCl 36%) transferred to a 25 ml volumetric flask and made up to the mark with distilled water, ensuring that the flask was thoroughly rinsed to include all of the sample. The sample was then aspirated and further dilutions prepared if necessary. Metal concentration in the sample was calculated according to the dilution factor where:-

$$\text{dilution factor} = \frac{\text{vol. sample in soln. in ml.}}{\text{vol. aliquot digested}} = \frac{25}{100} = 0.25$$

If a larger aliquot was taken, the calculation was adjusted accordingly.

All metal samples were run for total metal concentration. Soluble metal concentration may be assessed by filtering a fresh sample through a 450 mm glass fibre filter paper (GF/C Paper Whatmans Ltd., Maidstone) and membrane filter paper Nuflow 450 mm (Oxoid Ltd., London). Particulate matter metal content may then be determined by difference. Correction for any metal concentrations was obtained by

running a blank i.e. an acidified distilled water sample. Samples were then aspirated and results recorded on the flat bed recorder.

Detection limits for the Perkin-Elmer 306 are shown in Table 5.5 below:

TABLE 5.5 DETECTION LIMITS FOR METALS

Element	Unit mgl^{-1}
Cd	0.001
Cr	0.003
Cu	0.002
Fe	0.005
Zn	0.001
Pb	0.01
Ni	0.005

(Taken from Perkin-Elmer "Analytical Methods for Atomic Absorption Spectrophotometry."1976).

5.3.2.5. Copper Ion Determination

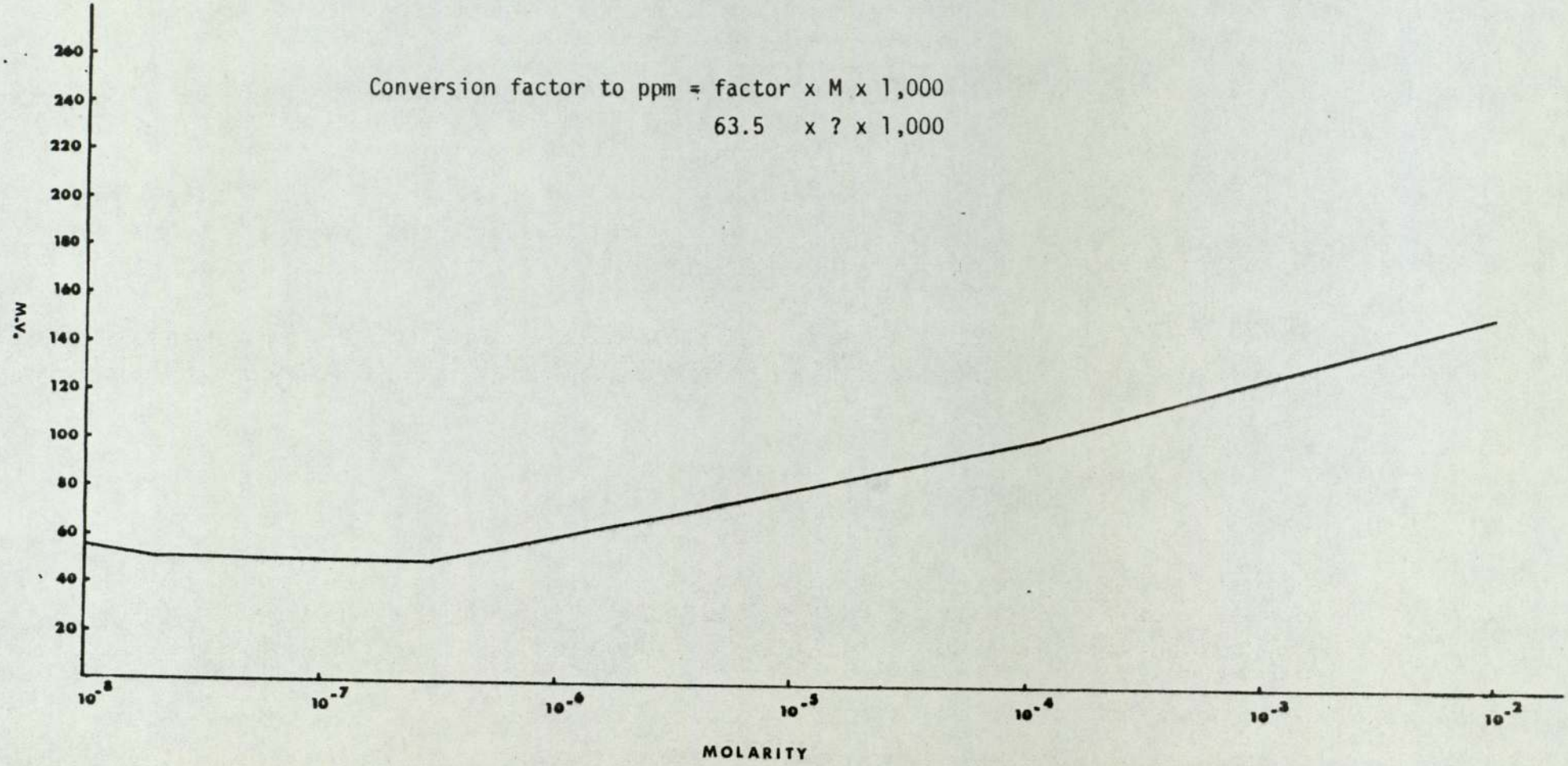
The cupric ion electrode (94-29) was set up together with an Orion Model (90-91) reference electrode. 0.5 M KCl, saturated with Ag^{+} was used as the reference electrode filling solution as recommended. Both electrodes were connected to the Corning-Eel Digital 100 meter and a calibration curve was constructed using the recommended Orion Standard solutions. The mV readings (linear axis) were plotted against concentration (log-axis) on semilogarithmic paper (Figure 5.9). A 100 ml sample of test solution was placed in a plastic non-adsorbative beaker. The sample was stirred on a magnetic stirrer and 2 ml 5M Analar Sodium nitrate was added (I.S.A). This ionic strength adjuster (I.S.A) added in these proportions gives a background ionic strength of 0.1M.

A millivolt reading was obtained, and the Cu^{++} concentration in each copper sample was calculated from the calibration curve. Problems

FIG. 5.9 CALIBRATION CURVE FOR Cu^{++} ELECTRODE

Conversion factor to ppm = factor x M x 1,000

63.5 x ? x 1,000



arise at the lower end of the scale if the sample is not on the acidic scale - fortunately, due to high copper concentrations used in the toxicity tests such interference was not encountered.

5.3.3. Experimental Design

The first stage in the toxicity testing was to perform ranging tests, using five H. angustipennis larvae in each of five widely spaced toxicant solutions. From the results of these tests, 96hr toxicity tests were performed using the proposed geometric dilutions. Acclimated animals were placed at random in the test chambers, which were set up in replicates as previously described. If larvae were particularly tolerant of even the highest concentration of toxicant, then a long term test was undertaken.

5.3.4. Methods of Processing Results

As the response of an animal to a toxicant is dependent upon the concentration of the poison and the period of exposure, the mortality of experimental animals can be expressed either as the number dead since the start of the experiment, or the rate of dying. Here the cumulative number of animals which died was recorded and expressed as a percentage value. Equal increases in percentage mortality are normally obtained when the time elapsed or concentration of poison is increased on a geometric rather than an arithmetic scale. Toxicity is usually described in terms of the concentration which will produce a specific effect in a specific proportion of the population in a specified time. The measured statistic is usually the median, therefore the following nomenclature is used to specify the type of effect and the length of time involved to gain that response:

e.g. 48 hr EC50 = (median effective concentration for 48 hours) from this the ET50 (median effective time) can be calculated.

When the response is death the EC50 $\equiv$ LC50 (i.e. Median Lethal Concentration). These concentrations are usually calculated for exposure period of 24, 48 and 96 hours. Similarly, the tests measuring

the time taken for the organisms to die = LT50.

If the relationship between 24, 48 and 96 hr. LC50's is plotted on log:log graph paper (concentration:response) and the result is curvilinear becoming asymptotic to the time axis, the asymptotic concentration may be extrapolated. This is often called the "threshold" or incipient median lethal concentration.

In static tests the term LC50 may only be used if the test concentration did not fall below 90% of the initial concentration. If the concentration was not maintained the term LC(I) 50 (Lloyd & Tooby, 1979) should be used to indicate that the concentration decreases throughout the test period. (I) then indicates that Initial Concentration (nominal) concentration. It is hoped that using this nomenclature will make it easier to judge these experiments in the correct context. Both mathematical and graphical methods may be employed to calculate LC50 and LT50 values (Litchfield & Wilcoxon, 1949).

The method used here is graphical, where values are plotted directly on to logarithmic probability paper. If 10 animals are used, for example, the response of the first is regarded as 5%, the second 15% and the last 95%. The reason for this is that the LC50 should not relate to the response time of the fifth animal, but to the response time between the fifth and sixth. Therefore, the lethal concentrations of five animals are then distributed on each side of the median. A line is fitted by eye to each set of data, giving greater weight to those values between 25% and 75% response, and the LC50 value interpolated. Lloyd (1960) employed this method when calculating the toxicity of zinc to rainbow trout in hard water.

5.4. COPPER TOXICITY

Introductory Review

Copper is found in natural waters as a trace metal at concentrations of less than 5 μgl^{-1} but it is often present at higher concentrations and associated with the mining of zinc, lead and tin, for example, R. Hayle, Cornwall 0.01 - 0.1 mgl^{-1} (Brown, 1976); R. Yswyth and R. Rheidol, Wales 0.03 and 0.06 mgl^{-1} respectively (Harmonised Monitoring Station D.O.E.1979); R. Molonglo, Australia 0.05 mgl^{-1} (Weatherley et al., 1967); R. Team, N. England 0.02 mgl^{-1} (Wehr et al. 1981).

In a recent survey of British rivers, 1980, copper levels were found to vary between undetectable -> 0.87 mgl^{-1}.

Copper compounds have been widely used in the aquatic environment both as algicides and molluscicides, particularly in tropical areas for control of schistosomaisis. In an industrial environment the use of copper is widespread for many engineering and industrial processes. The EEC directive on the quality of surface waters intended for the abstraction of drinking waters recommends a guideline level of copper 0.02 - 1.0 mgl^{-1}.

The sister document, an EEC directive on the quality of water needing protection or improvement in order to support fish life quotes the following concentrations for different water hardnesses for Salmonid and cyprinid fish, 0.005, 0.022, 0.04 and 0.112 mgl^{-1} dissolved copper at water hardnesses of 10, 50, 100 and 200 mgl^{-1} $CaCO_3$ respectively.

The chemical behaviour of copper in natural waters is complicated by the tendency to form complexes. The cupric ion Cu^{++} is thought to be the most toxic and the only stable form of copper in aerobic conditions in natural surface waters. This complexes readily with organic and inorganic agents (Stiff, 1971) with the result that the proportion of free cupric ion in solution is very small, approximately 1%

(E.I.F.A.C. 1976). In waters with heavy organic loads, or high pH (>7.5) it would be a smaller percentage. Only in waters of unusually low pH or very soft water could a significant proportion be present as the free ion. Evidence of this small percentage of copper as Cu^{++} is given in Table 5.6 (from Wilson, 1976). The table shows that only a very small proportion was Cu^{++} but often a high percentage of the dissolved copper carbonate complex was present.

Copper chemistry in unpolluted freshwater is basically copper in calcium bicarbonate solutions. Copper may be precipitated as cupric hydroxide, followed by conversion of hydroxide to oxide:

$$Cu^{++} + 2H_2O \longrightarrow Cu(OH)_2\downarrow + 2H^+ \longrightarrow CuO\downarrow + 2H^+ + H_2O$$

or as malachite

$$2\,Cu^{++} + 2H_2O + HCO_3^- \longrightarrow \underset{\text{(malachite)}}{Cu_2(OH)_2\,CO_3\downarrow} + 3H^+$$

The proportional amounts of Cu^{++}, $Cu(OH)_2$ and $Cu_2(OH)_2CO_3$ depend upon the pH and bicarbonate concentration of the solution. The concentration of Cu^{++} in equilibrium with malachite in the pH range 6.0 - 8.5 i.e. that of most freshwater is less than that in equilibrium with cupric hydroxide. Precipitation of malachite is a slow process and reaches equilibrium only after several days. In rivers where residence time is short equilibrium may never be attained, but this is one factor which must be taken into account when setting up toxicity tests. Complexes of copper which are soluble include carbonate, cyanide, amino acids and polypeptides, humic and fluvic acids.

In water receiving domestic and industrial effluents, natural chelating agents may be present plus synthetic chelating agents such as detergent builders (Herbert et al., 1965). Copper is at the top of the first transition series and the avidity for chelation rises from manganese through the periodic table up to copper, i.e.

TABLE 5.6 FORMS OF FILTERABLE COPPER IN ENGLISH RIVERS

River	Copper Added ($\mu g l^{-1}$)	Total Copper Found $\mu g l^{-1}$	% of Total Present as : Cu++	$CuCO_3^0$	Other * forms
Hiz	800	857	0.5	31	69
Lee (Harpenden)	800	792	1.2	38	61
Lee (Edmonton)	800	799	0.1	19	81
Tame	800	890	0.2	4.8	95
Tame	0	83.8	2.2	4.0	96
Anker	800	789	0.2	4.0	96
Arrow	800	820	1.3	53	46
Thames	800	880	0.5	33	66

From Wilson, 1976, W.R.C. Concentrations of trace metals in River Waters : A Review.

* Organic and inorganic forms included.

$$Mn^{++}, Fe^{++}, Co^{++}, Ni^{++}, Cu^{++} \longrightarrow$$

increasing avidity

and then slumps for Zn^{++}. The increase in avidity follows the decline in ionic radius as the series is ascended (Albert 1973).

Brungs et al. (1976) carried out 21 static acute toxicity tests at different times of the year on fathead minnows using water from a small stream. The 96 hr LC50 results were within the range 1.6 - 21.0 mgl^{-1} Cu. This variation was due largely to the water which was susceptible to organic pollution - hence varying amounts of chelating material available and altering the concentration of cupric ions.

Other factors affecting the toxicity of copper include the temperature (Cairns et al., 1978), dissolved oxygen (Petty, 1967; Clubb et al.(1975b) and presence of chelating agents (Muramoto, 1980). The level of copper and other heavy metals may be in different soluble and precipitated forms, complexed or ionic. It may be incorporated in the sediments, in the water or plant material, the metal then entering the food chain through the water or diet.

Toxicity tests on macroinvertebrates will generate data on acceptable concentrations consistent with the protection of aquatic fauna and establish water quality criteria for the protection of the aquatic environment generally. From a purely fishery point of view it may be argued that the removal of the most sensitive species by toxic pollution will not necessarily affect the dietary intake of fish as the more tolerant species tend to increase in abundance as competition is relaxed. Ultimately the acceptable environmental contamination level will depend upon the nature of the receiving water, the application rate (and the use of water resource). A biological 'no effect' concentration may be derived from such toxicity tests on invertebrates. High levels of heavy metals are known to bind to sediments and in certain circumstances macroinvertebrates have been shown to incorporate these into their body

tissues (Nehring, 1976), consequently they may provide an in situ bio-monitor of metal pollution.

Studies on the toxic effect of sediment bound versus dissolved or suspended metals may be useful, but data are very limited for such work at present (Nehring et al., 1979). Field studies by Anderson (1977) have shown that zinc and mercury are perhaps the only metals to be accumulated above the sediment levels by invertebrates. Analysis of animal tissue can indicate the biological availability of toxic metals, the high concentration of metals in one species may be correlated with the absence of more sensitive species.

Sensitivity to copper varies with species, as summarised by some of the experiments carried out on invertebrates and fish, their conditions and results. It has been found that the toxicity of heavy metals is dependent upon the oxygen available. The work of Clubb et al. (1975a) on the cased caddis Brachycentrus americanus showed 100% survival over a 96 hr TL_m at 17.5 mgl^{-1} Cd^{++} and 42.5 mgl^{-1} Cd^{++} when DO = 6.0 mgl^{-1}, minimum temperature = $10^{\circ}C \pm 2^{\circ}C$ and alkalinity 240mgl^{-1}. At changing oxygen levels the same workers (1975b) found an increase in cadmium toxicity with an increase in dissolved oxygen in the stonefly and mayfly, but NOT in B. americanus.

The "Pasteur effect" recorded by Petty (1967) whereby there is oxygen inhibition of metabolism, thus means that in some species heavy metals have a less toxic effect in an oxygen sparse environment.

Problems in comparing toxicity also arise due to the relative length of the life cycle of the species in the environment. Besch (1977) points out that a toxicity test of set duration using a macroinvertebrate represents a proportionally longer period than a test on fish. Generally, the less mature the animal, the greater the toxic effect. For example, Hubschman (1967a) found that the percentage mortality of newly hatched crayfish Orconectes rusticus was greater than in juveniles and in turn this was greater than in adults.

Similarly, Arthur & Leonard (1970) exposed newly hatched Gammarus pseudolimnaeus to copper solutions of concentrations 6.2 - 12.9 μgl^{-1} and all died, whereas adults were affected only when the concentration exceeded 10 μgl^{-1}. Invertebrates are also suspected of being tolerant to solutions of copper salts during ecolysis and pupa formation as concluded from work by Anderson (1944 & 1950) and Wurtz & Bridges (1961). Consequently, freshwater fisheries have realised that fish data and standards based on fish toxicity work may be inadequate to protect sensitive invertebrate species (E.I.F.A.C, 1976). The water quality of a river must be maintained to support a healthy and diverse invertebrate community. E.I.F.A.C. recommended copper concentrations are as follows:-

Values from the annual 50 and 95 percentiles range from 0.01 - 0.005 mgl^{-1} at water hardness 10 ppm $CaCO_3$ to 0.028 - 0.112 mgl^{-1} Cu at 300 ppm $CaCO_3$.

Water used for irrigation is recommended to be less than 0.2 mgl^{-1} Cu (W.R.C. , Wilson, 1976).

5.4.1. Laboratory Tests

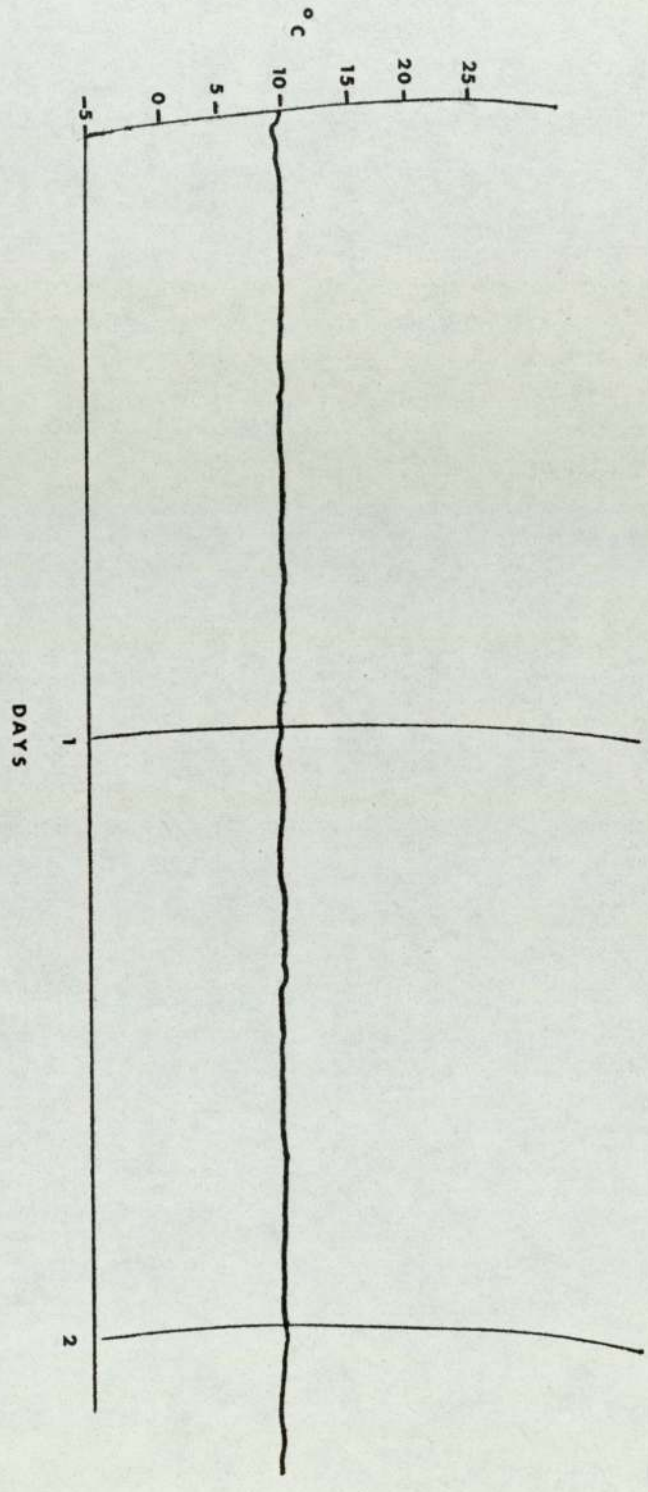
Evidence from previous workers showed that it was necessary to take into account not only total copper (Cu) concentrations, dissolved oxygen, pH and temperature but also to monitor water hardness and the resultant effect on copper ion (Cu^{++}) concentration.

5.4.2. Results

Temperature was constant, and maintained at 10°C in a temperature controlled cabinet as in Fig.5.10., a trace of the temperature recording for a one-week period. Similarly dissolved oxygen levels were constant, each test chamber receiving air continuously through a diffuser block.

Table 5.7 shows the change in pH at different concentrations of total copper. It may be seen that a slight increase in pH is associated with a decrease in copper concentration, but only drop 0.15 pH

Fig.5.10. Temperature record for temperature controlled cabinet



units from 560 mgl^{-1} Cu to 56 mgl^{-1} Cu. Measurement of pH in each of the toxicant solutions was carried out daily and found to be constant.

Figure 5.11 shows the variation in copper ion concentration, at each of the nominal total copper concentrations at three different water hardnesses. It is clear that an increase in calcium carbonate hardness at any concentration reduces the Cu^{++} concentration.

a. Ranging Tests

In five test chambers, five H. angustipennis larvae were exposed to copper solutions with total copper concentrations of 1000, 750, 500, 250 and 50 mgl^{-1} respectively and a further five animals were kept in a similar chamber filled with tap water as a control. Constant temperature and aeration was supplied and the test was of 24 hours duration.

TABLE 5.7. pH OF COPPER SOLUTIONS AT 19 ppm $CaCO_3$

Concentration mgl^{-1} Cu	pH
560	5.15
320	5.21
180	5.26
100	5.27
56	5.3
Control	6.4

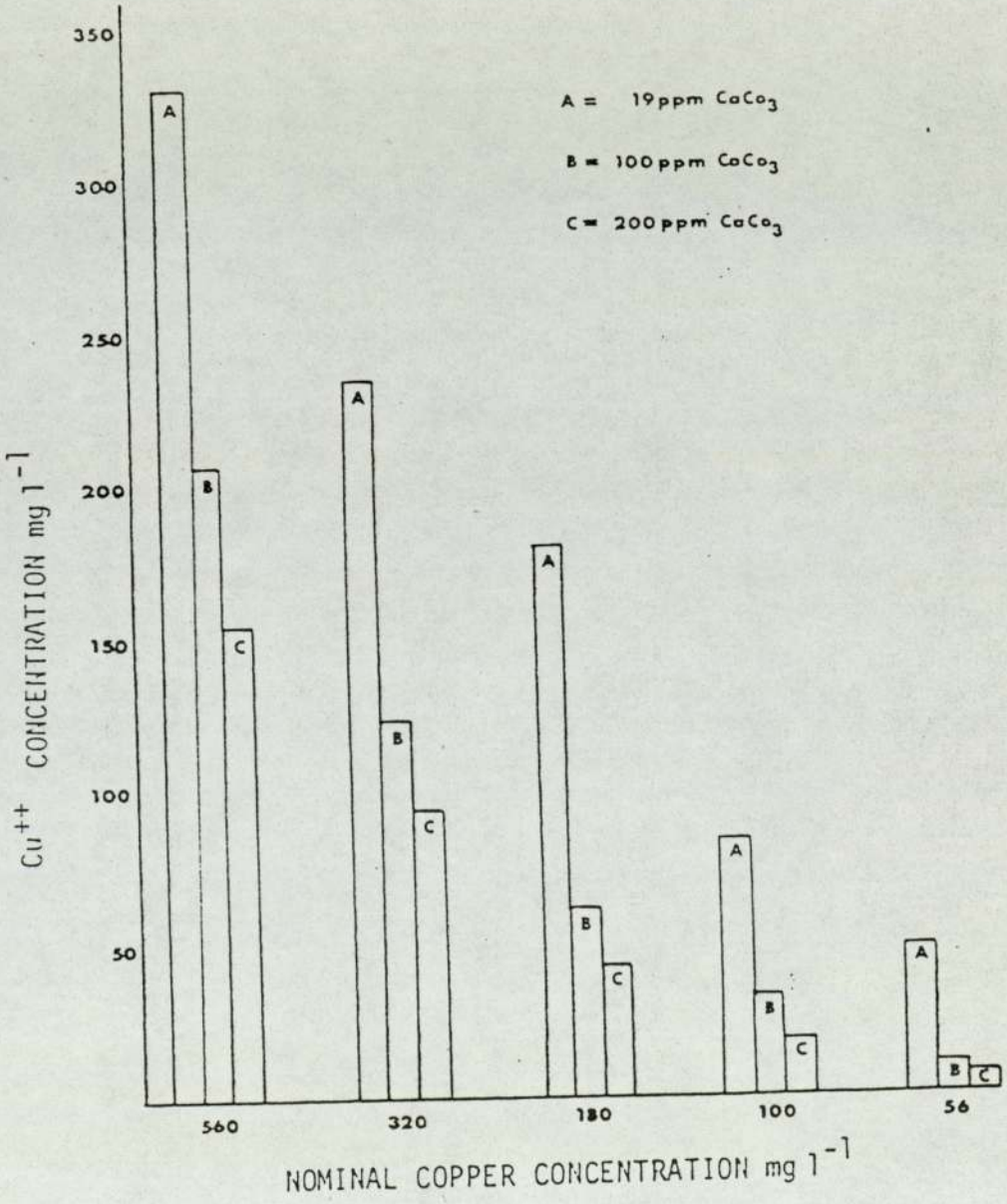
Results

It was found that 100% mortality occurred at 1000 and 750 mgl^{-1} Cu, 20% at 500 mgl^{-1} Cu but no deaths occurred at 250 and 50 mgl^{-1} Cu or in the control within the 24 hour period.

b. Definitive 96 hr. toxicity tests

In the light of the above ranging tests three 96 hr LC50 tests were performed, all under the conditions explained in the methods.

Fig.5.11. Concentrations of Cu^{++} at Varying Water Hardness



Experiment 1 was conducted using toxicants prepared with 'soft' water (19 ppm $CaCO_3$), Experiment 2 at 100 ppm $CaCO_3$ and Experiment 3 at 200 ppm $CaCO_3$. Table 5.8 records the mortalities of H. angustipennis in six toxicant concentrations. It may be seen that all individuals died in concentrations of 560 and 320 mgl^{-1} Cu within 48 hours of the start of the test. After 96 hrs. 80% had died at 180 mgl^{-1} Cu., 40% at 100 mgl^{-1} Cu and 10% at 56 mgl^{-1} Cu. No deaths had occurred in the control chamber. These percentage mortalities were then plotted as explained in section 5.3.4. to calculate 95 hr LC50 values and LT50 values. The results may be seen in Figs.5.12 and 5.13 respectively. These are quoted as total copper concentrations, but with assessment of Cu^{++} ions at each concentration, as seen in table 5.9 an equivalent Cu^{++} value may be assigned. Thus the 96 LC50 at 19 ppm $CaCO_3$ = 132 mgl^{-1} Cu 100 mgl^{-1} Cu^{++}.

TABLE 5.8. MORTALITY OF H. angustipennis IN SIX CONCENTRATIONS OF COPPER SULPHATE AT 19 ppm $CaCO_3$

Concentration -> Time ↓	560	320	180	100	56	Control
			ppm copper			
3/4 hr	0	0	0	0	0	0
1½ hr	0	0	0	0	0	0
3 hrs	0	0	0	0	0	0
6 hrs	0	0	0	0	0	0
12 hrs	0	0	0	0	0	0
24 hrs	0	0	0	0	0	0
31 hrs	40%	0	0	0		
48 hrs	100%	100%	50%	10%	0	0
72 hrs			70%	10%	0	0
96 hrs			80%	40%	10%	0

Fig.5.12 96 hr LC50 for H. angustipennis to copper sulphate at 10°C and 19 ppm $CaCO_3$

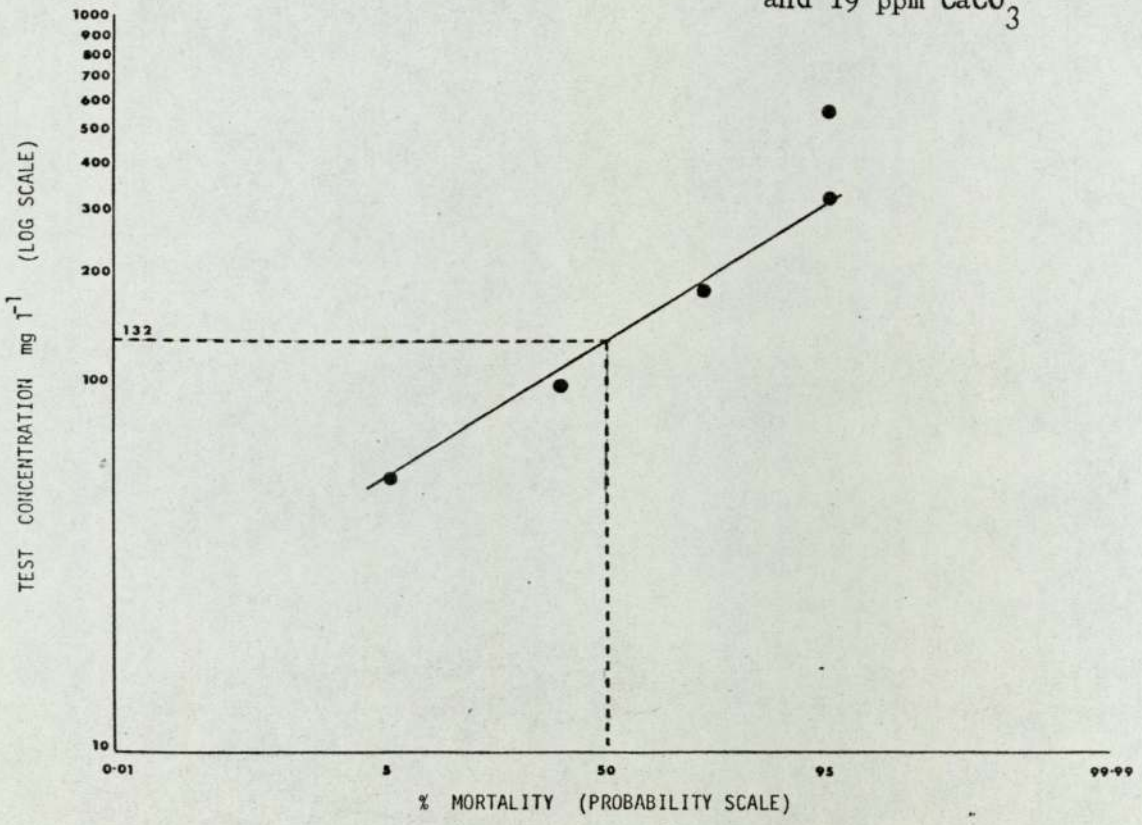


Fig.5.13 LT50 values for H. angustipennis to three concentrations of copper sulphate at 10°C and 19 ppm $CaCO_3$

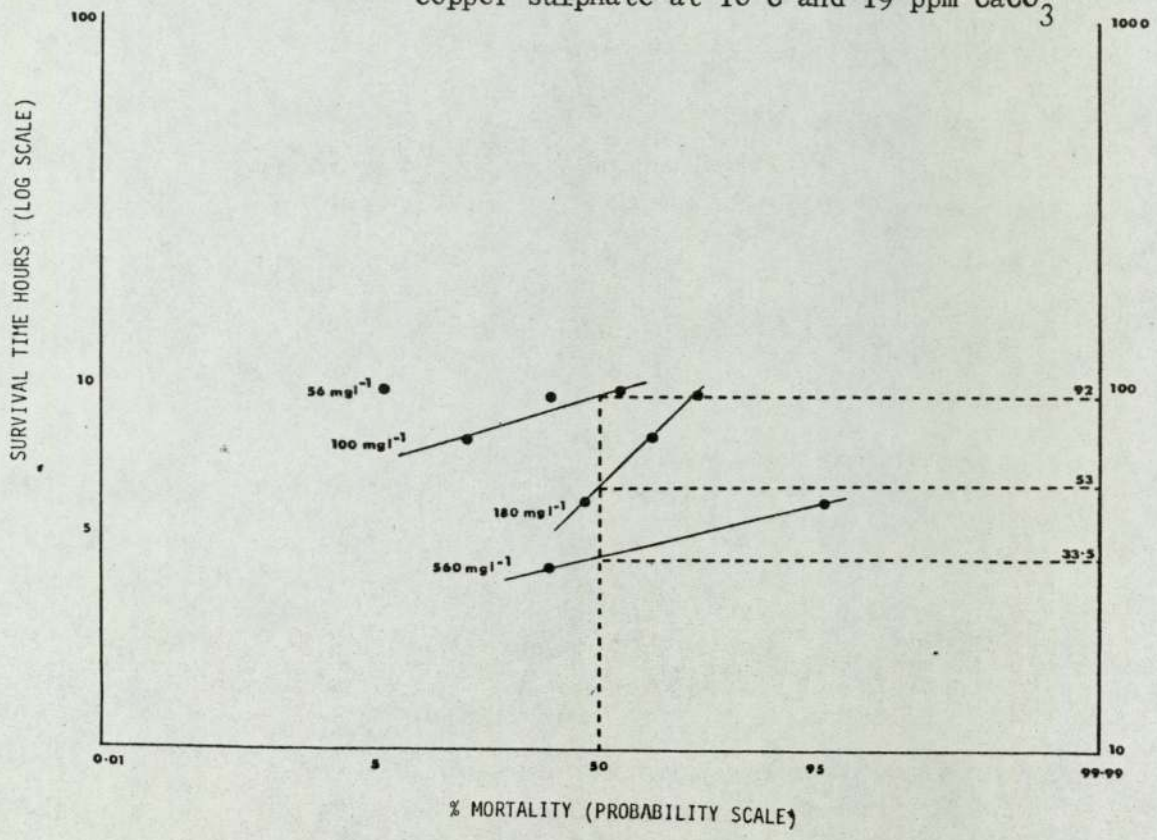


TABLE 5.9. COPPER AND COPPER ION CONCENTRATION at 19 ppm $CaCO_3$

Sample Nominal Cu Concentration mgl^{-1}	Cu^{++} mgl^{-1}
560	330.0
320	325.0
180	180.0
100	83.0
56	48.0
Control	Negligible

The results of Experiment 2 on copper toxicity in which toxicant solutions prepared with 100 ppm $CaCO_3$ water are recorded are shown below. It may be seen that in Table 5.10 the pH of the solutions is recorded; once again these remained constant. Similarly from tables 5.11 and 5.12 which record the millivolt readings of the copper ion electrode and the calculated Cu^{++} concentration respectively, it can be seen that copper concentrations were consistent over the test period. Table 5.13 shows Cu^{++} and total copper concentrations. The rate of mortalities of H. angustipennis larvae are recorded in Table 5.14 and once again 96 hr LC50 and LT50 results are presented in Figs.5.14 and 5.15.

TABLE 5.10 pH OF COPPER SULPHATE SOLUTIONS AT 100 ppm $CaCO_3$

Copper Concn. mgl^{-1}	pH
560	4.7
320	4.9
180	5.2
100	5.35
56	5.4
32	6.8
18	7.0
10	7.1
Control	7.5

TABLE 5.11 DAILY mV READINGS FROM COPPER SULPHATE SOLUTIONS

TIME	mv readings for nominal copper concentrations								
	560	320	180	100	56	32	18	10	Control
0	143	134	128	120	104	63	58	40	6
24 hrs	141	134	130	122	105	81	64	48	10
48 hrs	142	137	128	120	103	70	54	46	13
72 hrs	-	-	125	120	104	75	58	53	12
96 hrs	-	-	-	123	104	72	60	62	10
$\bar{x}$	142.0	135.0	127.7	121.0	104.0	72.2	58.8	49.8	10.2
S.D.	1.00	1.70	2.06	1.41	0.70	6.60	3.63	8.2	2.68

TABLE 5.12 COPPER ION CONCENTRATION mgl^{-1} OF COPPER SULPHATE SOLUTIONS

TIME	Concentrations of Cu^{++} at nominal copper concentrations								
	560	320	180	100	56	32	18	10	Control
0 hrs	222	127	63.5	28.0	7.0	0.1	0.04	neg	negligible
24 hrs	191	127	69.5	34.0	6.4	0.6	0.09	neg	negligible
48 hrs	209	114	63.5	28.0	6.9	0.2	0.03	neg	negligible
72 hrs	-	-	57.0	28.0	7.0	0.2	0.05	0.02	negligible
96 hrs	-	-	61.0	40.6	7.0	0.2	0.06	0.08	negligible
$\bar{x}$	207.3	122.6	62.9	31.7	6.86	0.26	0.054	0.05	
S.D.	15.56	7.50	4.54	5.60	0.26	0.19	0.02		

TABLE 5.13 SUMMARY OF DATA FOR WATER SAMPLES 96 hr TOXICITY TEST

at 100 ppm $CaCO_3$

Sample Nominal Cu Concentration	Cu^{++}	Total copper ppm
560	207.0	140.0
320	123.0	332.0
180	63.0	184.0
100	32.0	32.0
56	6.9	55.0
32	0.26	24.5
18	0.05	14.5
10	0.05	10.5
Control	negligible	0.3

TABLE 5.14 MORTALITY OF H. angustipennis IN SIX CONCENTRATIONS OF COPPER SULPHATE

at 100 ppm $CaCO_3$

Concentration time (hrs)	560	320	180	100	56	32	18	10	Control
			ppm approx. mgl^{-1}						
$\frac{3}{4}$	0	0	0	0	0	0	0	0	0
$1\frac{1}{2}$	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0
24	10%	0	0	0	1*	0	0	0	0
36	50%	10%	0	0	0	0	0	0	0
48	100%	100%	10%	0	0	0	0	0	0
72			20%	20%	0	0	0	0	0
96			50%	40%	30%	0	0	0	0

* lost due to cannabalism

Fig.5.14 96 hr LC50 for H. angustipennis to copper sulphate at 10°C and 100 ppm $CaCO_3$

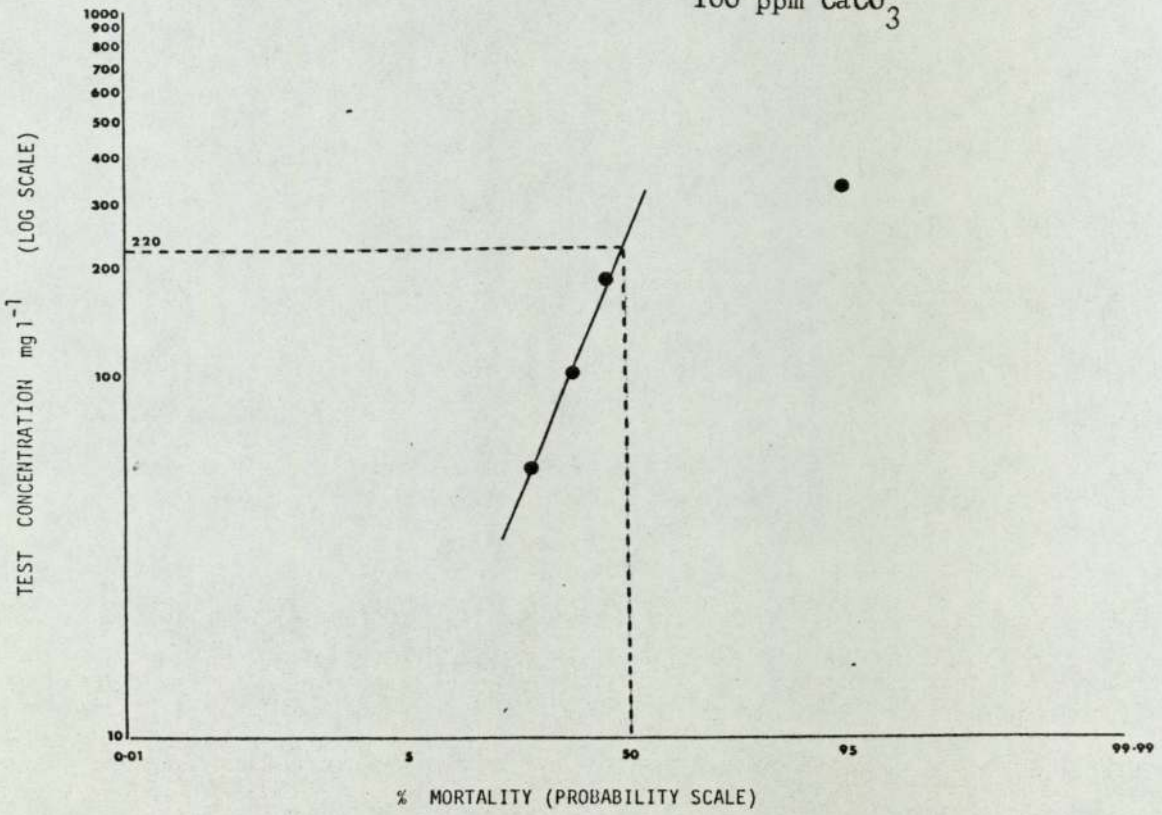
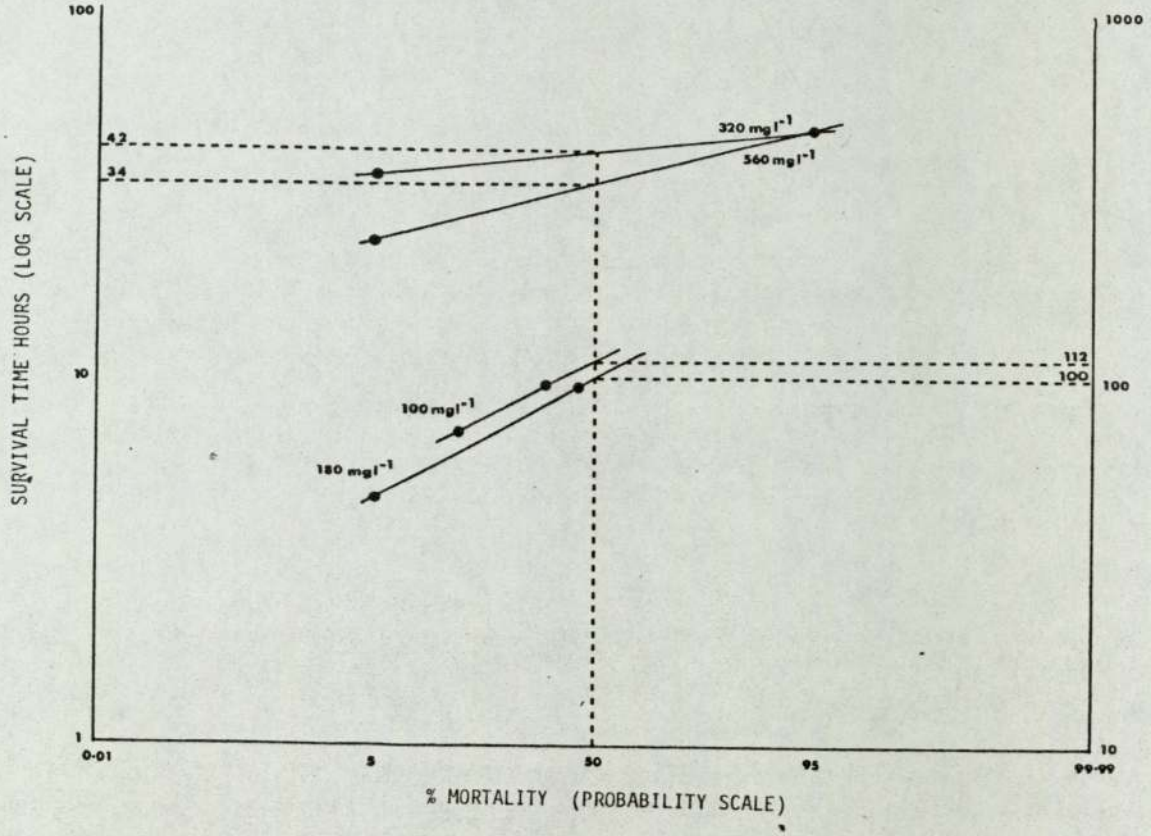


Fig. 5.15 LT50 values for H. angustipennis to four concentrations of copper sulphate at 10°C and 100 ppm $CaCO_3$



Finally, the results of Experiment 3 are presented, in which the copper toxicants were prepared using water with a hardness of 200 ppm $CaCO_3$. Table 5.15 presents data for copper concentrations in each of the toxicants.

TABLE 5.15 SUMMARY OF DATA FOR WATER SAMPLES 96 hr TOXICITY TEST at 200 ppm $CaCO_3$

SAMPLE Nominal Cu Concentration	Total Cu. A.A.S Cu^{++} mgl^{-1}	
560	158.8	585
320	95.3	330
180	41.3	220
100	19.05	65
Control	negligible	1.3

Table 5.16 records the mortalities of H. angustipennis in five concentrations of copper sulphate at 200 ppm $CaCO_3$. It may be seen that unlike the 100 mgl^{-1} Cu concentration in the previous two tests, there are no deaths in a solution with a hardness of 200 ppm $CaCO_3$ after 96 hours; in a similar trend the larvae have increased their rate of survival at 180 and 320 mgl^{-1} Cu, but show 100% mortality in 560 mgl^{-1} Cu after 48 hours in all the experiments. Figures 5.16 and 5.17 present 96 hr LC50 and LT50 results, LT50 values are summarised in Table 5.17. Using the median lethal time results from experiments 1, 2 and 3 with copper sulphate, an estimation by graphical methods may be made of LC50 values for both 48 hr LC50 and 96 hr LC50. Figures 5.18, 5.19 and 5.20 depict these results and the values are summarised in Table 5.18. As may be seen from Table 5.18, the softer the water the more acute the copper toxicant effect. This trend is similar if one calculates either 96 hr LC50 or 48 hr LC50 values.

TABLE 5.16 MORTALITY OF H. angustipennis in FIVE CONCENTRATIONS OF COPPER SULPHATE AT 200 ppm $CaCO_3$

Concentration time hrs.	560	320	180	100	Control
		mg $Cu l^{-1}$			
$\frac{3}{4}$	0	0	0	0	0
$1\frac{1}{2}$	0	0	0	0	0
3	0	0	0	0	0
6	0	0	0	0	0
12	0	0	0	0	0
24	25%	10%	0	0	0
48	100%	70%	10%	0	0
72		98%	30%	0	0
96		90%	40%	0	0

Fig.5.16 96 hr LC50 for H. angustipennis to copper sulphate at 10°C and 200 ppm $CaCO_3$

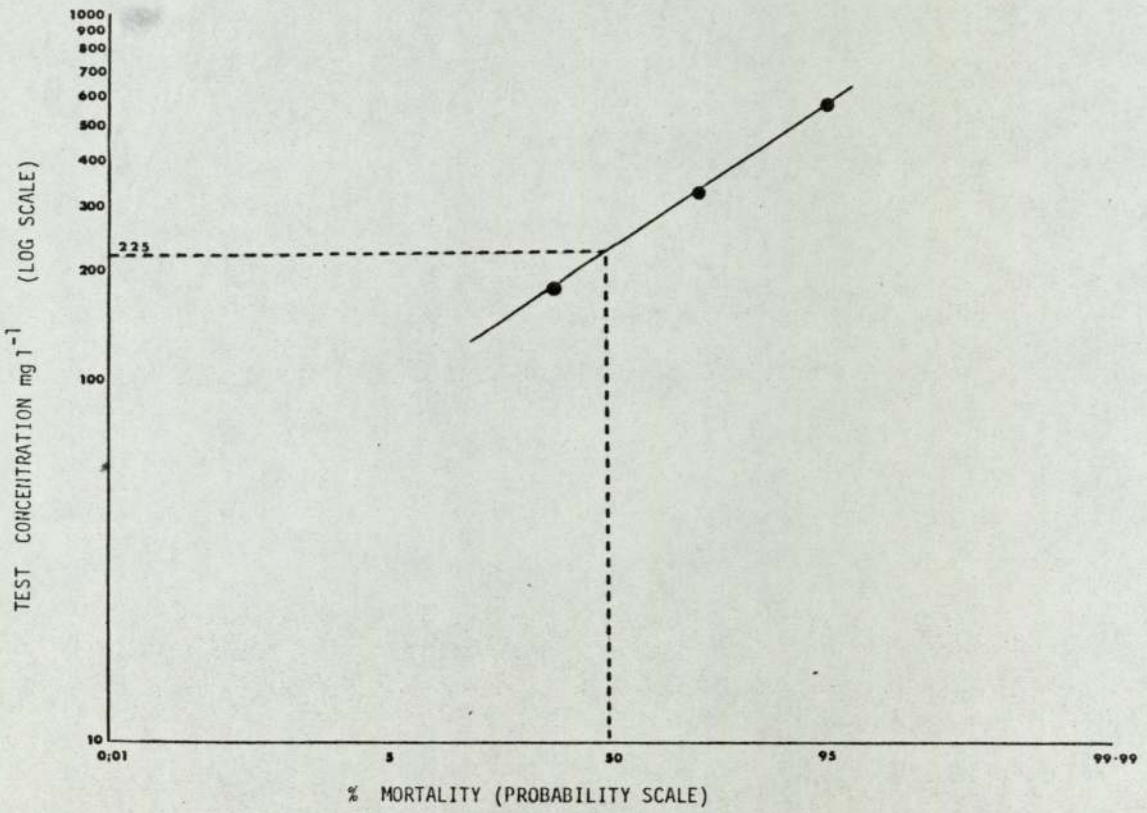


Fig.5.17 LT50 values for H. angustipennis to three concentrations of copper sulphate at 10°C and 200 ppm $CaCO_3$

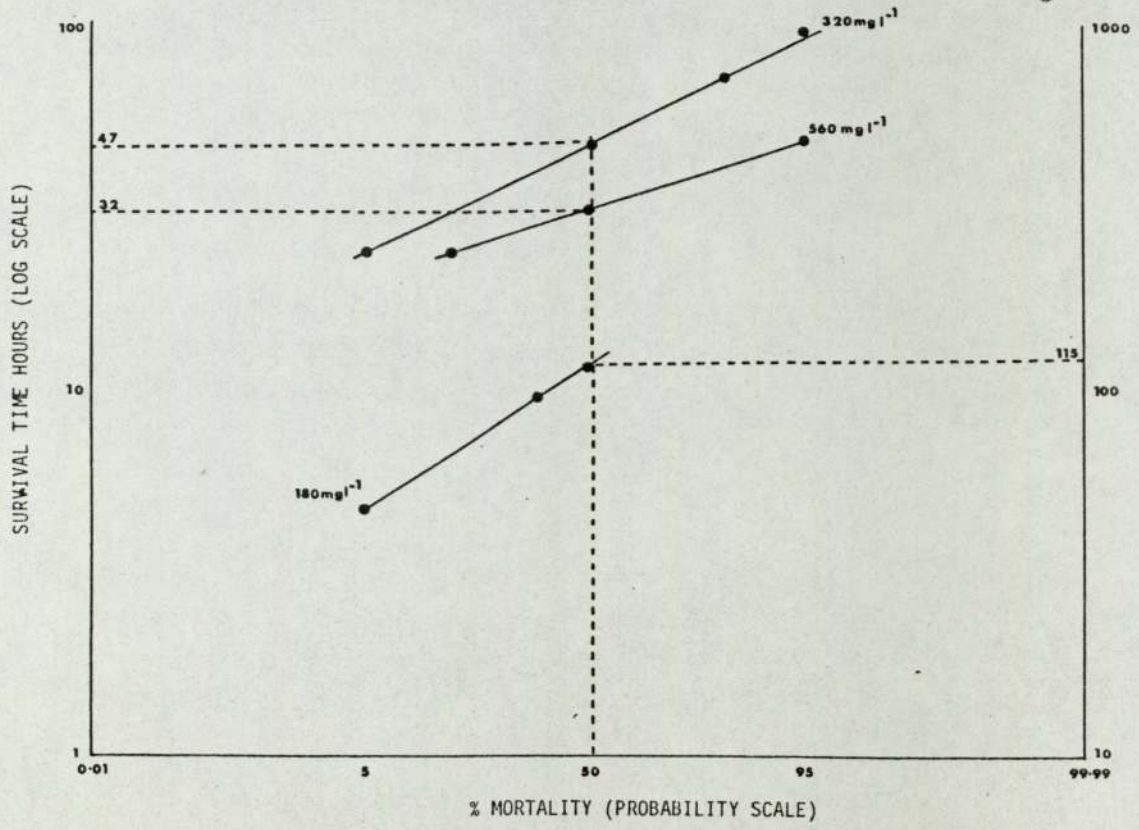


TABLE 5.17 RESULTS OF PLOTS OF LT50 AT VARYING COPPER CONCENTRATIONS AND WATER HARDNESS

WATER HARDNESS ppm $CaCO_3$	NOMINAL Cu Concn. ppm	LT50 (hrs)
19 ppm $CaCO_3$	560	$33\frac{1}{2}$
	180	53
	100	92
100 ppm	560	34
	320	42
	180	100
	100	112
200 ppm	560	32
	320	47
	180	115

Fig.5.18 Estimation of 96 hr and 48 hr LC50 values for H. angustipennis to copper sulphate at 10°C and 19 ppm $CaCO_3$

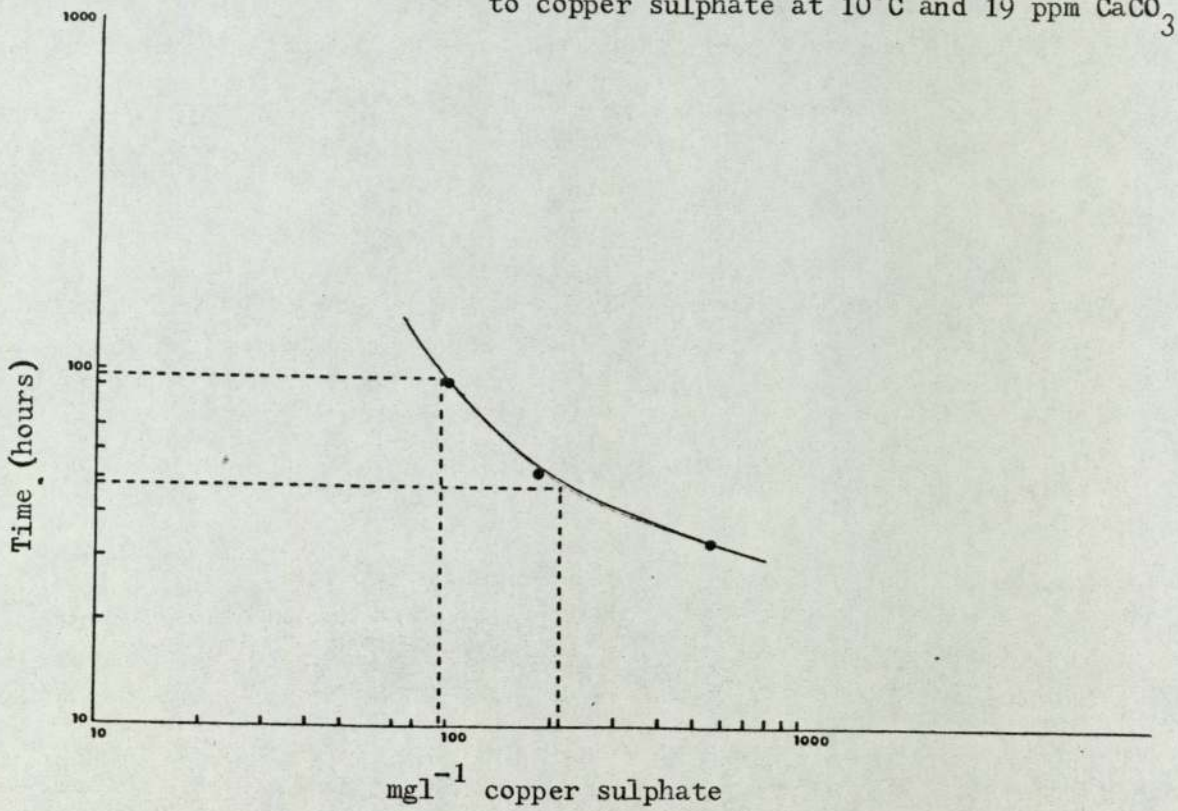


Fig.5.19 Estimation of 96 hr and 48 hr LC50 values for H. angustipennis to copper sulphate at 10°C and 100 ppm $CaCO_3$

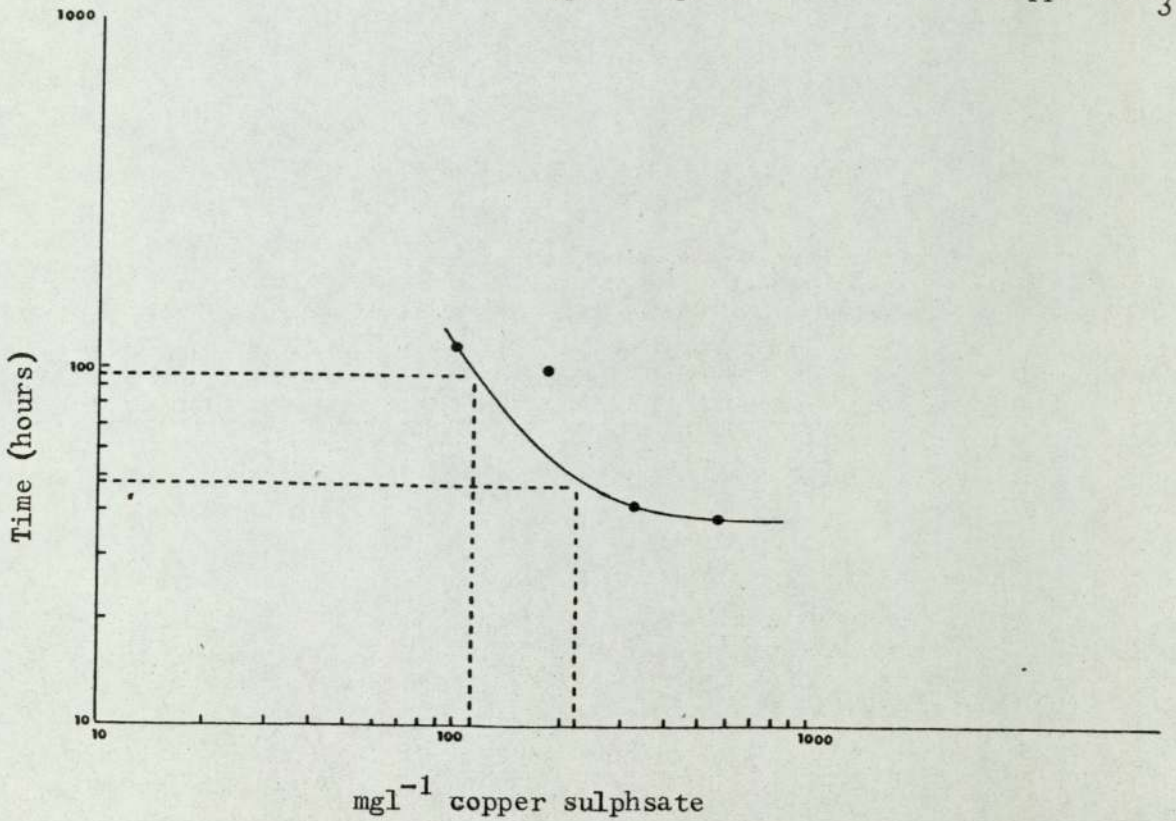


Fig.5.20 Estimation of 96 hr and 48 hr LC50 values for H. angustipennis to copper sulphate at 10°C and 200 ppm $CaCO_3$

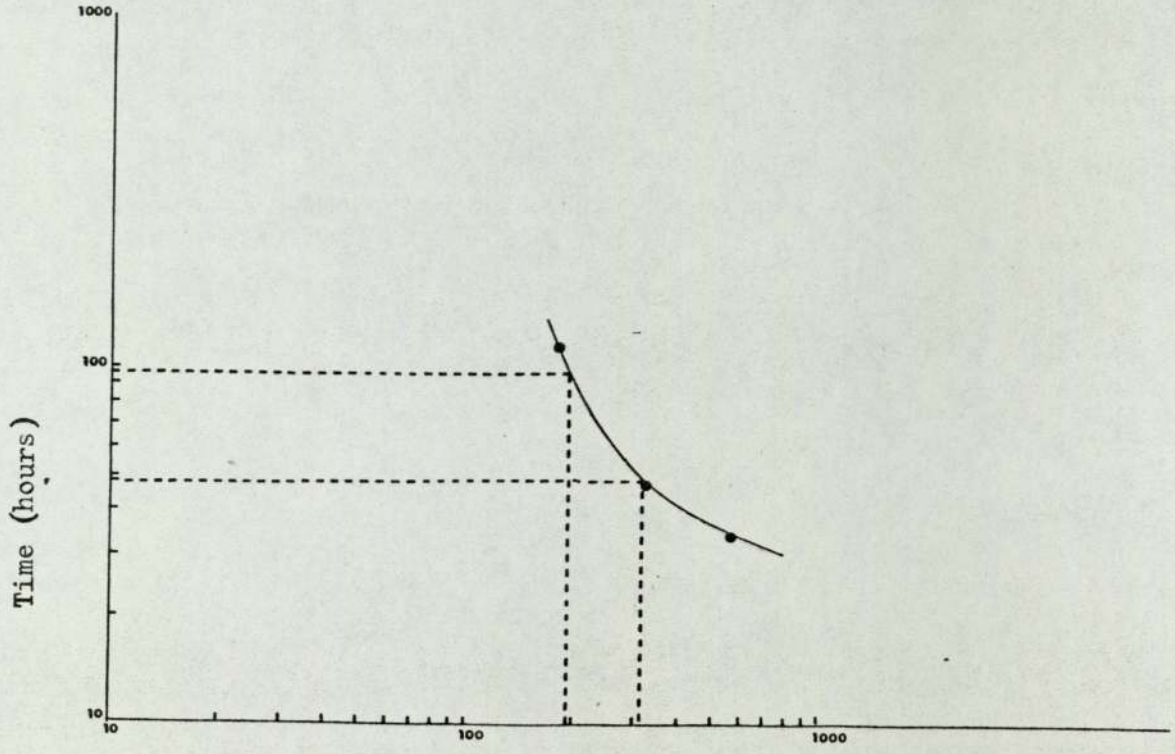


TABLE 5.18 ESTIMATED LC50 VALUES FOR H. angustipennis

Water Hardness ppm $CaCO_3$	Hours	Total concentration mg $Cu l^{-1}$
19 ppm $CaCO_3$	96 hr LC50	97
	48 hr LC50	210
100 ppm $CaCO_3$	96 hr LC50	112
	48 hr LC50	220
200 ppm $CaCO_3$	96 hr LC50	195
	48 hr LC50	310

5.4.3. DISCUSSION AND CONCLUSIONS

Comparing the 96hr LC50 results from the direct plots made after Experiments 1, 2 and 3 (Figs.5.12, 5.14 and 5.16) with the extrapolated 96hr LC50 results calculated from median lethal times (Figs. 5.18, 5.19 and 5.20) we may see from Table 5.18 that the estimated values are consistently lower than those from the direct LC50 tests. The discrepancy of 35 mgl^{-1} Cu at water hardnesses of 19 and 200 ppm $CaCO_3$, and also the larger difference at 100 ppm $CaCO_3$ is due to different experimental methodology, followed by extrapolation of experimental results, all of which may lead to increasing the margin of difference. It may be wise to recommend the lower values until further experiments have been carried out on the sensitivity of H. angustipennis to copper sulphate.

The 48hr LC50 values, as seen in Table 5.18 are proportionately higher than those at 96 hrs, as would be expected. However, the gradation of increasing sensitivity to decreasing water hardness remains the same throughout. This phenomenon, as previously discussed is due to increased concentration of ionic copper in softer waters as demonstrated by the data in table 5.19.

TABLE 5.19 SUMMARY OF LC50 VALUES FOR H. angustipennis

WATER HARDNESS ppm $CaCO_3$	96 hr LC50 (Actual Plot) mgl^{-1} Cu	96 hr LC50 (extrapolation from LT50) mgl^{-1} Cu
19 ppm	132	97
100 ppm	220	112
200 ppm	225	195
	48 hr LC50 (extrapolated)	96 hr LC50 (extrapolated)
19 ppm	210	97
100 ppm	220	112
200 ppm	310	195
	96 hr LC50 Actual Plot	equivalent mgl^{-1} Cu^{++}
19 ppm	132	100
100 ppm	220	50
200 ppm	225	54

The striking feature of all of these results is the extreme resilience of H. angustipennis to copper as a toxicant. It was found by Warnick and Bell (1969) that Hydropsyche bettini was extremely tolerant to copper. They recorded 50% mortality of larvae after 14 days in a copper solution of 32 mgl^{-1}. Clubb et al. (1975a) reported that another trichopteran Brachycentrus americanus was also highly resistant to cadmium. At an alkalinity of 240 ppm they recorded a 96 hr LC50 = 42.5 mgl^{-1} Cd^{++}.

In the natural river environment it is unlikely that such high concentrations of copper will be encountered, even in copper mining areas, but it is known that other factors working simultaneously cause an increased toxic effect in fish. This may also be true in invertebrate species, for example an elevation of temperature or a decrease in oxygen will increase the toxicity of heavy metals to rainbow trout. Altering these two parameters leads to stress in the trichopteran larvae. Presumably an additional stress, of a toxic heavy metal would result in limiting activity and premature death in the Hydropsyche species too. The presence of chelating agents, humic acids or excess calcium carbonate would result in a reduction in the amount of copper available in its most toxic form. In considering all these factors, it is most important to have knowledge of river water temperatures and other parameters as well as the expected levels of heavy metals likely to be released from an effluent when predicting the effect on the aquatic species present.

5.5. ZINC TOXICITY

Introduction

Zinc is an ubiquitous metal in the environment, in living organisms where it is used for nucleic acids (RNA and DNA)synthesis and in nature it occurs as sulphide, carbonate and hydrated silicate often associated with iron and cadmium.

Everyday items such as glassware, plastics and unpurified reagents contain zinc. It is used extensively in industry for galvanising in brass and alloys and other of its compounds such as the oxide, chloride, chromate and sulphide are widely used in other industries.

It is found in natural surface waters at background levels varying from 0.001 - 0.2 mgl^{-1} or even higher (O'Connor, 1968). Other sources quote a range from 0.002 - 10 mgl^{-1} Zn (data taken from a review of average river waters (Wilson, 1976). Levels quoted from EEC directives on quality of surface waters intended for the abstraction of drinking water recommend a guide level of 0.5 - 1.0 mgl^{-1} Zn and a mandatory level of 3-5 mgl^{-1}. Similarly, the directive on the quality of fresh water to support fish life quote the following levels

Total zinc mgl^{-1}	Water Hardness (mgl^{-1} $CaCO_3$)			
	10	50	100	500
Salmonid waters	0.03	0.2	0.3	0.5
Cyprinid waters	0.3	0.7	1.0	2.0

Zinc toxicity to aquatic organisms is mainly attributed to the ionic form, and perhaps also to particulate zinc, present as the basic carbonate or hydroxide held in suspension. Wastes containing zinc are often acidic with a high level of associated copper, lead cadmium and other heavy metals (O'Connor, 1968). The toxic effect is modified by water quality being reduced by an increase in hardness,

temperature, salinity and suspended solids and increased by a reduction in the concentration of dissolved oxygen. Addition of chelating agents such as NTA (nitrilotriacetic acid), EDTA (ethylenediaminetetraacetic acid) and DTPA (diethylentriamine penta-acetic acid) reduce lethal toxic effects when added to waters at neutral pH. Humic substances, amino acids, polypeptides and other organics may complex with zinc to reduce toxicity (Muramoto, 1980; Lloyd and Jordan, 1964). Alabaster and Lloyd (1980) also report that the addition of suspended solids may decrease the toxic effect of zinc, for example mine waters, when mixed with wastes from the flotation process which contains high concentrations of silica quartz and other finely divided materials adsorb the zinc from solution. Work by Lloyd (1960), Edwards and Brown (1967) show that brown and rainbow trout, respectively are able to acclimate to zinc. It is suspected that this phenomenon may also be true of invertebrates.

In Britain, the metal mining areas of West Wales exhibit some classic examples of zinc pollution, as seen from field work, section 4.6. A summary of toxicity test data on zinc to invertebrates and fish is found in Table 5.20.

Methods

Essentially the methods for the zinc experiments were the same as those for the copper toxicity work. Ranging tests were carried out over a 24 hour period, followed by LT50 tests at five zinc concentrations plus a control. The first set of LT50 tests were performed using final instar H. angustipennis (as with copper), but secondly a similar set of tests were carried out using 3rd. instar larvae with a head capsule size of 0.48 - 0.6 mm, to investigate toxicity with respect to age. Analar zinc sulphate was used to prepare toxicant solutions with a zinc concentration of 1,000, 560, 320, 180 and 100 mgl^{-1} in water of 19 ppm $CaCO_3$ hardness. All tests

TABLE 3.20 ZINC TOXICITY TO INVERTEBRATES AND FISH

Species	Toxicity Unit	Concn. of Metal	Conditions			Reference
			Temp.	pH	hardness	
G. pulex	48 hr LC50	3.2 mgl^{-1} Zn			1.00 ppm $CaCO_3$	Martin (1972)
G. pulex	8 days 50% dead	1.0 mgl^{-1} Zn				Herbst (1967)
	12½ " 50% dead	0.5 mgl^{-1} Zn				"
Rhyacophila sp. *Polycentropid sp.* *Hydropsyche sp.*	All survived 20 days	20.0 mgl^{-1} Zn				"
H. bettini	11 day LC50 Total survived	32.0 mgl^{-1}				Warnick & Bell (1969)
	14 day LC50 survived	32.0 mgl^{-1}				" "
S gairdnerii (Richardson)	Median survival time 285 mins	10.0 mgl^{-1}	15.0	7.97	320 ppm	Lloyd (1960)
	" 180 "	20.0 mgl^{-1}	15.0	7.96	320 ppm	"
	" 162 "	30.0 mgl^{-1}	15.0	7.94	320 ppm	"

were performed at 10°C.

5.5.2. Results

The ranging tests demonstrated that zinc was less toxic to H. angustipennis than copper over a 24-hr period.. There was only one dead larva in the 1,000 mgl^{-1} test beaker, all other larvae survived in the less concentrated zinc solutions and in the control. Consequently the 96hr LC50 test comprised 5 concentrations as above, plus a control. The mortalities are recorded in Table 5.21. The following tables 5.22 and 5.23 summarise the pH levels as established by daily electrode readings, and the zinc concentration as assessed by atomic absorption spectrophotometry respectively.

Table 5.22 demonstrates that the range of pH at any single zinc concentration was small and that the more concentrated the toxicant became the pH decreased slightly. A change of only 0.05 pH units between 100 - 1000 mgl^{-1} Zn. Similarly the zinc concentration was reasonably consistent at each concentration. As less than 50% mortality was observed within 96 hrs. in the most concentrated (1000 mgl^{-1} Zn) toxicant solution, it was impossible to construct a graph to calculate the LC50 value. However, a long term LT50 test was then performed at 10°C and 19 ppm $CaCO_3$ using the same range of zinc concentrations. Table 5.24 records the mortality rate of H. angustipennis over the 64 day experimental period. There were no mortalities in the three control chambers. During this test the zinc solution was replaced daily to maintain the toxic levels, at the same time as larval mortalities were counted. Approximately every five days the larvae were fed on flaked fish food. They spun nets in the test chambers, usually positioning themselves in the angle of the test beaker between the side and bottom, or slightly up the side.

Plots of LT50's are made by subtracting 5% for each reading as explained in section 5.4.2. Figures 5.21 - 5.25 show the LT50 cal-

TABLE 5.21 MORTALITY OF H. angustipennis in SIX CONCENTRATIONS OF ZINC SULPHATE

Concentration / Time (hrs)	1000	560	320	180	100	Control
		mgl^{-1} zinc				
$\frac{3}{4}$	0	0	0	0	0	0
$1\frac{1}{2}$	0	0	0	0	0	0
3	13%	0	0	0	0	0
6	20%	0	0	0	0	0
12	27%	0	0	0	0	0
24	27%	0	0	0	0	0
48	27%	0	0	0	0	0
72	47%	7%	7%	7%	7%	0
96	47%	13%	13%	13%	7%	0

TABLE 5.22 pH AT EACH ZINC CONCENTRATION

Concentration mgl^{-1}	pH	range
1000	5.86	(5.68-5.95)
560	5.87	(5.67-5.98)
320	5.93	(5.82-6.00)
180	5.95	(5.80-6.03)
100	5.91	(5.60-6.06)
Control	6.62	(6.40-6.71)

TABLE 5.23

Sample Nominal Concn. Zinc	Zinc concentration mgl^{-1}		
	$\bar{x}$	range	S.D.
1000	953.6	862.0-1166.0	124.4
560	514.6	493.0-541.0	21.7
320	287.1	262.8-301.5	15.3
180	165.7	152.4-172.0	8.1
100	92.9	89.9- 96.0	2.2
Control	1.0	0.01- 4.4	1.9

TABLE 5.24 OCCURRENCES OF MORTALITIES DURING ZINC LT50 EXPERIMENT

Concentration mgl^{-1} Zn	Time days	No. Dead	% Dead	% - 5
1000	4	1	6.6	1.6
	5	5	33.3	28.3
	7	7	46.6	41.6
	8	10	66.6	61.6
	13	11	73.3	68.3
	15	12	80.0	75.0
	20	13	86.6	81.6
	22	14	93.3	88.3
	42	15	100.0	95.0
560	2	1	6.6	1.6
	7	2	13.3	8.3
	9	6	40.0	35.0
	10	7	46.6	41.6
	13	8	60.0	55.0
	16	10	66.6	61.6
	20	12	80.0	75.0
	30	13	86.6	81.6
	34	14	93.3	88.3
	35	15	100.0	95.0
320	4	1	6.6	1.6
	6	3	20.0	15.0
	8	5	33.0	28.0
	13	6	40.0	35.0
	14	7	46.6	41.6
	21	8	53.3	48.3
	24	9	60.0	55.0
	27	11	73.0	68.0
	34	12	80.0	75.0
	36	14	93.3	88.3
	62	15	100.0	95.0

continued

TABLE 5.24 continued

Concentration mgl^{-1}	Time days	No. dead	% dead	% - 5
180	3	1	6.6	1.6
	4	2	13.3	8.3
	6	3	20.0	15.0
	8	5	33.0	28.0
	13	8	53.3	48.3
	14	10	66.6	61.6
	17	11	73.3	68.3
	23	13	86.6	81.6
	52	15	100.0	95.0
100	3	1	6.6	1.6
	8	3	20.0	15.0
	13	6	40.0	35.0
	17	7	46.6	41.6
	20	8	53.3	48.3
	21	9	60.0	55.0
	27	10	66.6	61.6
	28	11	73.3	68.3
	42	12	80.0	75.0
	49	14	93.3	88.3
	64	15	100.0	95.0

FIG.5.21 LT.50 value for H. angustipennis at 1000 mgl^{-1} Zn (final instar)

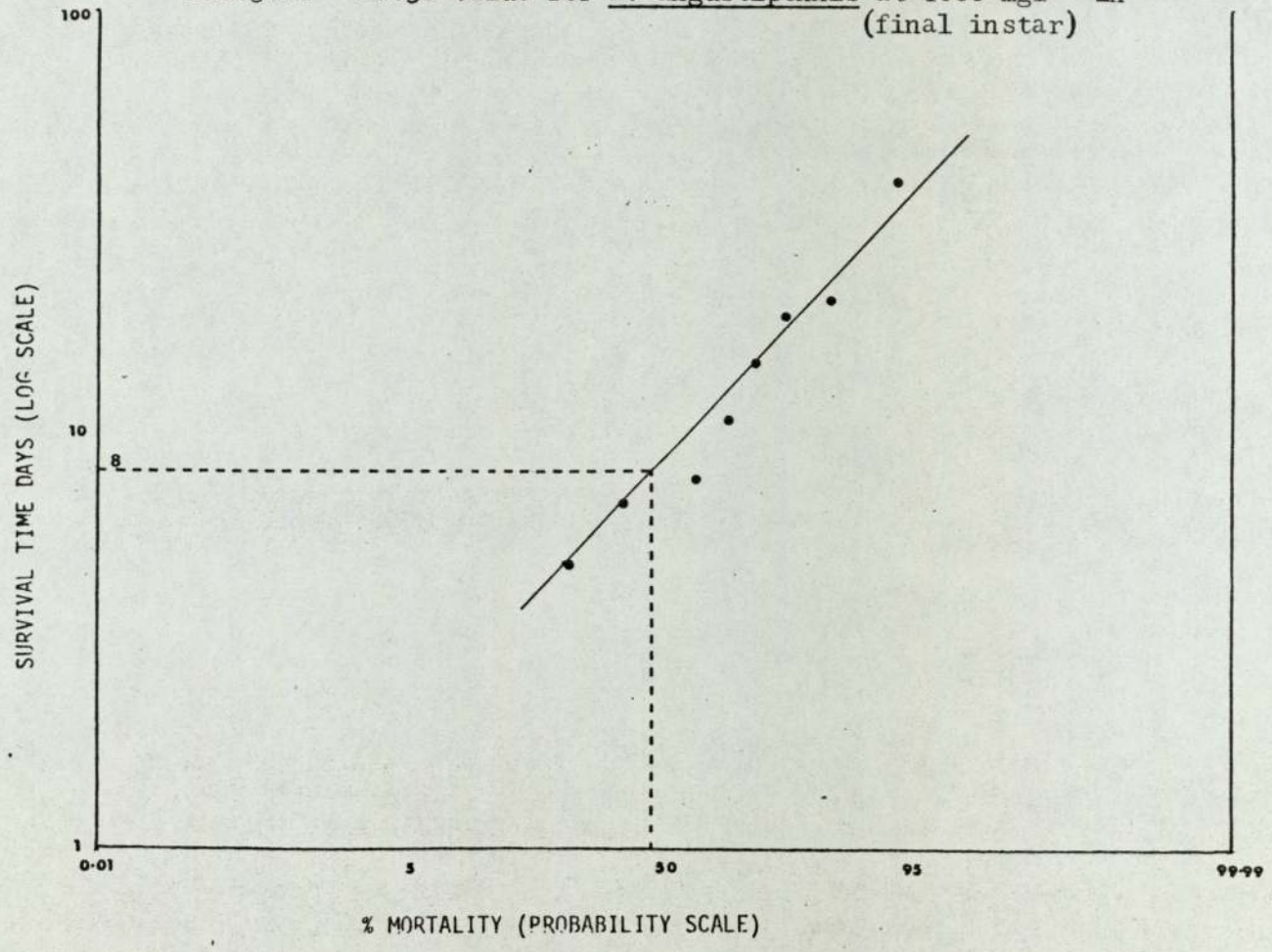


Fig.5.22. LT.50 value for H. angustipennis at 560 mgl^{-1} Zn (final instar)

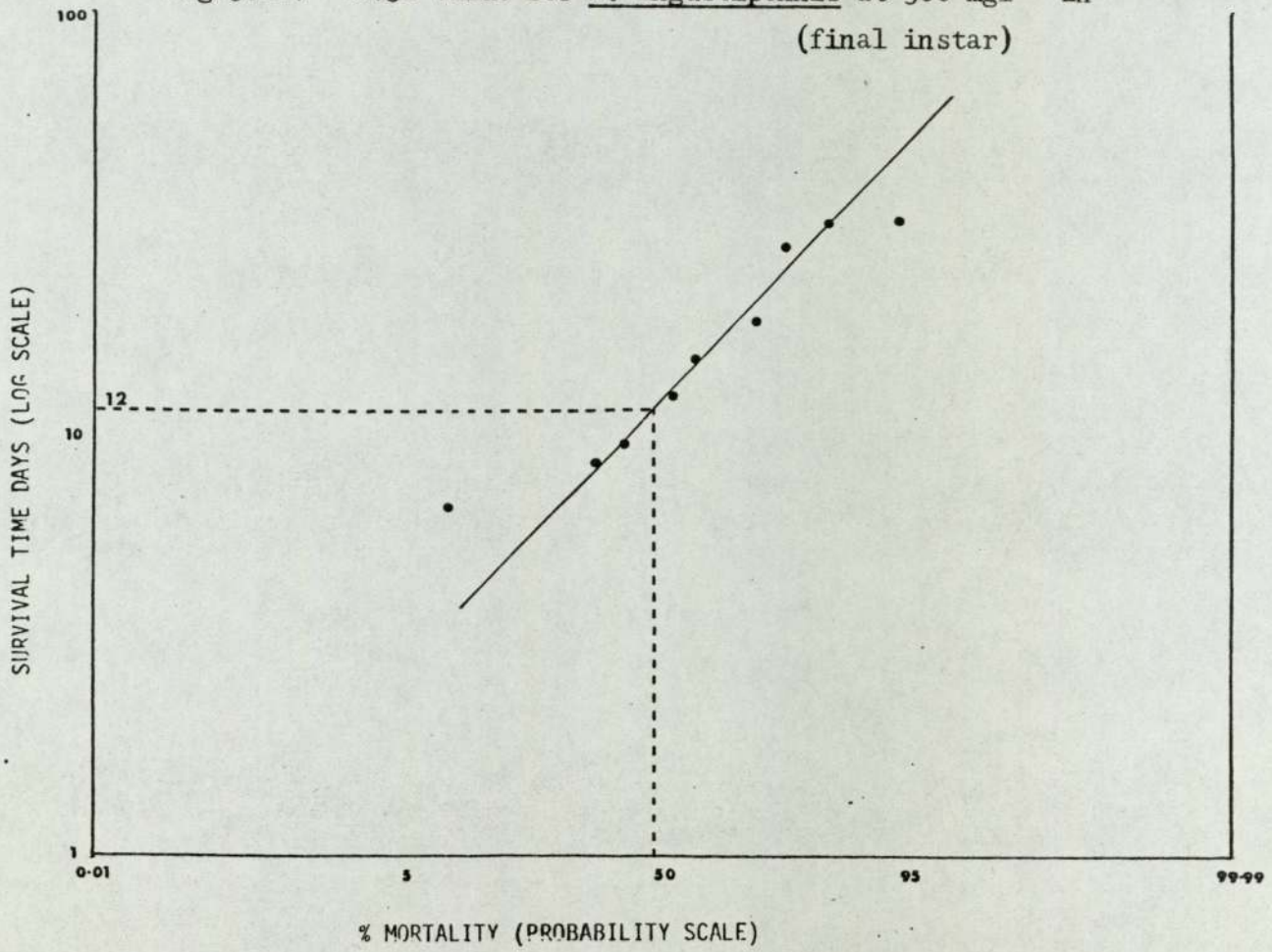


FIG.5.23 LT50 value for H. angustipennis at 320 mgl^{-1} Zn (final instar)

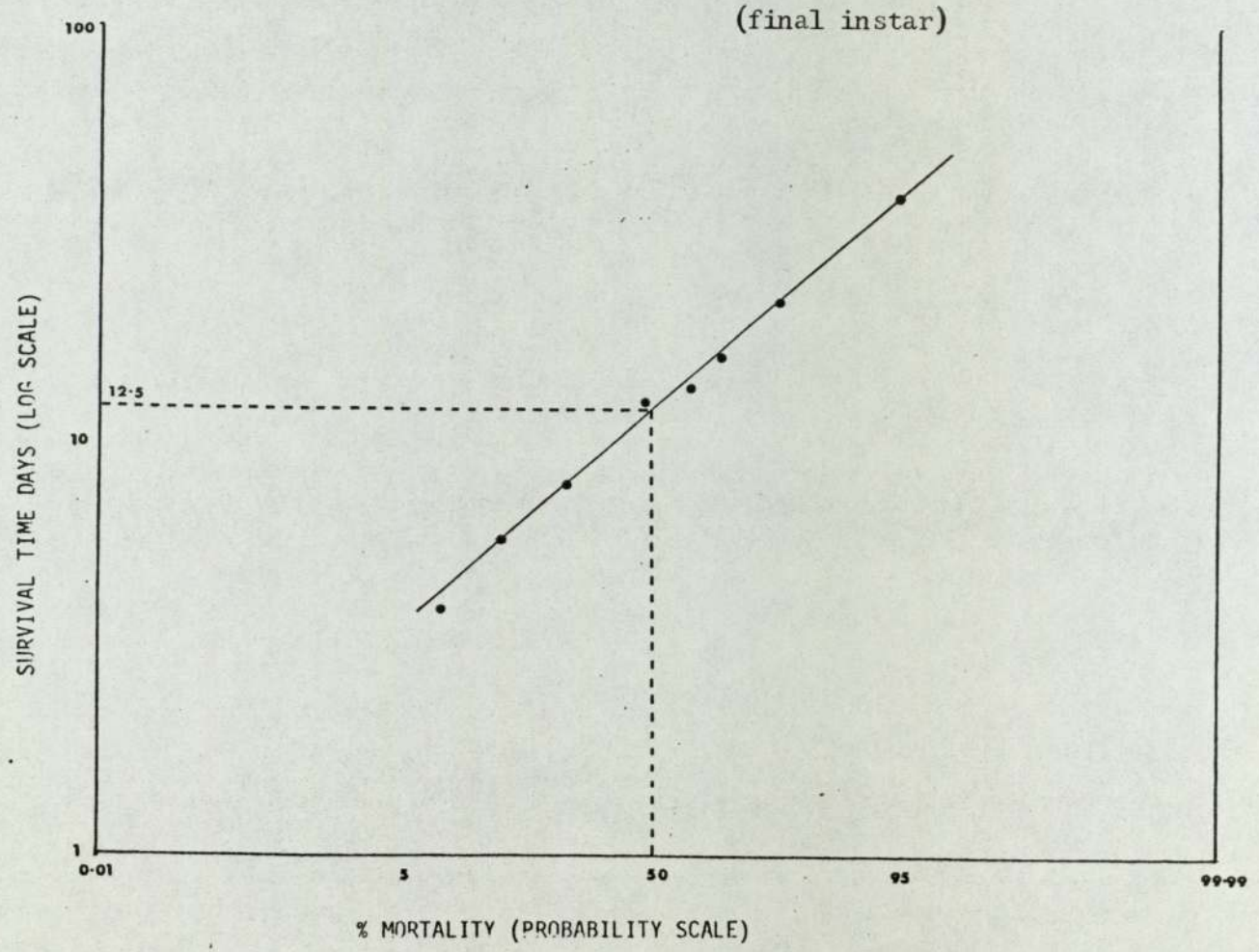


FIG. 5.24 LT50 value for H. angustipennis at 180 mgl^{-1} Zn (final instar)

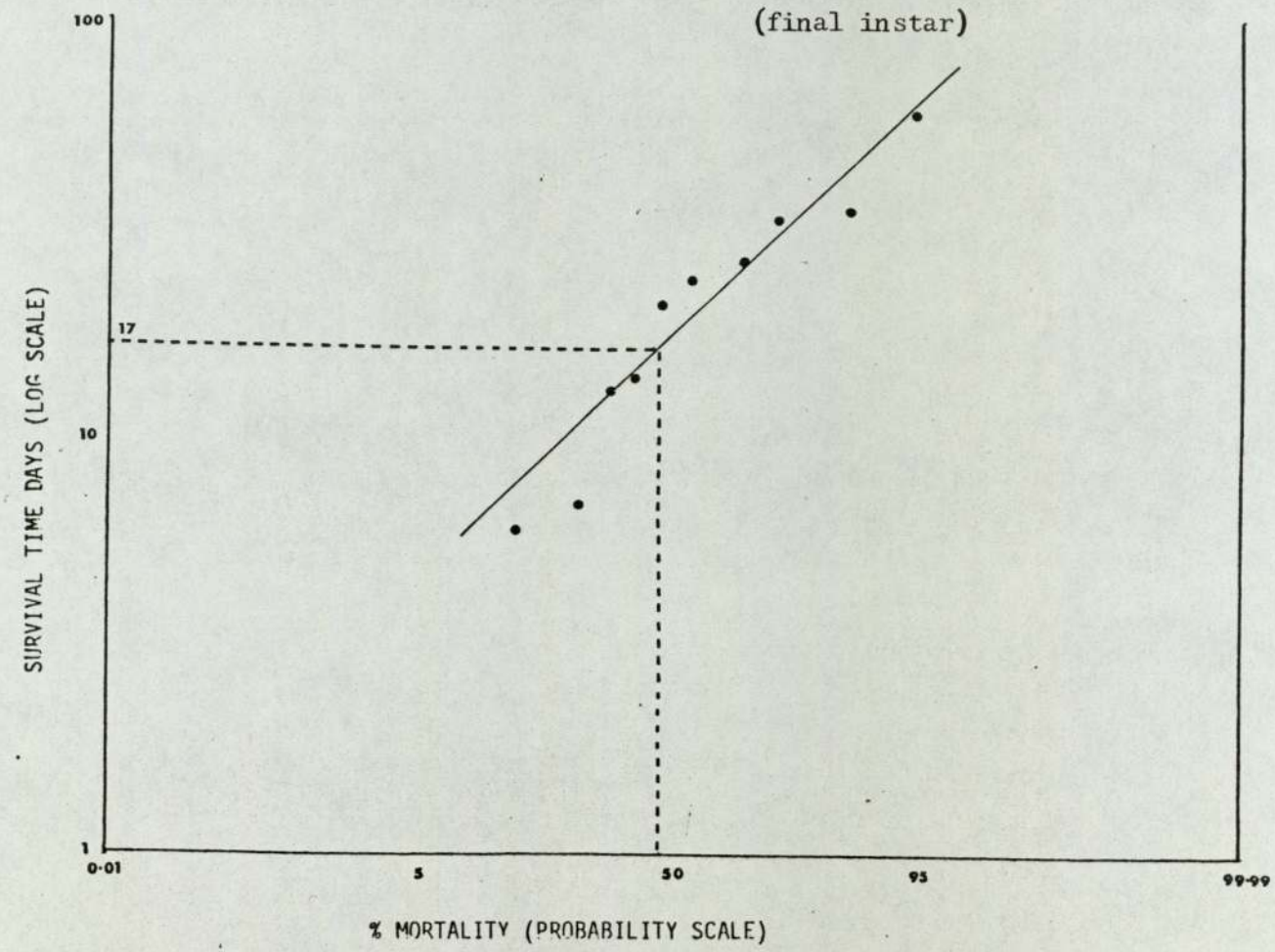
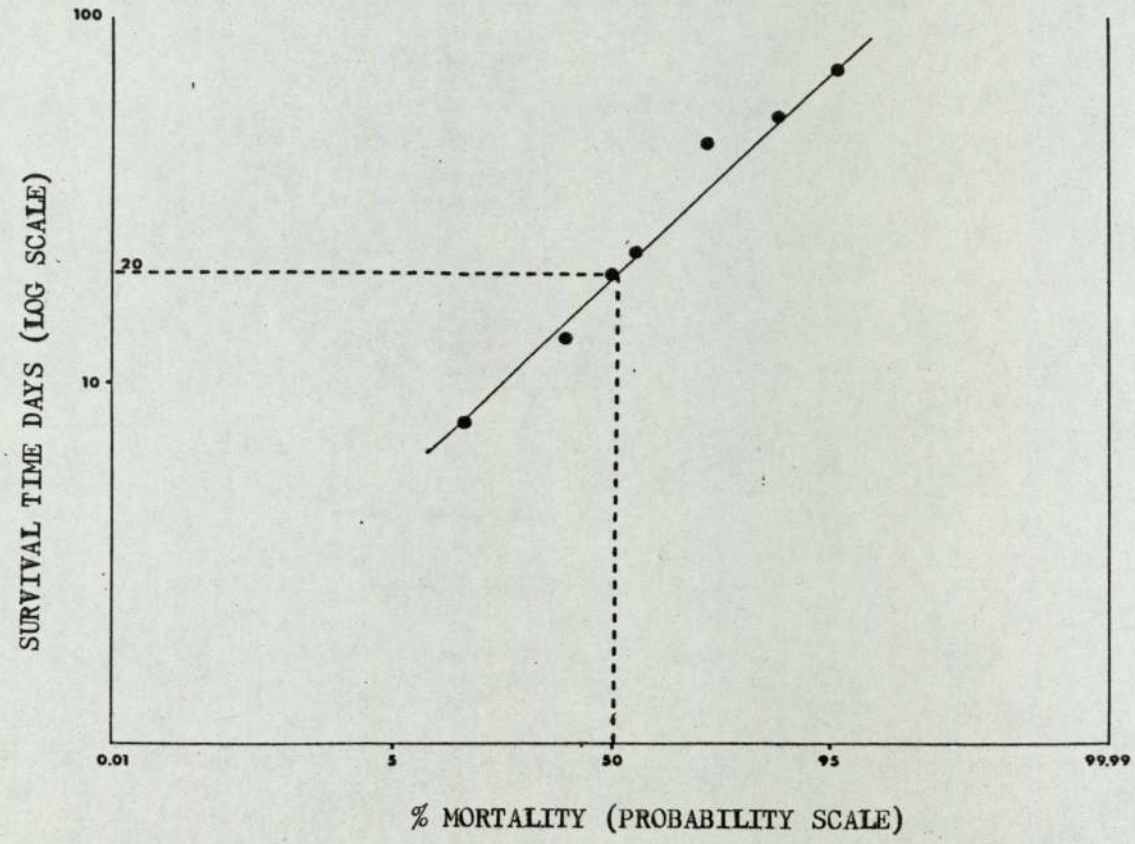


Fig.5.25. LT.50 value for H. angustipennis at 100 mgl^{-1} Zn (final instar)



culations at each experimental zinc sulphate concentration. The results are summarised in Table 5.25 below.

TABLE 5.25. LT50 VALUES FOR H. angustipennis AT FIVE CONCENTRATIONS OF ZINC SULPHATE

Concentration mgl^{-1}	Median Survival Time (days)
1000	8.5
560	12.0
320	12.5
180	17.5
100	20.0

By estimation using graphical means, this would give H. angustipennis a 96 hr LC50 value slightly in excess of 900 mgl^{-1}.

Results of LT50 tests on third instar H. angustipennis are recorded in Figs. 5.26 - 5.30. The values are summarised below (Table 5.26) and compared with copper and zinc results for final instar larvae

TABLE 5.26. LT50 VALUES FOR H. angustipennis TO $CuSO_4$ and $ZnSO_4$ AT FIVE CONCENTRATIONS, 10°C and 19 ppm $CaCO_3$

Concentration mgl^{-1}	Cu LT50 hours final instar	Zn LT50 days final instar	Zn LT50 days 3rd. instar
1000	-	8.5	3.0
560	33.5	12.0	7.5
320	42.5	12.5	7.6
180	53.0	17.5	13.0
100	92.0	20.0	18.0

Fig.5.26 LT50 value for H. angustipennis at 1000 mgl^{-1} Zn

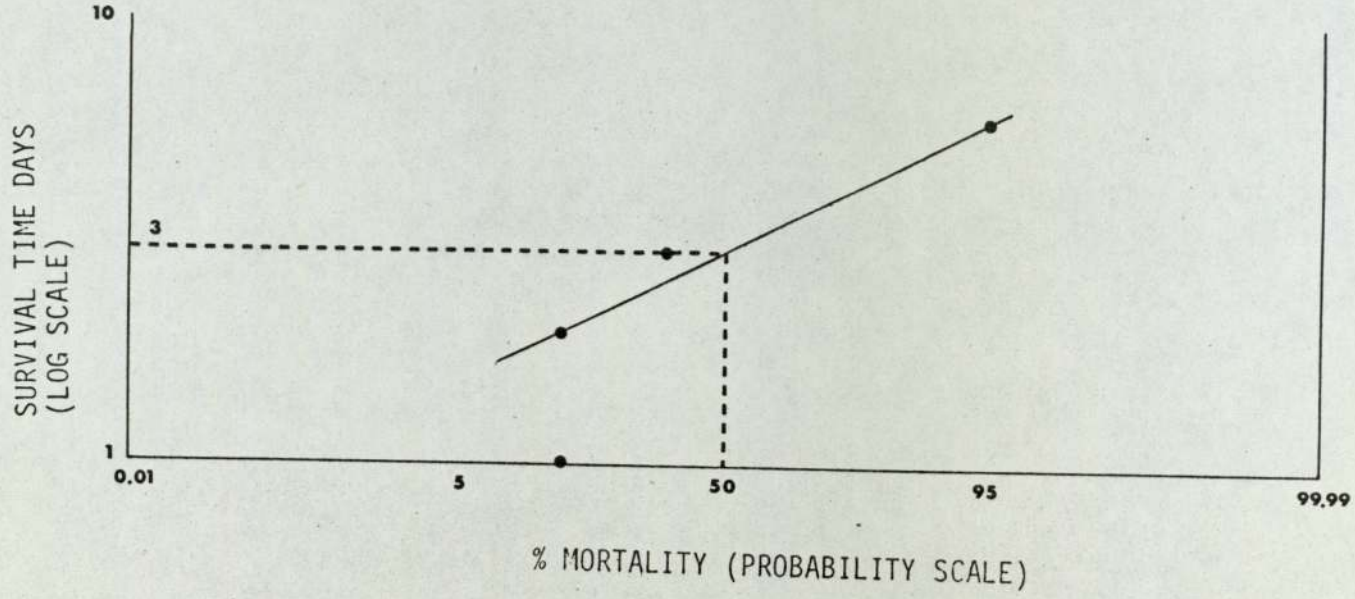


Fig. 5.27 LT50 value for H. angustipennis at 560 mgl^{-1} Zn

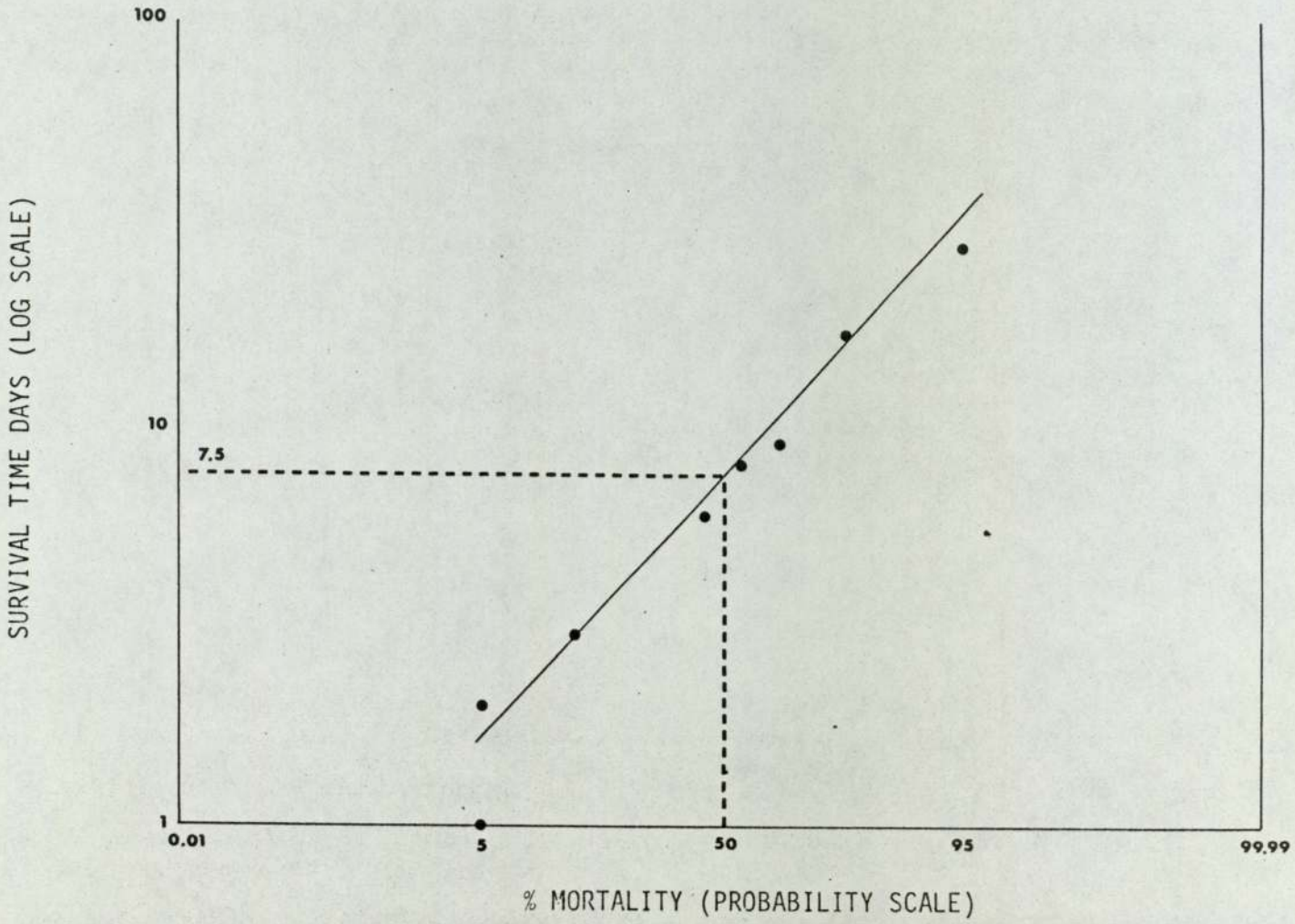


Fig. 5.28. LT50 value of H. angustipennis at 320 mgl^{-1} Zn

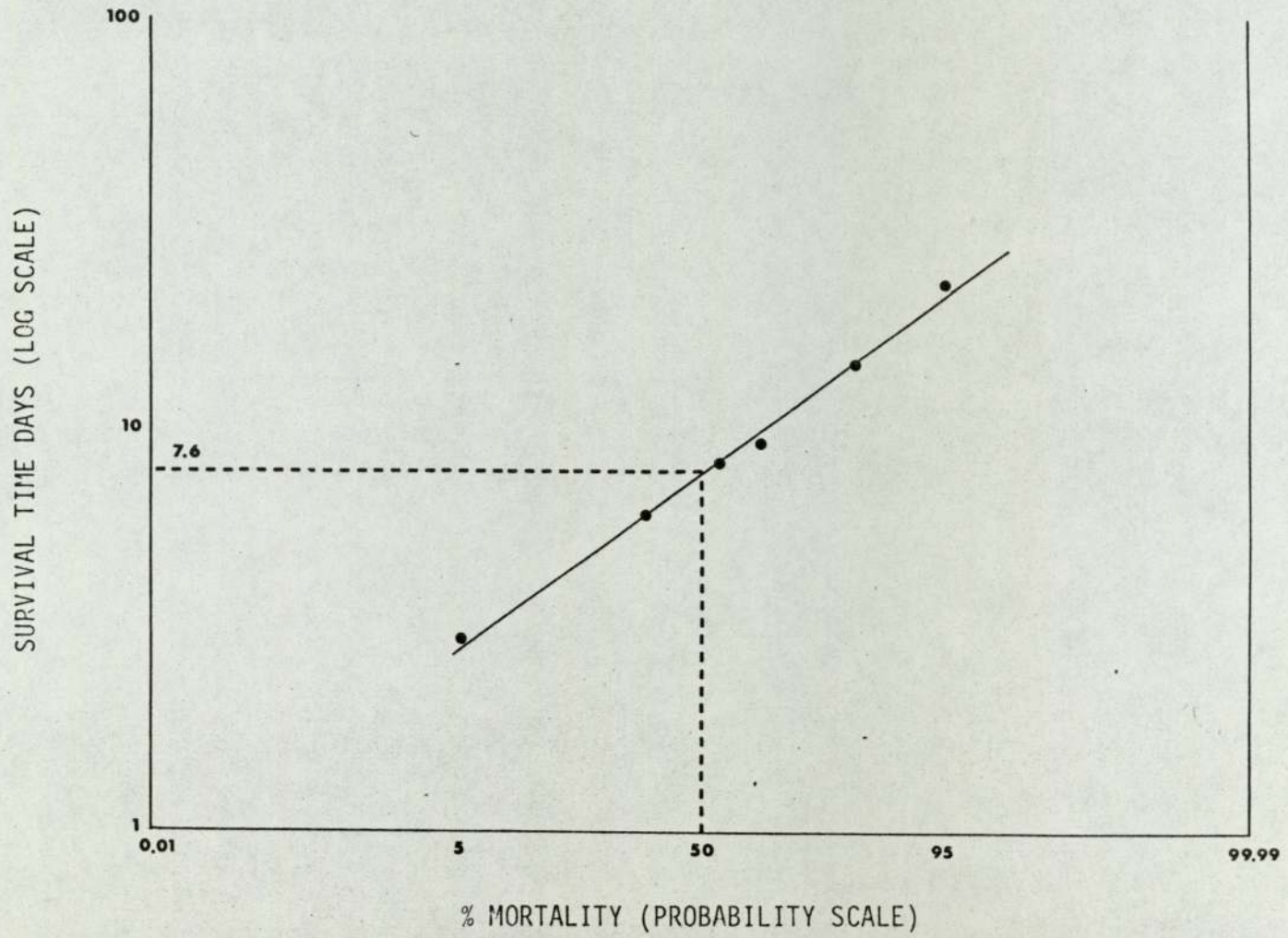


Fig. 5.29 LT50 value for H. angustipennis at 180 mgl^{-1} Zn

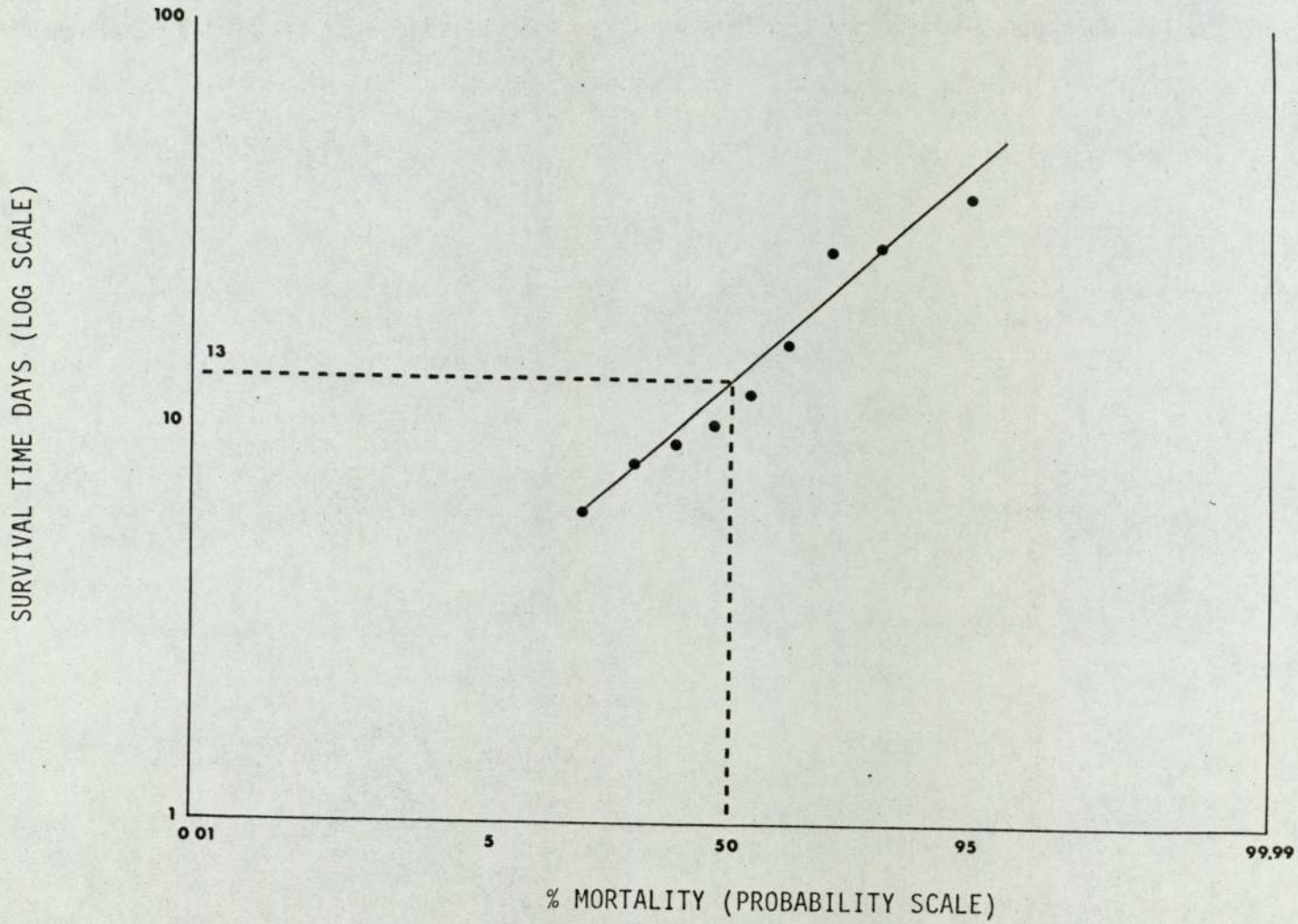
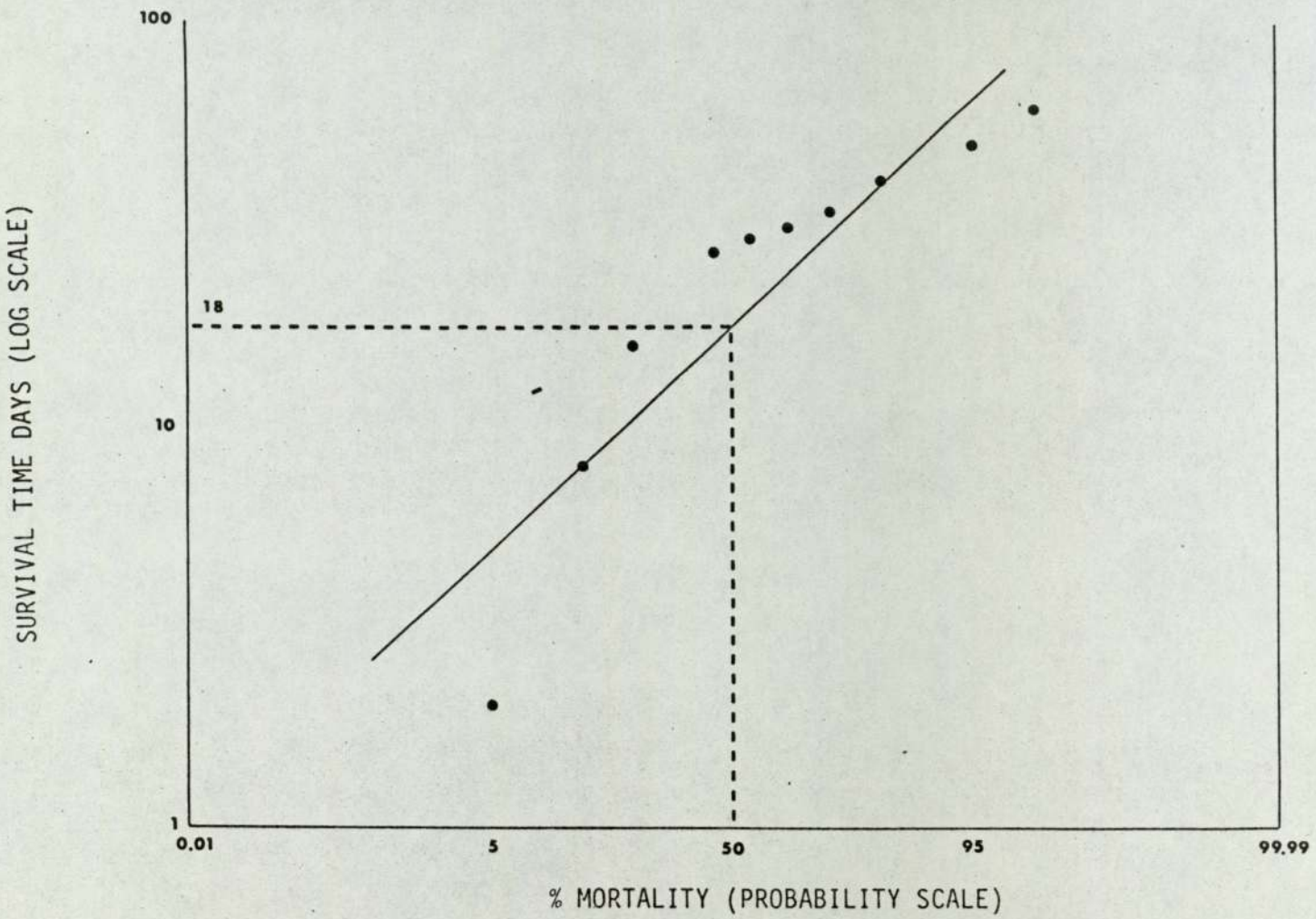


Fig. 5.30 LT50 value for H. angustipennis at 100 mgl^{-1} Zn



5.5.3. Conclusions

It may be seen that third instar H. angustipennis are more sensitive than final instar larvae to comparable concentrations of zinc. At all concentrations, comparing final instar larvae copper is far more toxic than zinc.

Thus, stage of life cycle and specific toxicant must be carefully considered when quoting values for toxicity of heavy metals.

5.6. AMMONIA TOXICITY

Introductory Review

The toxicity of ammonia to aquatic species is related to pH value amd the temperature of the water, due to the fact that the unionized fraction of ammonia is the major toxicant. Concentrations of ammonia are described using differing terminology, so to clarify:

"ionized ammonia" = NH_4^+

"unionized ammonia" = NH_3

"ammonia" = $(NH_3 + NH_4^+)$

For calculating the percentage of unionized ammonia present in an ammonia solution the following formula may be used:

$$\% \text{ unionized ammonia} = \frac{100}{1 + \text{antilog } (pKa - pH)}$$

where pKa = negative log of the ionisation constant.

The value of pKa depends on temperature and appropriate values can be taken from Emerson et al. (1975) and are very close to those of Bates and Pinching (1950).

Ammonia is present in most waters as a natural degradation product of proteins. The most common source of ammonia in rivers is from sewage effluents, although large quantities may be produced by industries such as coal gas, coke and fertilizers. Ammonia gas dissolves in water, thus

$$NH_3 + H_2O \quad NH_4^+ + OH^- .$$

This equilibrium is dependent upon pH and temperature - higher values of each favouring the existance of the unionized form (NH_3).

Toxicity of ammonia may also be modified by such factors as hardness, alkalinity, free CO_2 and salinity. Consequently, when experiments on ammonia toxicity are performed, strict controls are necessitated.

Numerous toxicity studies involving fish have been carried out. Ball (1967) setting out rigorous testing systems with fish.

Studies on invertebrate organisms have been fewer and apart from studies by Malacea (1966) on D. magna, and Davis (1971) workers have not differentiated between undissociated and dissociated forms. Davis (1971) tested eight species, R. dorsalis, E. dispar, G. pulex, H. angustipennis, A. aquaticus, E. octoculata, E. testacea and H. stagnalis classifying the first two as clean water species, G. pulex and H. angustipennis as mild organic pollution species and the last four species as tolerating severe organic pollution. He tested at dissolved oxygen tensions of 10, 2 and 1 ppm and unionized ammonia concentrations of 0.25, 0.75, 1.5 and 3.0 ppm as N at 10°C. His experiments revealed that R. dorsalis and H. angustipennis were far more tolerant of unionized ammonia than the other invertebrate species. 50% of H. angustipennis survived when DO = 2 mgl^{-1} and NH_3-N = 3.0 ppm. R. dorsalis, although tolerant was killed at NH_3-N = 3.0 ppm at both 10 mgl^{-1} DO and 2.0 mgl^{-1} DO. All individuals survived at an NH_3-N concentration of 1.5 ppm at 10 mgl^{-1} DO although they died at this ammonia concentration at 2.0 mgl^{-1} DO.

The chironomid species Davis used in his similar ammonia experiments C. riparius, P. olivacea and Brillia longifurca were also very tolerant but he reported them to be poor experimental animals once they were removed from their tubes, as they rarely survived the full experimental programme.

Continuing the gradation of tolerance of these species, the leeches were the next most tolerant, then Asellus followed by the most sensitive Ecdyonurus and Gammarus. When he repeated the experiments at 18°C the trichopteran species were still the most tolerant and G. pulex and E. dispar the most sensitive. Generally decreasing the oxygen concentration increases the toxic effect of the unionized ammonia.

The concentrations of ammonia present in some British rivers are

almost certainly toxic to some forms of freshwater life. Recommended maximum concentrations of undissociated ammonia for freshwaters range from 0.005 - 0.025 mgl^{-1} (E.IFAC 1970; EPA, 1976). The H.M. Scheme in 1976 gave a mean value for 229 sites for total ammonia as 0.899 mgl^{-1} N. The range of values for the ten worst sites over the period 1974 - 1978 was 13.1 - 42.9 mgl^{-1} N (from Murphy, 1979). Obviously, there is a need for more complete data on freshwater macro-invertebrate species and their sensitivity to ammonia under different conditions. In the following experiments, Hydropsyche angustipennis continues to be investigated.

5.6.1. Methods

Essentially the method for these experiments was the same as for copper and zinc studies. Analar ammonium sulphate $(NH_4)_2SO_4$ was used for making up stock solutions. Replicates were made at each concentration of 1000, 560, 320, 180 and 100 with a control of tap water (19 ppm $CaCO_3$). The pH was measured daily using a pH electrode and daily samples of the ammonium sulphate solution were taken, preserved with 6 drops conc. HCl and then analysed on the autoanalyser. Temperature in the cabinet where the experiments were performed was kept at a steady 10°C. pH readings were taken daily.

Ranging tests were carried out and five LT50 tests performed. From these the 96 hr LC50 value was calculated.

5.6.2. Results

The mean pH values in each of the toxicant concentrations are recorded in Table 5.27 below:

TABLE 5.27 MEAN pH VALUES IN SIX CONCENTRATIONS OF AMMONIUM SULPHATE

Sample Concentration mgl^{-1}	$\bar{x}$ pH
1000	8.0
560	7.9
320	7.8
180	7.8
100	7.7
Control	6.9

As may be seen from these results, the addition of ammonium sulphate has the effect of increasing the pH in the test chamber, As this in turn affects the concentration of unionized ammonia in the sample, the toxicant solutions were checked on the autoanalyser for total concentration and the percentage of NH_3 calculated from a standard table.

The results may be seen in Table 5.28.

TABLE 5.28. NH_3 CONCENTRATIONS USED IN TOXICITY TEST AT 10°C

Nominal Total level	Actual Total level	pH	% NH_3 from table	ppm NH_3
100	106	7.7	0.925	0.98
180	187	7.8	1.16	2.17
320	347	7.8	1.16	4.02
560	615	7.9	1.46	8.98
1000	1120	8.0	1.83	20.49

The LT50 values resultant from the tests may be seen in figures 5.31 - 5.35. These are summarised in Table 5.29.

TABLE 5.29 LT50 VALUES FOR H. angustipennis AT FIVE AMMONIUM SULPHATE CONCENTRATIONS

Concentration (Total Ammonia)	Concentration NH_3	LT50 days
1000 mgl^{-1}	20.49	1.2
560 mgl^{-1}	8.98	3.25
320 mgl^{-1}	4.02	4.25
180 mgl^{-1}	2.17	11.0
100 mgl^{-1}	0.98	15.0

Fig.5.36 shows the calculated 96hr LC50 value for H. angustipennis to be 350 mgl^{-1} which is equivalent to 3.23 mgl^{-1} NH_3. Once again, this is an extremely high tolerance to ammonia for an aquatic invertebrate species.

5.6.3. DISCUSSION AND CONCLUSIONS

These data are comparable with those of Davis (1971) in which H. angustipennis exposed to a concentration of 1.5 ppm NH_3 survived up to 300 hrs. (12.5 days).

The caseless caddis are extremely tolerant of unionized ammonia in even oxygen-poor environments and will be markers of heavily organically polluted sites. They are more tolerant of ammonia toxicity than fish, in which the mode of toxic action is through gill surfaces, reducing the oxygen carrying capacity of the blood, and increasing the respiratory rate. However, it is difficult to quote toxic levels for fish, as various workers have reported differential resistance to eggs, alevins and fingerlings. Short exposure does not affect some fish but data for larger exposures is not yet available. To further complicate the issue, workers report different levels of toxicity in fish theoretically undergoing toxicity experiments at the same concentrations.

Fig. 5.31. LT50 value for H. angustipennis to 1000 mgl^{-1} ammonium sulphate

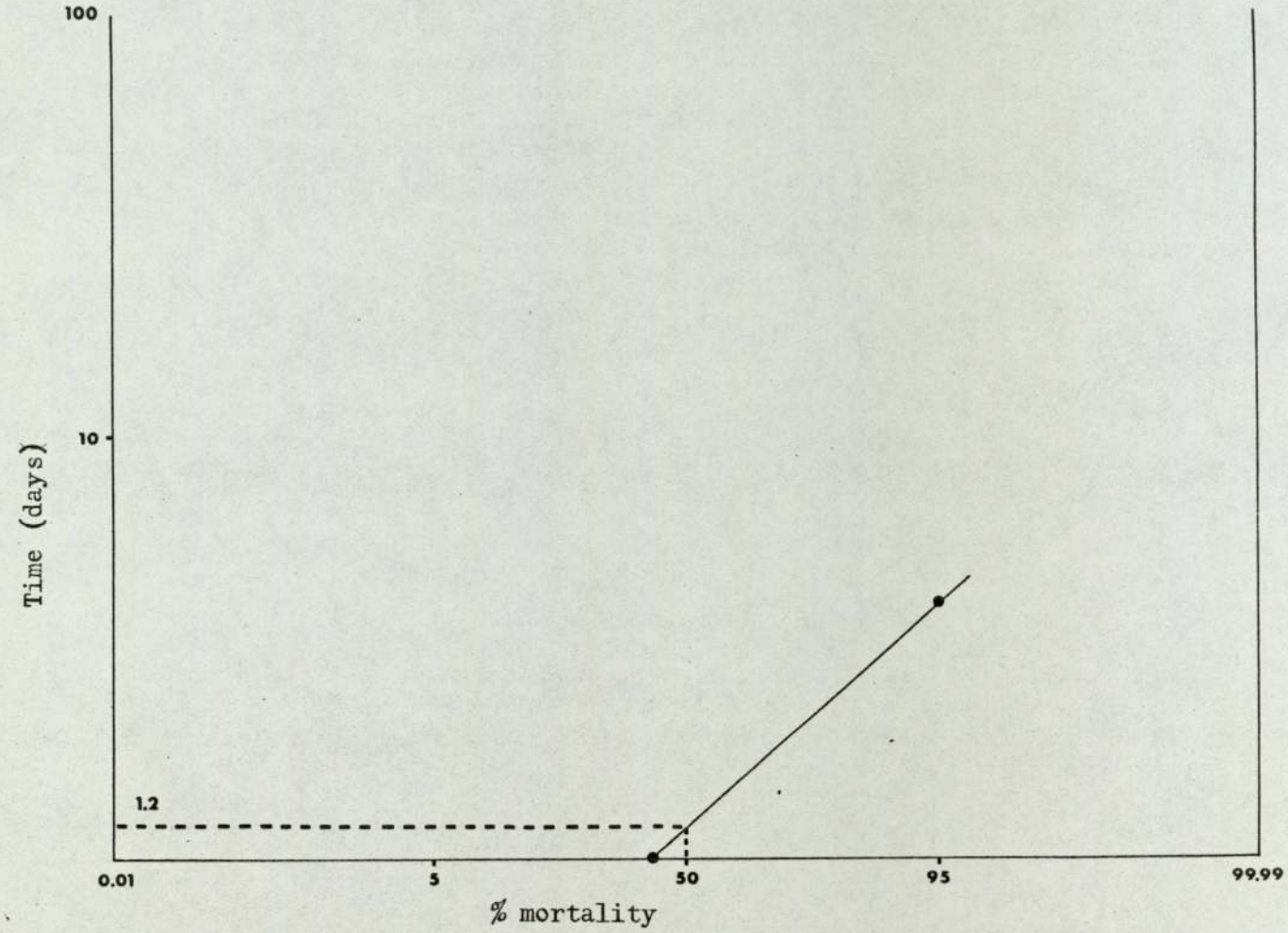


Fig.5.32 LT50 value for H. angustipennis to 560 mgl^{-1} ammonium sulphate

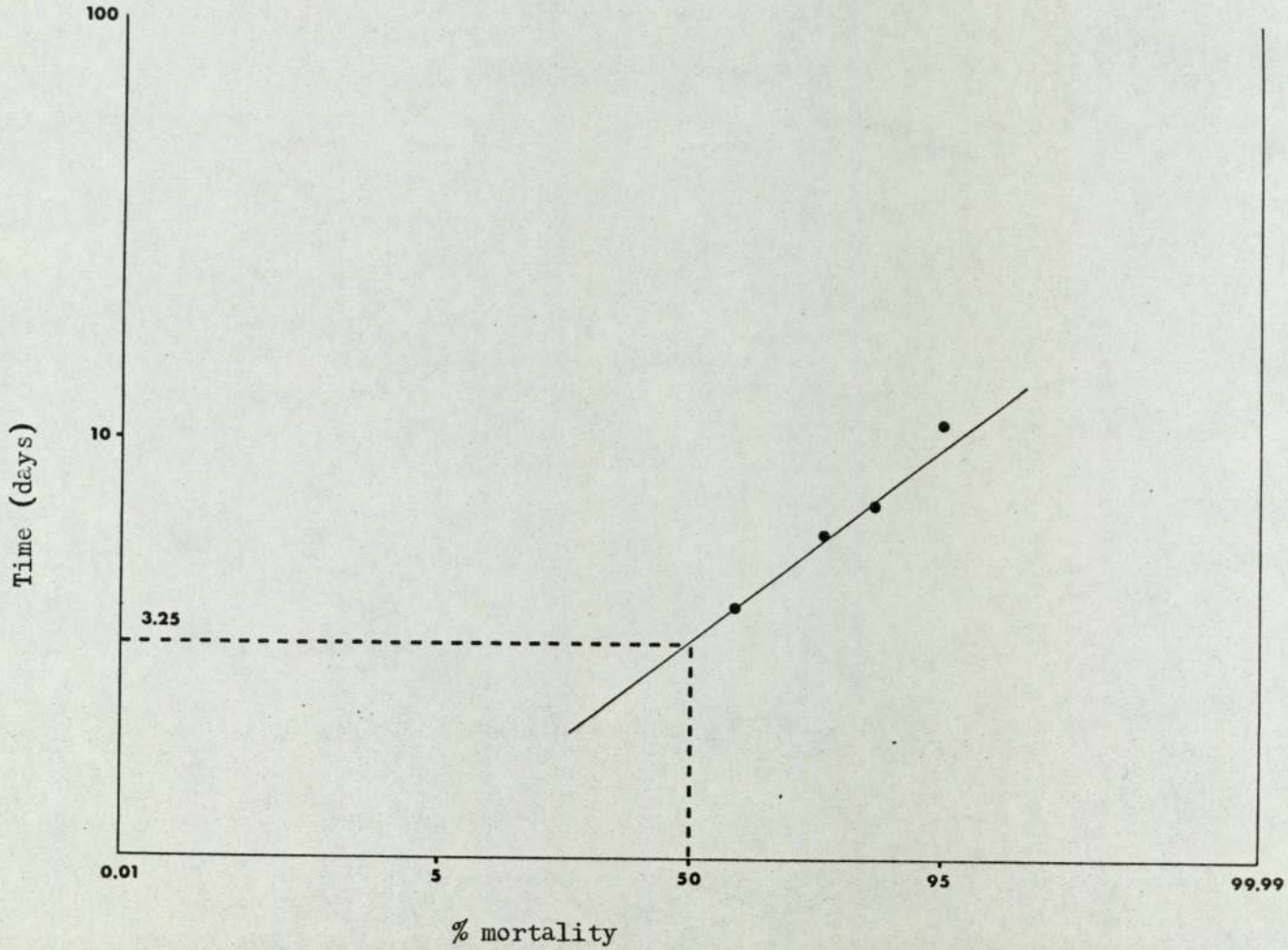


Fig.5.33 LT50 value for H. angustipennis to 320 mgl^{-1} ammonium sulphate

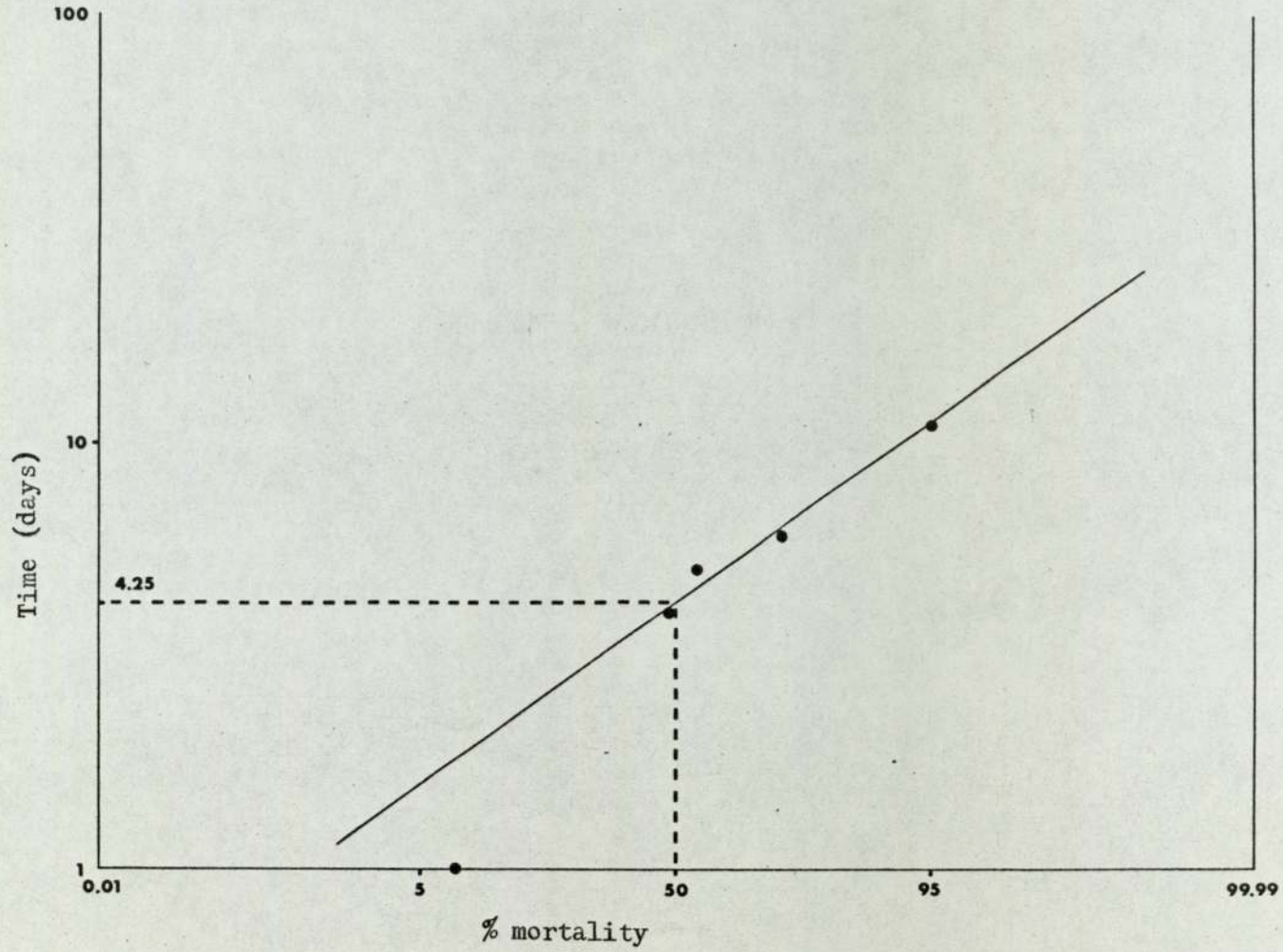


Fig. 5.34 LT50 value for H. angustipennis to 180 mgl^{-1} ammonium sulphate

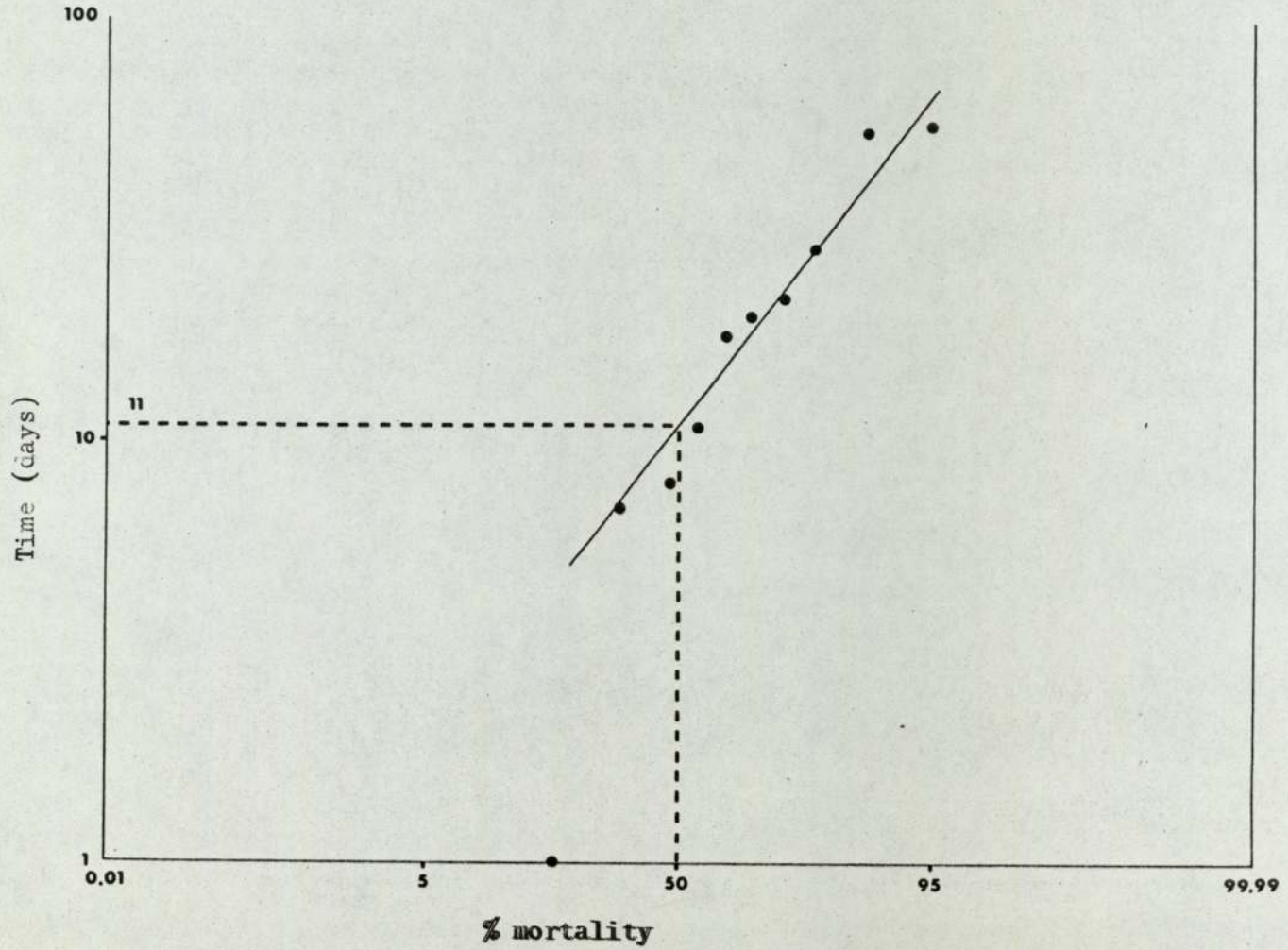


Fig. 5.35 LT50 value for H. angustipennis to 100 mgl^{-1} ammonium sulphate

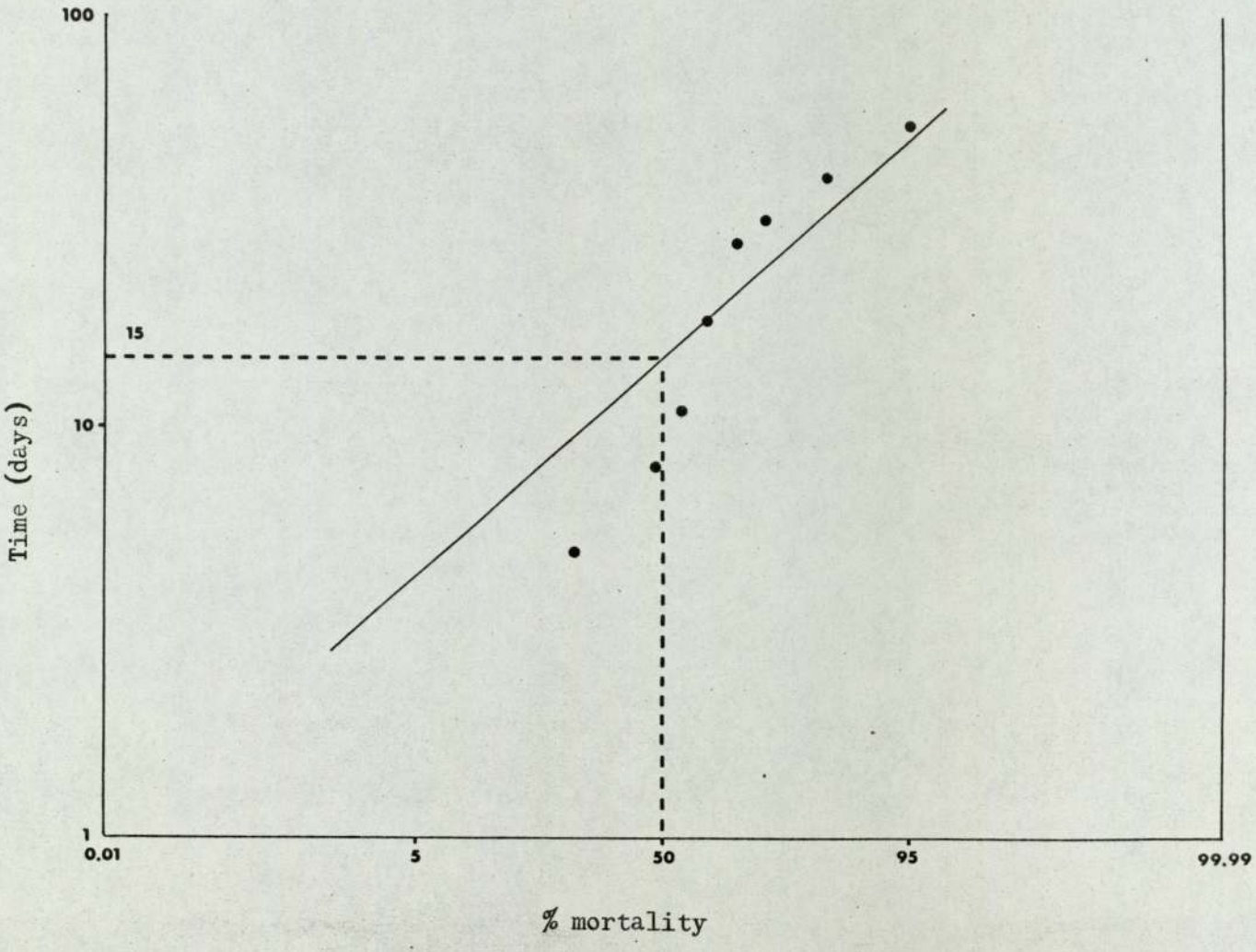
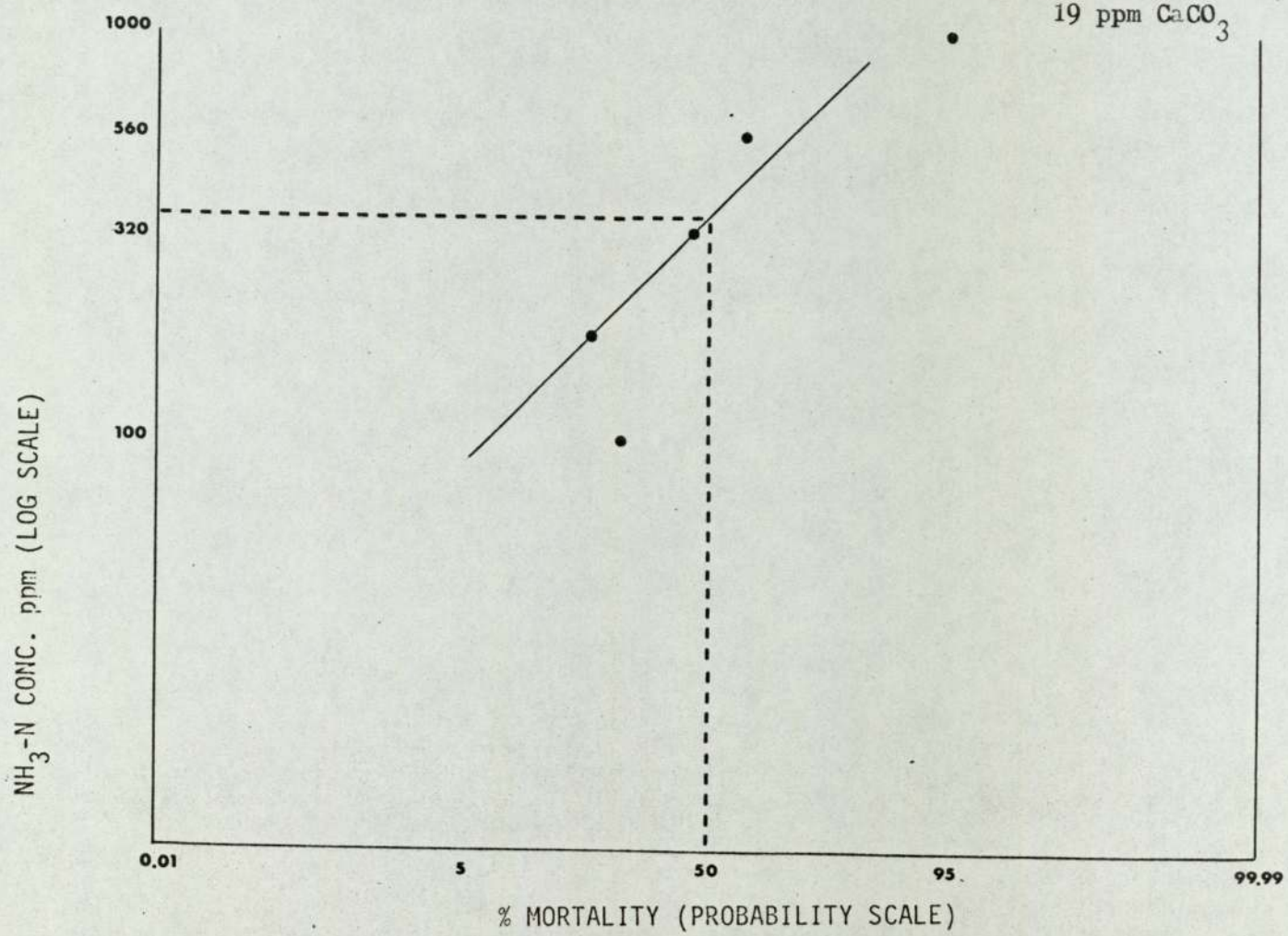


Fig. 5.36 96 hr LC50 of H. angustipennis to ammonium sulphate at 10°C, 19 ppm $CaCO_3$



Conclusions were that differences in handling techniques before the start of the fish experiments may cause variations in the results.

Natural fluctuations in diurnal ammonia concentration with changes in pH and temperature which occur in both clear and polluted waters also distorts a direct explanation of ammonia toxicity. In addition it is likely that there may be other toxicants in the water which may exert a further effect, and thus summing of toxicities as carried out by Herbert and Shurben (1964) zinc with ammonia on rainbow trout may be necessary.

However, other workers, Brown et al. (1969) showed that at low concentrations the toxic effect was lower then predicted. Thus, ammonia toxicity is a complex area requiring far more work.

Aquatic macroinvertebrates appear to demonstrate a gradation of sensitivity which may be very useful in assessing organic pollutants and may prove easier to interpret than fish data at present.

As H. angustipennis is so tolerant, it may not be of primary use as an indicator organism in the laboratory, but perhaps an easily maintained species such as G. pulex could be used as a standard indicator species for ammonia toxicity testing in the future.

5.7. HERBICIDE TOXICITY

Introductory Review

Pesticides used in the aquatic environment to control aquatic macrophytes, algae, and riverside vegetation are monitored by the Pesticides Safety Precaution Scheme (P.S.P.S).

Under this scheme only nine herbicides are currently cleared for use in the aquatic situation. Of the nine only two have algicidal properties. These are:

i. Terbutryne (Clarosan$^{(R)}$ Ciba-Geigy)

ii. diquat (Reglone 40$^{(C)}$ I.C.I.Plant Protection)

The remaining seven preparations are:-

1. Chlorthiamiol
2. 2,4-Damine
3. Dalapon
4. Dichlabenil
5. Glyphosate
6. Maleic hydrazide
7. Paraquat

As these herbicides are used in aquatic environments, they may also have deleterious repercussions on the aquatic biota, particularly on the species associated with the filamentous algae.

It was decided to use the two algicides Clarosan and Reglone 40 in standard toxicity texts on the larvae of Hydropsyche angustipennis. The two preparations are quite different in indication and time of action. Only limited toxicological data are available for both.

Diquat is a fast acting (24-hr) non-selective herbicide. It acts on a limited algal spectrum and on vascular plants.

Terbutryne, a granular formulation as opposed to the liquid diquat has a longer exposure period (7 days) and being non-selective

will kill many submerged vascular plants. In certain instances, where a large quantity of vegetation is killed, there may be deoxygenation problems. Terbutryne has a widerspectrum than diquat, but Vaucheria spp., often a severe problem of many drainage channels is only suppressed at the highest permitted 0.1 mgl^{-1} a.i. Lack of success of terbutryne is often due to increasing amounts of water being abstracted, artificial flow being created in drainage channels and thus the herbicide not being in contact with the algal biomass for long enough to be effective, even for algal species normally susceptible in still or sluggish waters.

COMPARISON OR TERBUTRYNE AND DIQUAT

1. TERBUTRYNE (Clarosan$^{(R)}$ Ciba-Geigy)

BSI name = (2-ethylamino-4-methylthio-6-t-butylamine-1,3,5-triazine)

Granular formulation 1% w/v active ingredient (a.i)

Toxicological data available (from M.A.F.F.Booklet)

Acute oral LD50 rat = 2400 mg/kg

96 hr LC50 fish = 3.5 ppm rainbow trout

96 hr LC50 fish = 4.0 ppm carp

48 hr LC50 invert = 1.4 ppm D. magna

Maximum permitted concentration used 0.1 mgl^{-1} a.i.

2. DIQUAT (Reglone 40$^{(R)}$ I.C.I.Plant Protection Division

BSI name = 9,10-dihydro-8a,10a-diazoniaphenolthrene)

Solution formulation with 20% w/v active ingredient

Application - to foliage

Dose - 4.4 kg/ha (4lb/acre)

Permitted concn. - 0.1 ppm

Time of application - June - August

Toxicological Data available

Acute oral LD50 rat = 5000 mg/kg

96 hr LC50 fish = 5000 ppm rainbow trout

96 hr LC50 fish = 5000 ppm channel catfish

(no time quoted) LC50 inverts.

Lymnaea sp.	17,000 ppm
Gammarus sp.	17,000 ppm
Chironomid (L)	31,600 ppm
Tubificids	31,600 ppm

48 hr LC50

Limnephilus sp. = 65 mgl^{-1} larvae static test

15°C 64 ppm $CaCO_3$ pH 6.9

26 hr LC50 D. magna = 7.1 mgl^{-1} static test. 1st. instar

21°C

Notes on Reglone 40 from I.C.I.

Used as a preharvest crop desiccant and weedkiller (e.g. oilseed rape, peas for harvesting, dry clover for seed, field beans, laid barley, oats and potatoes.) Also for control of some submerged and floating weeds and algae. Main species controlled are Cladophora, Canadian pondweed, rigid hornwort and pondweed.

It is best used in still or slow water as in muddy waters Reglone 40 will be inactivated on contact with soil particles, therefore avoid stirring up mud on treatment. The manufacturers also note to use diquat on about a quarter of the weed if growth is heavy as large quantities of decaying plant material may lead to depleted oxygen conditions and fish kills.

The PSPS aquatic herbicides are subject to Acts of Parliament:

(a) Rivers (Prevention of Pollution) Acts 1951 and 1965

(b) Rivers (Prevention of Pollution)(Scotland) Acts 1951 and 1965

(c) Northern Ireland Water Act 1972.

The Acts apply when aquatic herbicides are used to control weeds growing in or by reservoirs, rivers, ditches and drains. Acts do not apply to herbicides when used to control weeds growing in ponds or

lakes not discharging to a water course.

Permission must first be obtained from any Water Authority to use a selected herbicide in their area. The Guidelines for Use of Herbicides In or Near Watercourses MAFF Booklet 2078 (1980) outlines methods employed.

5.7.1. Methods.

In the light of the information above, it was decided to use Clarosan and Reglone 40 in static toxicity tests at 10°C using final instar H. angustipennis as the test animal. A 24-hr acute ranging test was set up with both herbicides tested at 100, 50, 10, 1.0 and 0.1 ppm a.i. with a tap water control. Each beaker, containing 200 mls. of test solution was given constant aeration and maintained at 10°C. Five animals were used in each replicate beaker. This was felt to be appropriate initially as the exposure period for diquat is only recommended to be 24 hrs. H. angustipennis are frequently associated with Cladophora blankets in the field situation but very limited invertbrate data are available on these herbicides.

5.7.2. Results.

Tabulated results of the ranging tests are found overleaf (Table 5.30). As none of the larvae in the granular terbutryne test were dead within the 24 hour period, a long term test was continued, using the same concentrations of active ingredient in order to discover the effect over a prolonged time period. The results of this test, which was discontinued after 59 days are shown in Table 5.31.

These results are in marked contrast to the 80% kill of animals by 0.1 ppm of diquat within a 24-hour period.

5.7.3. Conclusions

Diquat administration at MAFF recommended concentration would precipitate a kill of a large proportion of the H. angustipennis population associated with any Cladophora in a river situation. As there are

TABLE 5.30. RESULTS OF RANGING TESTS SHOWING NUMBERS OF *H. angustipennis* larvae

Toxicant: Diquat

Concn.-> Time ↓	100	50	10	1.0	0.1	control
			mgl^{-1} a.i			
0 hrs	0	0	0	0	0	0
$\frac{3}{4}$ hr	5	5	5	0	0	0
$1\frac{1}{2}$ hrs				3	0	0
3 hrs				5	0	0
6 hrs					0	0
12 hrs					1	0
24 hrs					4	0
Toxicant: Terbutryne						
0 hrs	0	0	0	0	0	0
$\frac{3}{4}$ hr	0	0	0	0	0	0
$1\frac{1}{2}$ hrs	0	0	0	0	0	0
3 hrs	0	0	0	0	0	0
6 hrs	0	0	0	0	0	0
12 hrs	0	0^{+}	0^{+}	0	0	0
24 hrs	0	0	0	0	0	0

+ = *H. angustipennis* incorporating granules of terbutryne into their nets

TABLE 5.31 TERBUTRYNE - RESULTS OF LONG TERM TOXICITY TEST

Concn. -> / Time ↓	10	50	1.0	1.0	0.1	Control
Day 1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	2 on backs 0	0	0	0	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	2	1	0	0	0	0
↓ 14	3	5	0	0	0	0
↓ 21	3		0	0	0	0
↓ 34	5		0	0	0	0
↓ 42			1	0	0	0
↓ 59			2	1	1	0

Test Discontinued

no complicating factors in the laboratory experiments such as silt/mud to adsorb and inactivate the herbicide or large quantities of decomposing vegetation to decrease the oxygen concentration, the herbicide must be directly responsible for the toxic nature of the reaction. The 65 mgl^{-1} for a 48 hr LC50 test on the cased Limnephilus sp. as quoted by ICI, although not directly comparable because of the different conditions of the static test may indicate that the cased caddis is afforded some protection by its case, perhaps in a similar manner to mud 'inactivating' the diquat by adsorption. Terbutryne is far less toxic to the caseless caddis as demonstrated in the long term test and at the

0.1 ppm a.i. concentration would be harmless to H. angustipennis and according to the other toxicological data quoted to D. magna and fish.

If allowed to remain in a still/sluggish stretch of water for 7 days as recommended, providing deoxygenation due to decomposition is not too severe, the caddis and fish population should be unaffected.

In conclusion, terbutryne would be the preferable and less damaging type of herbicide to caseless caddis for use in a field situation.

5.8. BIOCONCENTRATION STUDIES

The first part of these investigations was a direct continuation of the acute toxicity work carried out in Section 5.4 and 5.5.

Larvae of H. angustipennis which had been used in 96hr LC50 tests with copper and zinc were retrieved from the test chambers and at the end of the experiment dried to constant weight and analysed as to the quantity of metal that had bioconcentrated. In further experiments an alternative approach, using the radioisotope of zinc (^{65}Zn) was utilised to assess the rate of zinc accumulation at differing water hardness, the anatomical sites of metal uptake and the ability of the larvae to eliminate the zinc. At each stage it was attempted to relate laboratory findings to the field situation.

5.8.1. Introductory Review

Much of the relevant work relating to the bioconcentration of copper and zinc by H. angustipennis and other freshwater macroinvertebrates has been discussed in Section 4.6. From field studies already documented it is apparent that the caseless caddis larvae are extremely tolerant to heavy metal pollution, being more tolerant of zinc than copper as a general rule. These experiments seek to investigate these phenomena in greater depth with the larvae of H. angustipennis. Atomic Absorption Spectrophotometry and concentration factor techniques have already been employed (Nehring et al., 1979) but the use of radioisotopes in this area of study is limited.

The behaviour of copper in natural waters has been documented by Stiff (1971). Not only is increasing hardness of water responsible for a reduction in toxicity, but alkalinity is also important in determining the activity of certain heavy metals such as copper and lead. The hardness of water is caused by divalent metal ions, primarily calcium (Ca^{2+}) and magnesium (Mg^{2+}) and is normally expressed in mgl^{-1} $CaCO_3$. Total Hardness refers to the combined calcium and magnesium hardness.

Determination of calcium hardness by titrimetric methods with ethylene-diaminetetraacetic acid (E.D.T.A) enables one to calculate the remaining hardness due to magnesium.

Total hardness - Calcium Hardness = magnesium hardness (TH - CH = MH) (Sawyer & McCarty, 1978).

The role of alkalinity must also be taken into account when discussing copper toxicity. Alkalinity is due to bicarbonate, carbonate and hydroxide compounds of calcium, magnesium, sodium and potassium. The part of the total hardness that is chemically equivalent to the bicarbonate plus carbonate alkalinities present in water is considered to be carbonate hardness. Since alkalinity and hardness are both expressed in terms of $CaCO_3$ the carbonate hardness can be found as follows:

When alkalinity < total hardness, Carbonate hardness = alkalinity

When alkalinity $\geq$ total hardness, Carbonate hardness = total hardness.

Carbonate hardness was formerly called "temporary hardness" because it can be caused to precipitate by prolonged boiling. Noncarbonate hardness can be calculated by simple subtraction:

Total Hardness - carbonate hardness = non-carbonate hardness.

The noncarbonate hardness was formerly called "permanent hardness" because it cannot be removed by boiling, these cations are usually associated with sulphate, chloride and nitrate anions. Thus, it becomes apparent that the equilibria involved in hardness and alkalinity characteristics of water may have an effect on the copper in the system. The toxicity of copper is reduced, not so much by increasing hardness as by increasing alkalinity as the metal ions complex with the bicarbonate ions. Therefore, in any toxicity experiment with copper, it is advisable to measure free Cu^{2+} ions with an ion specific electrode wherever possible. In the experiments carried out in this work, waters of differing total hardness were achieved by mixing Shustoke Reservoir Water with Birmingham Tap Water. The relative proportions of calcium

and magnesium hardness differed, so Cu^{++} ions were measured directly.

In contrast to the effect of alkalinity on copper, the toxicity of zinc is more dependent upon the hardness of the water. Murphy (1978) reviews papers which suggest that zinc competes with magnesium and calcium ions for binding sites on or in target organisms, so that increasing water hardness reduces toxicity by this method. The chemical difference in activity between copper and zinc in natural waters may go a long way to explaining the differing toxic effect of the two metals.

Work investigating the accumulation of zinc by aquatic invertebrates using radioisotope techniques is limited. Dean (1974) studied the accumulation of ^{65}Zn by tubificid worms, and concluded that worms readily accumulated from water but did not accumulate measurable amounts of zinc that were bound to sediments.

In their study of Simulium larvae, Carter and Nicholas (1978) investigated the uptake and loss of ^{65}Zn. They looked at the chemical groups binding zinc by investigating the chemical bonding. Repeatedly washing fractions in a series of buffers of decreasing pH, and using substances which displace zinc assisted in this study. Two water hardnesses of 50 and 100 ppm $CaCO_3$ were used, and Carter and Nicholas (1978) found that calcium had no effect on zinc accumulation in Simulium at high or low concentrations. This does not support the theory, which has been proposed in fish by Skidmore (1964) that hardness reduces the toxicity of heavy metals by reducing the permeability of membranes.

Neither zinc nor calcium in the external medium affected the rate of zinc loss or the quantity lost in Simulium ornatipes larvae. Carter and Nicholas explain this by suggesting that zinc is actively excreted rather than exchanged. Divalent ions in the water would be expected to increase loss if exchange were important. The conclusions from the above work contrast with results of Kormandy (1965) who studied uptake

of trace levels of ^{65}Zn by the larvae of the dragonfly Plathemis lydia. He concluded that the surface adsorption rather than metabolism was the major factor in zinc uptake. However, Bryan (1973) suggested that accumulation of trace metals by scallops Pecten maximus and Chlamys opercularis was related to metabolism. The presence of a stable zinc pool, shown in the Carter and Nicholas (1978) work, may result in the incorporation of zinc into pupal and imaginal tissue. Simulium larvae living in streams subject to fluctuating levels of zinc will retain a significant amount after 24 hours in zinc free water, and thus might continue to provide a potential source of zinc to predators. Further conclusions were that zinc becomes progressively incorporated into cuticle and water soluble proteins as actively growing larvae will be synthesising such compounds.

Perhac (1972) in a study of the distribution of metals in dissolved and particulate solids from two streams in Tennessee found that generally over 90% of each metal occurs in the solution state, less than 10% occurs with the coarse particulates and less than 1% as colloids. Thus zinc will be available to aquatic macroinvertebrates such as H. angustipennis in the field situation.

5.8.2. Methods and Materials

Determination of Metal Content of Animal Tissues

Both animal samples from metal toxicity tests and from field sampling were treated in the same manner for analysis of metal content. The methods of determination of metals in animal tissues were extensively reviewed by Huddart in an internal report from the Applied Hydrobiology Research Station at Checkley. Dry Ashing and Wet Digestion methods were compared from available published papers. In conclusion, the most effective method of extracting metal from fish and invertebrate tissues was by using a wet digestion method with a nitric/perchloric acid and he quotes Smeyers Verbeke et al. (1962) using this method to determine

copper and zinc levels in biological material. However, due to the hazardous nature of this digestion method, a more straightforward nitric acid digestion was performed with quite satisfactory results for zinc and copper.

The larval samples were generated from larvae used in each of the 96hr toxicity tests (i.e. 15 individuals, 5 from three replicates were retained on death during the test, or at the end of the test). Bulked samples of 15 larvae were kept in individual containers according to the concentration of metal solution in which they had been immersed. The bulked sample (15 larvae) was dried to constant weight at 105°C and then transferred to an Erlenmeyer flask. 5 ml. of concentrated nitric acid was added, and the flask covered with a watch-glass. Samples were left to soak for 24 hrs. before being placed on a hotplate for gentle refluxing until the yellow colour was lost and the solution was practically colourless. The sample was then heated to dryness followed by dissolution in 2 ml 50% HCl and then made up to 25 ml with distilled water in a volumetric flask. This resultant sample was then aspirated in the same way as the river water samples using the Perkin-Elmer Atomic Absorption spectrophotometer. Calculation was made for the amount of copper or zinc in mg metal per kg dry weight of larval tissue, from which, with a knowledge of metal concentrations in solutions, a concentration factor could be calculated.

Table 5.32 lists the experiments from which animal material was taken and measured for bioconcentration studies.

TABLE 5.32 List of Toxicity Tests which were the source of animal material for bioconcentration studies

Metal	Water Hardness	Period
Copper	Distilled Water (control)	96 hr LC50
Copper	19 ppm $CaCO_3$	96 hr LC50
Copper	100 ppm $CaCO_3$	96 hr LC50
Copper	200 ppm $CaCO_3$	96 hr LC50
Zinc	19 ppm $CaCO_3$	96 hr LC50
Zinc	19 ppm $CaCO_3$	64 days

The second stage of experimentation involved radioactive tracer methodology. Radioactive zinc (^{65}Zn, Amersham International Ltd.) which emits γ (gamma) radiation, with a half life of 245 days was used to investigate zinc uptake by final instar larvae of H. angustipennis. Analar zinc sulphate was used to make up a 'cold' stock solution of 500 ppm zinc in distilled water.

20 µl of ^{65}Zn tracer was added to 100 mls of the 500 ppm stock solution in a beaker, to provide 0.865 µCi/100 mls.

Animals were immersed in replicate beakers containing 100 mls toxicant for 7 days. Each beaker was provided with a standard aerator and lid. Each day animals were removed from beaker, and placed in an LP3 tube and counted in an ICN GS-5024-TP gamma counter.

A 200 µl sample of tracer solution was counted at the start of each experiment, and thereafter on a daily basis.

Calculations were made daily to assess the rate of ^{65}Zn accumulation, the site of maximum uptake and the rate of elimination of the metal when transferred to a zinc free environment.

Experiments were designed to compare washed v unwashed animals, the rate of uptake in soft v hard water, major sites of accumulation and the rate of elimination of zinc when animals were transferred to

tap water. In the final experiment animals were fed fish flakes, and the effect of this was noted.

Table 5.33 lists the series of ^{65}Zn tracer experiments undertaken.

TABLE 5.33 List of ^{65} Zn Tracer Experiments

Expt. 1 (a) 5 animals, 7 days immersion

(b) 500 ppm Zn cold soln. made up with distilled water. 20 µl tracer added

(c) Animals blotted before counting

Expt. 2 (a) 5 animals, 7 days immersion

(b) Solution as in Experiment 1

(c) Animals washed in distilled water, then blotted dry before counting.

Expt. 3 As Expt. 1 but 10 animals

Expt. 4 As Expt. 2 but 10 animals

Expt. 5 Stock solution 500 ppm Zn, artificially hardened by adding saturated $CaSO_4$ solution to give a final water hardness of 223 ppm $CaCO_3$.

10 animals immersed

Expt. 6 Dissection of 10 animals after 7 days immersion in standard 500 ppm Zn solution and 20 µl tracer

Expt. 7) 2 x 10 animals in hardened water allowed to absorb Zn for
) 7 days, then transferred to tap water and elimination rate
Expt. 8) plotted.

Also fed on fish flakes in this experiment.

5.8.3.1. RESULTS (Copper and Zinc Accumulation by H. angustipennis after Toxicity Tests).

COPPER

The data presented in Table 5.34 shows nominal and actual concentrations (assessed by A.A.S) for copper sulphate solutions prepared with distilled water. Also presented are the concentrations of metal in the larval tissue (mg $Cu kg^{-1}$) after 96 hr immersion in the respective test solutions. These concentrations were calculated after drying larvae to constant weight, followed by accurate weighing, acid digestion

TABLE 5.34. Results of bioconcentration experiment using distilled water

Nominal Concentration Cu mgl^{-1} in water	Actual Concentration A.A.S result in water	Concentration of copper in Animal Tissue $mg\ kg^{-1}$
100	91.3	3,363
56	45.4	1,371
32	29.4	676
18	16.8	641
10	8.9	541

and A.A.S assessment of metal content.

Table 5.35 summarises results from toxicity tests carried out at these different water hardnesses and compares them to distilled water values. Copper ion levels are recorded and the right hand columns record the concentration of copper in animal tissue, and the concentration factor calculated from actual metal values: concentration in animal tissue.

In the distilled water experiment the uptake of copper in the larvae increased with the increased copper concentrations in the solution. The concentration factors were calculated from actual copper concentrations measured by A.A.S rather than from nominal values. It appears that H. angustipennis larvae concentrate copper more avidly from lower concentrations in the environment.

The results for copper from the 96 hr toxicity tests at 19, 100 and 200 ppm $CaCO_3$ have a few anomalies, especially when comparing nominal copper concentrations to actual values (Table 5.35). These discrepancies may be due to experimental error in either making up solutions, evaporation or loss in preparation of the digested sample before aspiration. The effect of hardness and alkalinity in these natural waters, as discussed in the introductory section 5.8.1 also reduces the amount of total copper in each solution. Consequently the values for free copper ions are given, and these show the expected trends; firstly that with decreasing total concentrations at the same hardness, the proportion of Cu^{++} decreases. Similarly, at increasing water hardness, at the same nominal concentration the Cu^{++} concentration decreases. As free copper ions are thought to be the direct toxicant these values demonstrate how the levels would change in the field situation depending upon the receiving water. In almost every block of results there are higher concentrations of copper measured in the animal tissue, at higher levels of copper in solution. (There are some anomalies which will be

TABLE 5.35 Summary of measured levels of copper in solutions and animal tissues

Nominal Concentration mgl^{-1} Cu	Actual Concentration mgl^{-1} Cu (A.A.S)	Cu^{++} concentration mgl^{-1} (Electrode)	Cu concn. $mg\ kg^{-1}$ in tissue	Concn. Factor
Distilled Water				
100	91	-	3363	36.95
56	45	-	1371	30.46
32	29	-	676	23.3
18	16	-	641	40.06
10	9	-	541	60.11
Control	0.05	-		
19 ppm $CaCO_3$ Hardness				
560	600	330	3370	5.6
320	312	325	4545	14.5
180	346	180	6470	18.6
100	105	83	4421	42.1
56	14.5	48	3171	218.6
100 ppm $CaCO_3$ Hardness				
560	540	270	7952	14.7
320	332	123	4119	12.4
180	184	63	3982	21.6
100	140	32	3525	25.1
56	55	6.9	2608	47.4
32	24	0.26	2315	96.4
18	14	0.05	2290	163.5
10	10	0.05	744	74.4
200 ppm $CaCO_3$ Hardness				
560	585	158	5815	9.94
320	330	95	6093	18.46
180	220	41	4696	21.34
100	65	19.5	3557	54.72
56	-	5.4	-	

discussed in the following paragraph). Also, as a general trend, concentration factors calculated from actual values and tissue levels are greater at the lower levels of copper in the environment.

Examining the distilled water system (Table 5.35) where there is a minimal effect of complexing in suppressing the Cu^{++} ions, it follows that the copper present would be potentially most toxic. It may be that the increased toxic effect reduces the ability of the larvae to assimilate and bioconcentrate the metal (cf. Distilled with 100 ppm $CaCO_3$ at 56, 32, 18 and 10 mgl^{-1} Cu). Unfortunately this does not hold true for copper at the nominal 100 mgl^{-1} level, but actual levels were recorded as 91 and 140 mgl^{-1} respectively so these differences may have some effect on the resultant concentration factor.

The anomalies of lower copper concentration in tissues of animals immersed in copper solutions of higher concentrations, for example at 19 ppm $CaCO_3$ - 560 and 320 mgl^{-1} Cu, and at 200 ppm $CaCO_3$ at 560 mgl^{-1} Cu may be explained in the following manner. Although theoretically there is more copper available to be assimilated and bioconcentrated, it is also far more toxic. Thus, deaths of some larvae occurred before the end of the 96 hr period, so they therefore stopped accumulating copper. On death they were removed from the test vessel and retained until the end of the experiment, when the remaining larvae from the same concentration were bulked with them. Obviously, the longer the live larvae stay in copper solution, the more they may assimilate but equally they are becoming more greatly intoxicated and closer to the lethal level.

Comparisons of Laboratory and Field Conditions

Obviously copper toxicity to H. angustipennis in distilled water is a very artificial situation, so a comparison of field data with laboratory work was attempted.

The concentration of copper in Langley Brook is approximately in the same range as Cu in the channels at Checkley. Consequently, the copper levels of the control animals - which in effect have been acclimated at 0.01 - 0.02 mgl^{-1} Cu can be tentatively compared with the invertebrates analysed from the three Checkley Channels, river water, 25 and 50% effluent having 0.002-0.008, 0.012-0.031, 0.018-0.048 mgl^{-1} Cu respectively (these are ranges of median values over a 2 year period 1975-1977)(Checkley Summary Report).

Table 5.36 gives comparative water quality data for Checkley Channels (A=river water, B=25% effluent, C=50% effluent) and Langley Brook. The average copper concentration of Langley Brook water - calculated from eleven weekly samples was 0.0217 mgl^{-1}. Both Langley Brook and the channels have a hard-water characteristic, which will undoubtedly affect the copper toxicity, also high organic content. The copper content of other benthic macroinvertebrates has been noted from work carried out at Checkley and the relative concentrations tentatively compared. Animal samples from Checkley were treated in exactly the same manner, being digested in nitric acid and auto-analysed for metal content, but it must be remembered that these animals were taken directly from the stream/channel and have not been starved; therefore gut contents may be a source of error.

Table 5.37 summarises the metal levels in macroinvertebrates taken from the various environments. These results will be examined more closely in Section 5.8.4 - Discussion and Conclusions.

ZINC

In a similar manner to the copper experiments, larvae from the 96hrLC50 zinc sulphate experiment were digested and their metal content measured. Table 5.38 shows the dry weights and concentrations of zinc in the animal tissue after these tests. These values are much lower than those for copper over a 96 hr period, the respective

TABLE 5.36 Water Quality Data from Checkley Channels and Langley Brook

Parameter	Channel A			Channel B			Channel C			Langley Brook	
	AUGUST			NOVEMBER							
Percentile	5	50	95	5	50	95	5	50	95	Sept. 1981	March 1982
Temp.	6.5	9.0	12.0	7.5	10.5	12.7	9.5	12.0	13.5	16.0	8.5
D.O.	9.7	11.0	12.5	8.9	10.0	11.5	7.0	8.5	10.0	5.5	11.3
pH	7.9	8.1	8.3	7.4	7.6	7.8	7.2	7.4	7.8	6.8	7.4
Total H	245	315	340	300	340	375	310	350	395	252	310
Ca	-	-	-	-	-	-	-	-	-	134	184
Mg	-	-	-	-	-	-	-	-	-	118	136
Alkalinity	-	-	-	-	-	-	-	-	-	0	0
Total Alk.	-	150	170	-	151	175	-	175	200	120	-
$N-NO_3$	2.50	3.52	4.15	6.40	9.46	11.35	10.10	13.52	14.70	14.4	17.0
$N-NH_3$	0.05	0.1	0.25	0.1	0.15	0.65	0.10	0.20	0.75	1.0	0.2
$P-PO_4$	0.08	0.35	1.50	2.10	2.89	3.90	3.10	4.58	6.30	12.9	8.9
Cu	0.001	0.003	0.008	0.001	0.016	0.025	0.020	0.024	0.038	0.01	0.02

TABLE 5.37 Metal levels in Macroinvertebrates

Animal	Environment	Cu level mg (μkg^{-1})
H. angustipennis	Control Distilled Water	297.0
H. angustipennis	Control 19 ppm $CaCO_3$	311.0
G. pulex	Channel A (River Water)	120.0[1] 85.0*
Simulium sp.	Channel A (River Water)	47.6[1] 18.0*
Simulium sp.	Channel B (25% effluent)	128.5[1] 159.6*
Simulium sp.	Channel C (50% effluent)	110.3[1] 203.0*
Asellus aquaticus	Channel A (River Water)	281.0 141.1[1]
Asellus aquaticus	Channel B (25% effluent)	342.2[1] 325.0* 246.9°
Asellus aquaticus	Channel C (50% effluent)	432.0[1] 466.6* 377.0°

1 - data from T. Wardle (1979)

* - Data from Checkley Applied Hydrobiology Research Station July 1976

o - Data from Checkley, September 1976

Table 5.38. Weights and metal content of H. angustipennis taken from zinc solutions prepared with water at 19 ppm $CaCO_3$

Sample nominal Zn concentration mgl^{-1}	Zinc in tissue $mg\ kg^{-1}$
1000	209.1
560	73.8
320	40.6
180	35.7
100	20.5
Control	16.0

Table 5.39 Concentration factors calculated for H. angustipennis over 96 hrs at 19 ppm $CaCO_3$

Concentration	Concentration Factor	
	Copper	Zinc
1000 mgl^{-1}	-	0.219
560 mgl^{-1}	5.6	0.143
320 mgl^{-1}	14.5	0.141
180 mgl^{-1}	18.6	0.215
100 mgl^{-1}	42.1	0.220

TABLE 5.40 Metal content of solutions, metal in tissues and concentration factors for H. angustipennis after LT50 test with zinc sulphate of 64 days duration

Nominal Concn. mgl^{-1} Zn	Actual concn. mgl^{-1} (A.A.S)	Concn. in tissue mg kg^{-1} Zn	Concn. Factor
1000	1057.5	5196	4.9
560	572.0	4077	7.1
320	330.5	3144	9.4
180	206.0	2799	13.5
100	99.0	2735	27.6
Control	0.75	344	

concentration factors are given in Table 5.39.

Zinc is bioconcentrated at a much slower rate than copper in H. angustipennis and this might well explain the higher LT50 values for comparable experiments in Sections 5.4 and 5.5. During the zinc LT50 experiments an extended test, which ran for 64 days was carried out. Again, larvae from these test chambers were analysed on completion of the test. Table 5.40 presents the results.

It may be seen that over an extended period, the zinc uptake increases at all experimental concentrations of the toxicant, suggesting long term resistance to zinc pollution by final instar H. angustipennis.

5.8.3.2. RESULTS (Radioisotope studies with ^{65}Zn)

The uptake by final instar H. angustipennis larvae immersed in radioactive zinc solution for 7 days was calculated as follows:-

Counts for the test solution are made at time 0 and thereafter on a daily basis (Table 5.41). Any renewal of solution is accordingly modified in the calculations. Due to the long half life of ^{65}Zn the decay rate and thus the difference in counts is relatively low.

Table 5.41. Counts for test solution and larvae of H. angustipennis

n/s = not significant

Day	No. counts in 200 µl test solution	Total No. counts for 5 larvae
1	1907	n/s
2	-	n/s
3	-	n/s
4	2747	627
5	2946	1499
6 New solution	1769	1626
7	2061	1627

1. Concentration 1907 dpm per 200 µl

= 9535 dpm per ml

= 1.907×10^6 c.p.m. for 200 mls

$\therefore$ = 0.9535×10^6 c.p.m per 100 mls (i.e. in beaker)

2. Assume 50% counting efficiency

at 100% 1µCi = 2.2×10^6 d.p.m

at 50% 1µCi = 1.1×10^6 d.p.m

So $\frac{1.907}{1.1} = 10^6$ c.p.m. = 1.73 µCi

$\frac{0.9535 \times 10^6}{1.1 \times 10^6}$ c.p.m. = 0.865 µCi/100 mls

Day 1 No significant uptake

Day 2 " " "

Day 3 " " "

Day 4 Counts per 5 animals = 627

$\frac{6.27 \times 10^2}{1.1 \times 10^6} = 5.7 \times 10^{-4}$ µCi

What is the specific activity? Zn solution 500 ppm = 500 mgl^{-1}

i.e. 50 mg/100 ml

$\therefore$ Specific Activity = 0.865 µCi/50 mg

$\frac{5.7}{0.865} \times 50 = 329.4 \times 10^{-4}$

$= 3.29 \times 10^{-2}$

= 32.0 µg Zn per 5 animals

$\therefore$ 6.58 µg Zn per animal

Dry wts:

Wt. of foil = 0.1577 g

Wt. of foil + dry animals = 0.1809 g

$\therefore$ Wt. 5 animals = 0.0232 g

$\therefore$ 0.0232 g animal takes up 139.3 µg Zn in 7 days

Table 5.4.2 shows the results of the first four experiments using ^{65}Zn.

Experiments 1 and 3 used 5 and 10 animals respectively and animals were simply blotted before counting commenced in the gamma counter. To compare, experiments 2 and 4 (using 5 and 10 animals

respectively) involved washing the animals in distilled water, followed by blotting before measuring the zinc taken up.

TABLE 5.42. Results of zinc uptake (µg) per animal from four experiments comparing blotted only to washed and blotted specimens

Time Days	Expt.1 Uptake of Zinc (µg) Blotted only	Expt.2 Uptake of Zinc (µg) Washed and Blotted	Expt.3 Uptake of Zinc (µg) Blotted only	Expt.4 Uptake of Zinc (ug) Washed and Blotted
1	0	0	0	0
2	n/s	n/s	-	-
3	n/s	n/s	-	-
4	6.58	13.67	-	-
5	26.20	13.82	-	-
6	27.60	14.80	28.6	-
7	27.86	-	34.3	16.7

n/s = no significant uptake

Fig.5.37 shows the comparison of zinc uptake (µg/g dry wt) between larvae which were simply blotted after immersion, compared to those which were washed with distilled water, blotted and then counted. These results demonstrate that animals taken directly from the zinc solution and blotted had approximately twice the concentration of zinc to the group of larvae which were washed. This suggests that the zinc was only loosely bound to the animals, presumably at least half of the zinc being adsorbed on to their surface.

Experiment 5 was designed to assess the effect of zinc uptake from hard water, 223 ppm $CaCO_3$ compared with that of animals from Experiment 2 where distilled water was used to make up the zinc solution.

Table 5.43 summarises the results of the uptake per animal.

Fig.5.37. Zinc uptake in washed and unwashed specimens of H. angustipennis

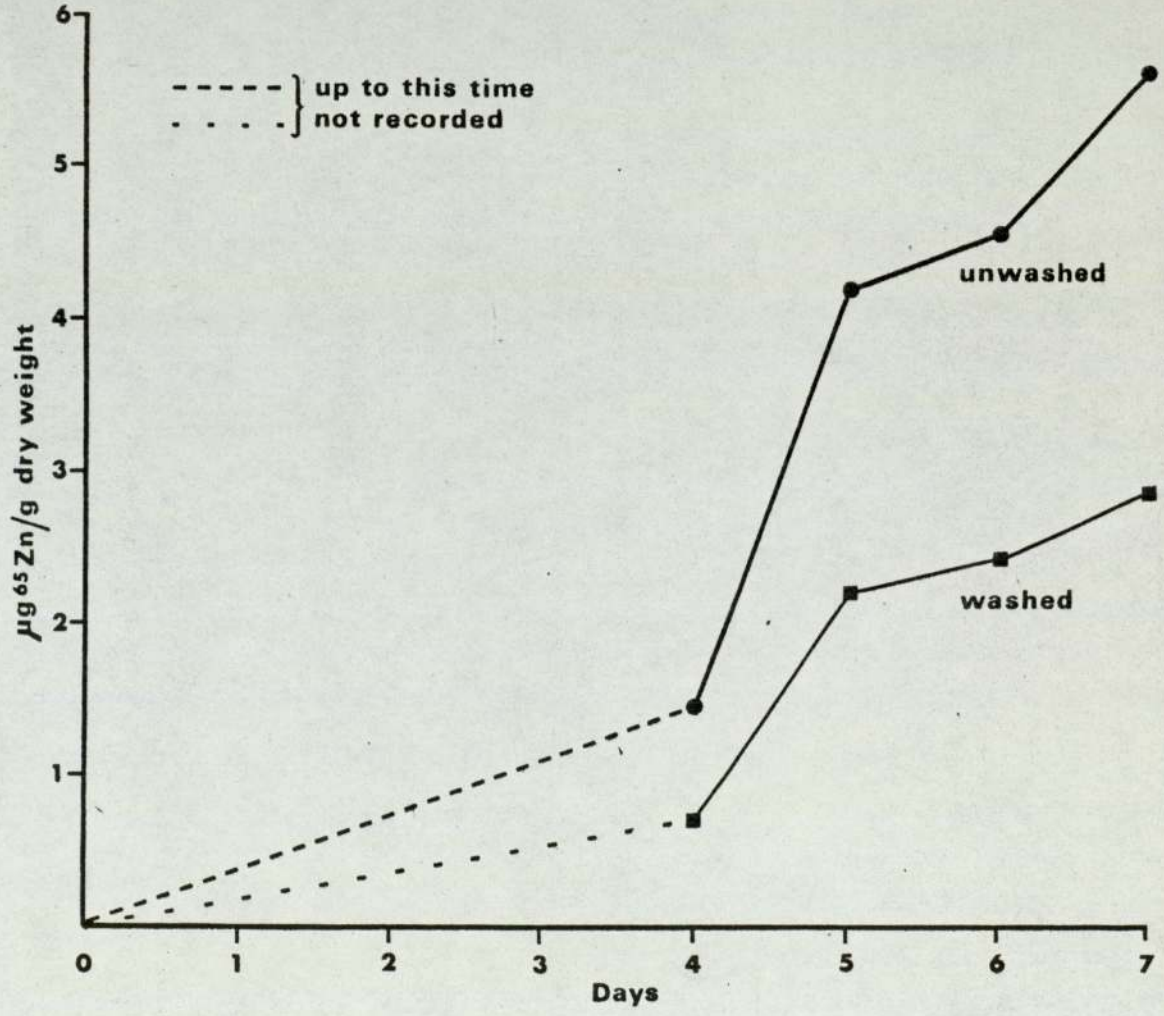


TABLE 5.43 Results of Experiment 5

	Uptake of Zn µg per animal
Day 1	n/s
2	2.07
3	3.03
4	-
5	8.04
6	10.35

Fig.5.38 shows the comparative values of larvae from Experiment 2 (soft water) to those from Experiment 5 (hard water) in µg. Zn per g dry weight.

Increasing the hardness of the water reduces the concentration of zinc which the larvae accumulate from solution by approximately half over 6 days. e.g. Final instar larvae from the soft water at day 5 has accumulated 13.82 µgZn compared to 8.04 µg from the harder water.

Experiment 6 was a slightly different type of experiment, designed to allow the larvae to accumulate zinc from the same solution containing tracer ^{65}Zn for 7 days.

From 10 animals dissections were prepared of

(a) gills only
(b) head and thorax
(c) abdomen only without gills

Each of these portions were weighed and counts made on the γ-counter to assess the concentration of zinc.

It was calculated that:

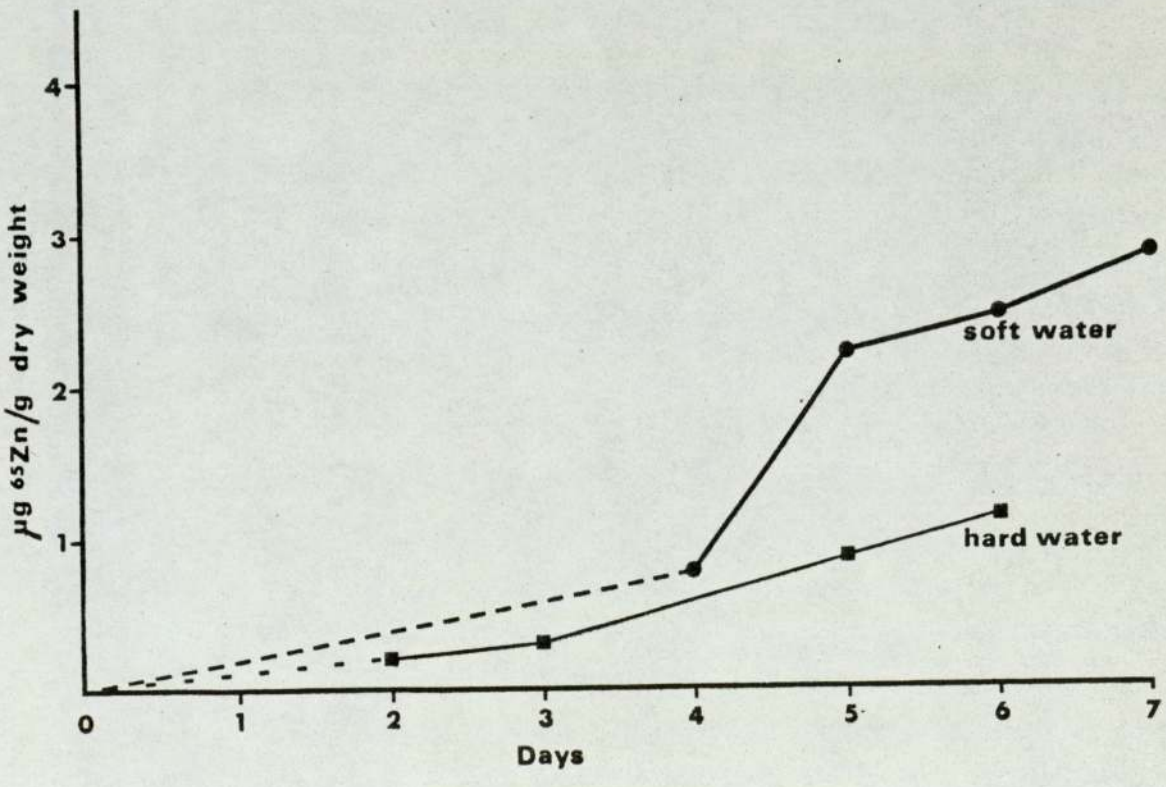
Approximately 0.6% zinc in gill tissue
60.8% zinc in abdomen
15.6% zinc in head and thorax
76.2%

The difference is due to leakage during dissection.

Conclusion: The uptake of zinc is predominantly by the soft tissues

Fig.5.38. Zinc uptake in soft v hard water in

H. angustipennis



of the body.

There is a relatively small proportion of zinc in the sclerotised area.

Generally the uptake is by adsorption and diffusion as opposed to active uptake via the gills.

Experiment 7, the accumulation and elimination of zinc was investigated in this study. As it extended over a 14 day period, the larvae were also fed at Day 5 on flaked fish food. Fig.5.39 demonstrates the pattern of accumulation of zinc from the solution containing 500 ppm Zn + 20 µl tracer ^{65}Zn.

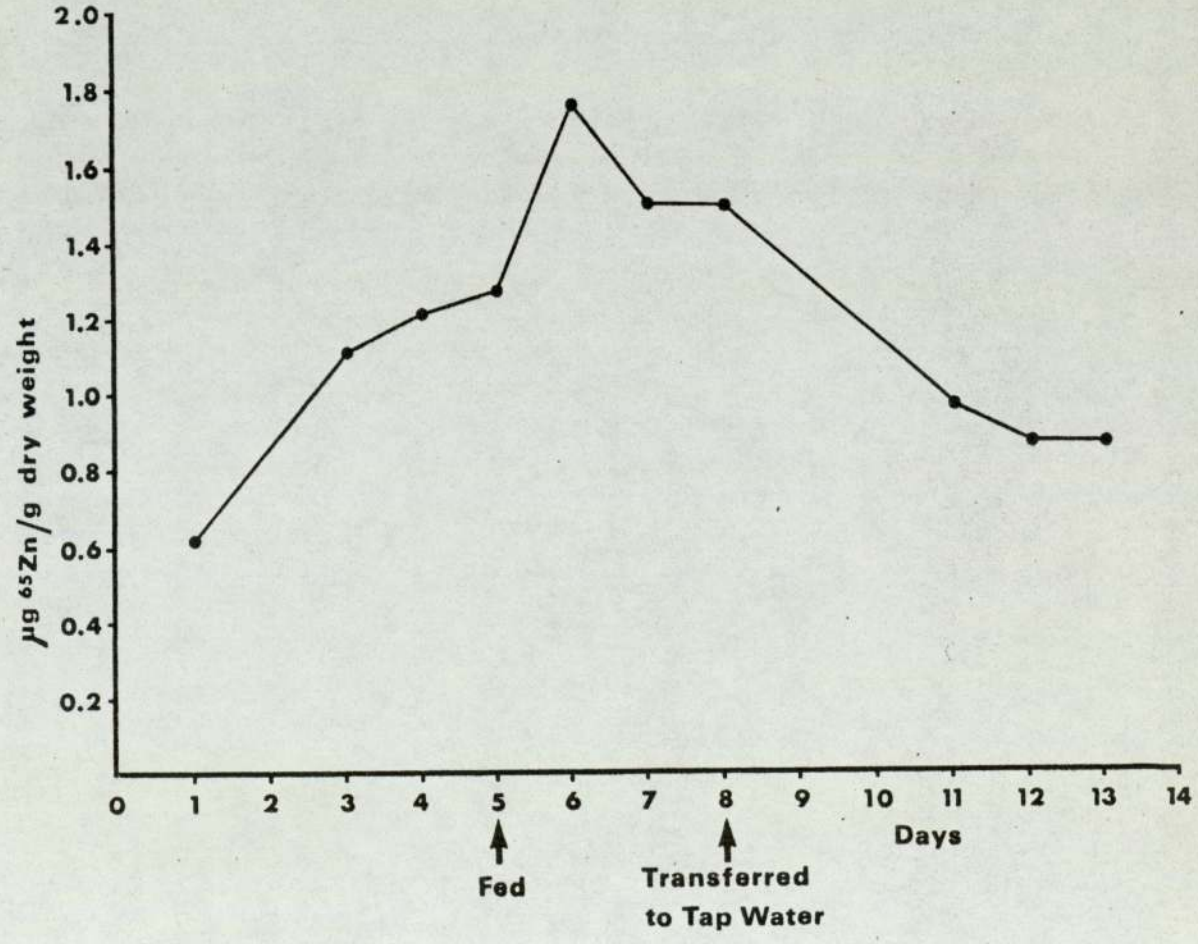
Feeding rapidly increases the rate of zinc uptake, over the 24 hr period from day 5 - 6; presumably because the zinc is adsorbed on to the food flakes and transported into the gut. A similarly sharp decrease over the next 24 hrs. occurs, as defaecation occurs although the larvae are still in the zinc solution. Day 7 - 8 the concentration is static, but on transfer to tap water a steady rate of elimination of zinc occurs over the next 4 days. The concentration then stabilises but at a slightly higher value than the initial concentration. This suggests that incorporation of zinc has taken place into the tissues over this two week period, showing a net bioconcentration of zinc from the surrounding water.

Substantiation of these facts came from measurements made from field collections of larvae (R. Ystwyth, Section 4) which demonstrates the ability of H. angustipennis to take up zinc from the environment.

5.8.4. Discussion and Conclusions

It has been demonstrated by these studies that the larvae of H. angustipennis have the ability to bioconcentrate both copper and zinc from the environment both at realistic field concentrations or at abnormally high experimental levels. Tentatively comparing H. angustipennis in the field situation with other macroinvertebrates (Table 5.37)

Fig.5.39. Accumulation and Elimination rate of zinc by H. angustipennis



it may be seen that H. angustipennis bioconcentrates copper at a similar rate to Asellus aquaticus in river water and 25% effluent and a little more avidly than Simulium sp. For Simulium sp. and A. aquaticus the more metal available in the environment (i.e. as the effluent concentration increases, the greater the amount of copper therein), the more copper is accumulated by the animal. This trend generally was found to be true (see Table 5.39) with some exceptions. In a study by Brown (1977) on the zinc and copper polluted R. Hayle (Cornwall), where the zinc concentration was approximately ten times that of copper, she reports that in the "free-living" Trichoptera larvae, concentrations of zinc and copper in the tissues appeared to follow copper and zinc levels in the water. A further study by Brown (1977) on the isopod A. aquaticus, showed that copper was accumulated from solution and the diet. Weiser (1961) reports a prime site for copper accumulation to be the hepatopancreas in terrestrial isopods. Histochemical and X-ray microanalysis revealed this site to store copper and a marked increase in sulphur was noted in this area too. A parallel was drawn between the production of sulphydryl groups and copper tolerance in yeasts (Ashida et al., 1962), higher plants (Antonovics et al., 1971) and these isopods.

Using the radioisotope technique, the major site for zinc accumulation appeared to be in the soft tissues of the abdomen of H. angustipennis. More accurate centres of accumulation were not pinpointed in this study.

It was noted that occasionally large concentrations of copper were lodged between dorsal sclerites, but this was dislodged when the animal moved its thoracic segments. From work on the amphipod G. pulex with zinc, Bullock (pers. comm.) and copepods with copper (Martin, 1970) it has been demonstrated that a large proportion of heavy metal may be removed by moulting. Since crustacea molt several times,

the exoskeletons may play an important role in eliminating the metal.

H. angustipennis may have an elimination method for removing metals, as demonstrated in the zinc tracer study, but as only a small proportion of the exoskeleton is sclerotised it is unlikely that molting plays a primary role in removal of metal from these larvae. Overall there may be a net increase in metal in the tissues over the life cycle of H. angustipennis, i.e. bioaccumulation may occur and as these larvae have been shown to be extremely tolerant of low concentrations of metals they may be useful as monitors in the terms outlined by Butler et al. (1971). As the distribution of Hydropsyche spp. is widespread, as shown by the survey data, they may be useful tools for monitoring zinc and copper levels in the aqueous environment in a surveillance/monitoring situation.

5.9. RESPIROMETRY INVESTIGATIONS

5.9.1. Introductory Review

As a logical progression from the field work results, respiratory measurements on H. angustipennis were carried out to establish the oxygen requirements of this apparently tolerant caddis larva.

It was suggested as long ago as 1734 by Reaumur that the tracheal gills of Phryganea (Trichoptera) larvae are respiratory organs, and this interpretation was accepted by Dufour (1947) for a hydropsychid larva. Sleight (1913) however, came to the conclusion that this was not true for all caddisfly larvae, some species of which rely exclusively on cutaneous respiration. On removal of the entire complement of tracheal gills of Macronema zebratum, Morgan and O'Neil (1931) found this had little or no effect on oxygen consumption. Indeed, gill-less larvae still built cases and pupated. Hydropsychids require oxygen for respiration by both methods, either direct diffusion through the cuticle or through specially adapted tracheal gills.

It is already known that current velocity has a great influence on respiratory rate, and affects the distribution of species in a river. Ambuhl (1959) showed H. angustipennis to favour a flow of 60 cm sec^{-1}, Scott (1958), H. fluvipes 40 - 50 $cm.sec^{-1}$, R. dorsalis, 80 - 90 $cm.sec^{-1}$ and S. stellatus 0 - 10 cm sec^{-1} and Chutter (1969) Cheumotopsyche thomasetti and C. afra to favour flow in excess of 50 cm sec^{-1}.

Feldmeth (1970b), investigating the respiratory energetics of two Limnephild species, Pycnopsyche guttifer and P. lepida, a slow and fast flowing species respectively, when anaesthetised had the same metabolic rate at currents down to 2 cm/sec. Below this, the rate decreases, due to the formation of a boundary layer around the animal which retards diffusion of respiratory gases. In unanaesthetised larvae, each species exhibits greatest amount of locomotor activity and thus

greatest oxygen consumption for the velocity range in which it is found in the field. For P. lepida oxygen consumption is maximal at a current velocity of 20 cm sec^{-1}, whereas P. guttifer reaches its maximum at 4 cm sec^{-1}, these values being approximately 1.2 and 1.65 mgl^{-1} O_2/g. dry wt./hr.

Similarly, Hildrew and Edington (1979) demonstrated that Hydropsychids and Polycentropids are distributed in riffle and pool sections respectively, due to preferences imposed by their respiratory physiology and conditions for successfully catching food in their nets.

Temperature and oxygen tension of the water are the other two factors which affect respiration. Typical temperature-respiration response, whereby the respiratory rate of insects is low at low temperatures, increasing rapidly through mid-range and breaking sharply as lethal temperatures are approached is the same for both terrestrial and aquatic larvae. This is discussed fully in Keister and Buck's account of Respiration: some exogenous and endogenous effects on rate of respiration in Chapter 7, Rockstein(1974).

Such investigations to ascertain "the incipient limiting point" for an organism provides ecological information which may help in assessing the tolerance of the organism to decreasing oxygen tensions.

Aquatic animals, living in an environment where oxygen tensions can be variable, have to regulate their respiration and metabolic rate. Some, known as "conformers" or "dependent" have metabolic rates which decline with decrease in oxygen tension. Others, known as "regulators" or "independent" are able to maintain their metabolic rate until a critical level of oxygen tension is reached, below which they also become "conformers". At the other end of the scale, the metabolic rate of regulators remains steady with increase in oxygen tension above air saturation while that of conformers rises until some level is reached at which they in turn start to regulate (Mill, 1972). The tension at

which this occurs can be referred to as the "critical level" but is often not very precise.

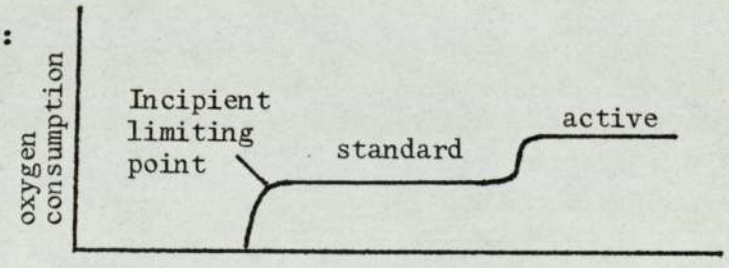
Fox et al. (1937) studied the relationship at 10°C between metabolic rate and environmental oxygen concentrations in ephemeropteran larvae. They found small Baetis larvae to be conformers whilst Leptophlebia and Cloëon were regulators. Intermediates also occur, for example Ephemera. Leptophlebia and Cloëon both live in fairly static water, where an ability to regulate metabolism is an advantage, since fluctuations in oxygen tension are to be expected. Baetis, however, lives in fast flowing streams and does not normally encounter low oxygen tensions. Petty (1967) found that all year class nymphs of the stoneflies Pteronarcys californica and Acroneuria pacifica were conformers i.e. the more oxygen they had available, the more they used and vice versa. Edwards & Learner (1960) in their studies of oxygen consumption on Asellus aquaticus and A. meridianus described their function as "regulatory" or "independent"between oxygen concentrations of 8.3 - 1.5 ppm O_2. They report from various sources that locomotory activity decreases and pleopod activity increases as the oxygen concentration falls. Differences in activity pattern have been described by Walshe (1948) for Chironomus plumosus, where the proportion of time spent feeding decreases and that spent in respiratory irrigation increases below 3 ppm. The assumption that an independent type of oxygen consumption is favourable to an organism (Beng and Ockelmann, 1959) implies maintenance of full metabolic function is involved wherever such changes in activity pattern take place, because the "scope" for activity" (Fry, 1947) decreases. Thus animals with such a behaviour pattern can survive only relatively short periods of exposure to low oxygen concentrations unless they can withstand longer periods of starvation.

Generally it seems to be true that the respiratory rate of still water animals is of the "independent" type, i.e. it remains more or less constant as the oxygen content falls, and begins to decline only at quite low oxygen tensions. That of stream-dwelling animals tends to be "dependent", at least at low and medium concentrations the respiratory rate falls as the oxygen content falls (Hynes, 1970).

The least amount of dissolved oxygen at which the animal can maintain itself is referred to as the "incipient limiting point", often described as being autecologically significant in the distribution of the animal.

There are three theoretical levels which may be identified. These are, firstly the active rate when the animal is moving, the standard rate, not moving and the incipient limiting point where the animal is at basal maintenance level.

Thus:



In the field situation, if the animal encountered conditions which caused it to reach the "incipient limiting point", for example deoxygenated conditions due to organic pollution, then it is likely to die.

More realistically the survival level may come at the point of inflexion where active rate moves to standard rate of metabolism. If the animal is unable to move, its food collection, growth and reproduction will be impaired (i.e. in terminology used for fish studies, the "scope for activity will be severely reduced"). Often the incipient limiting point, and these three identifiable levels on the curve are disrupted. Information from Warren (1971) shows that in fish

there is often an increase in the standard rate, thus increasing oxygen consumption just before the incipient level is reached. This is caused by increased respiratory activity necessary at low oxygen tensions. Investigations into the respiratory rate, and oxygen consumption of H. angustipennis under differing conditions have been carried out in this section of the laboratory investigations.

5.9.2. Methods and Materials

Fig.5.40 illustrates the apparatus which was designed and built for the respirometric studies. More detail may be seen from Photographs 6, 7, 8 and 9.

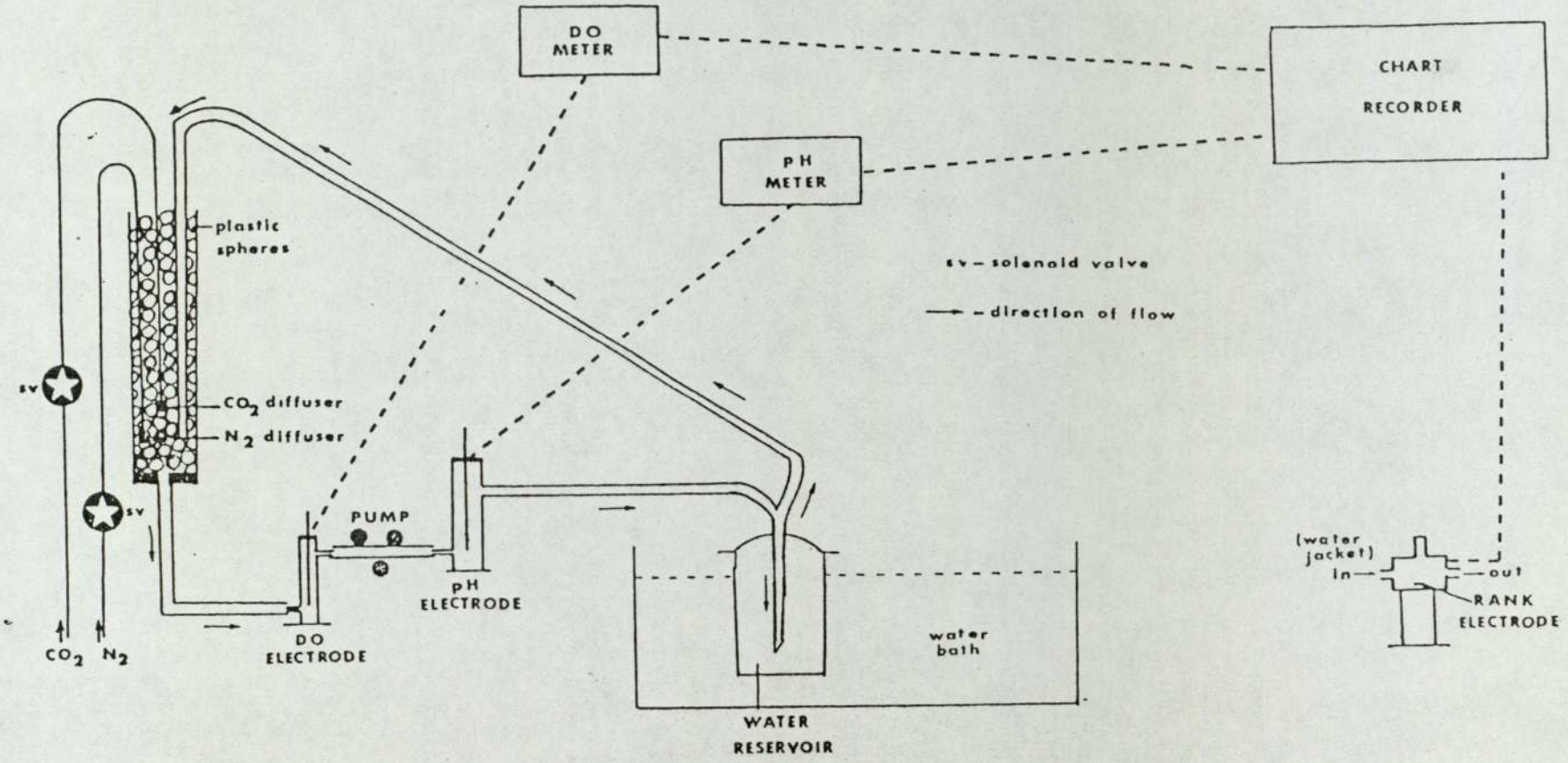
The water preparation system involves a gaseous exchange column filled with plastic spheres, through which water is circulated to regulate the oxygen at the required level. Oxygen and pH electrodes in the system monitor the levels of gases and limit switches operate solenoid valves so that nitrogen can be supplied to the system from a diffuser block in the column. The nitrogen strips off oxygen, but this has the effect of increasing pH so CO_2 can also be automatically added when required to correct and maintain the pH level.

Water prepared in this manner was used in the respirometry chamber. This was a modified Rank Electrode (Rank Bros., Cambridge) bored out to approximately 15 ml volume. A magnetic stirrer (Rank Bros.) supplied a continuous current at constant speed. In the base of the chamber the silver-platinum oxygen electrode was housed. This was linked to a J.J. Chart Recorder so that the oxygen used by the animal over the two hour experimental period could be continually recorded.

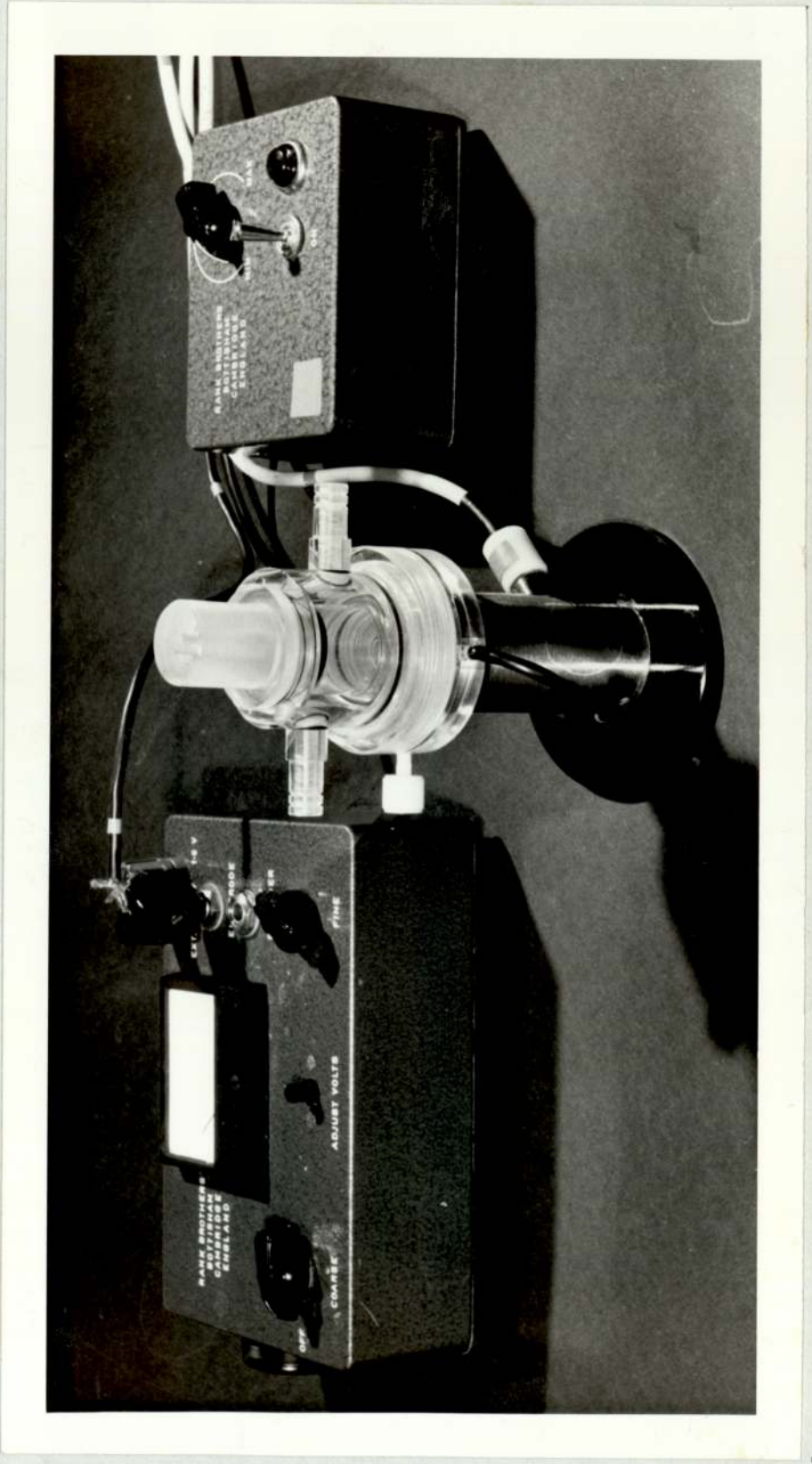
The entire apparatus was housed in a temperature controlled cabinet and maintained at $\pm 1^{\circ}C$. In order to ensure even temperature in the respirometry chamber a continuous flow of water was pumped through the outer sleeve of the chamber.

Individual final instar larvae of H. angustipennis were used for each experimental run. Animals collected from Langley Brook were

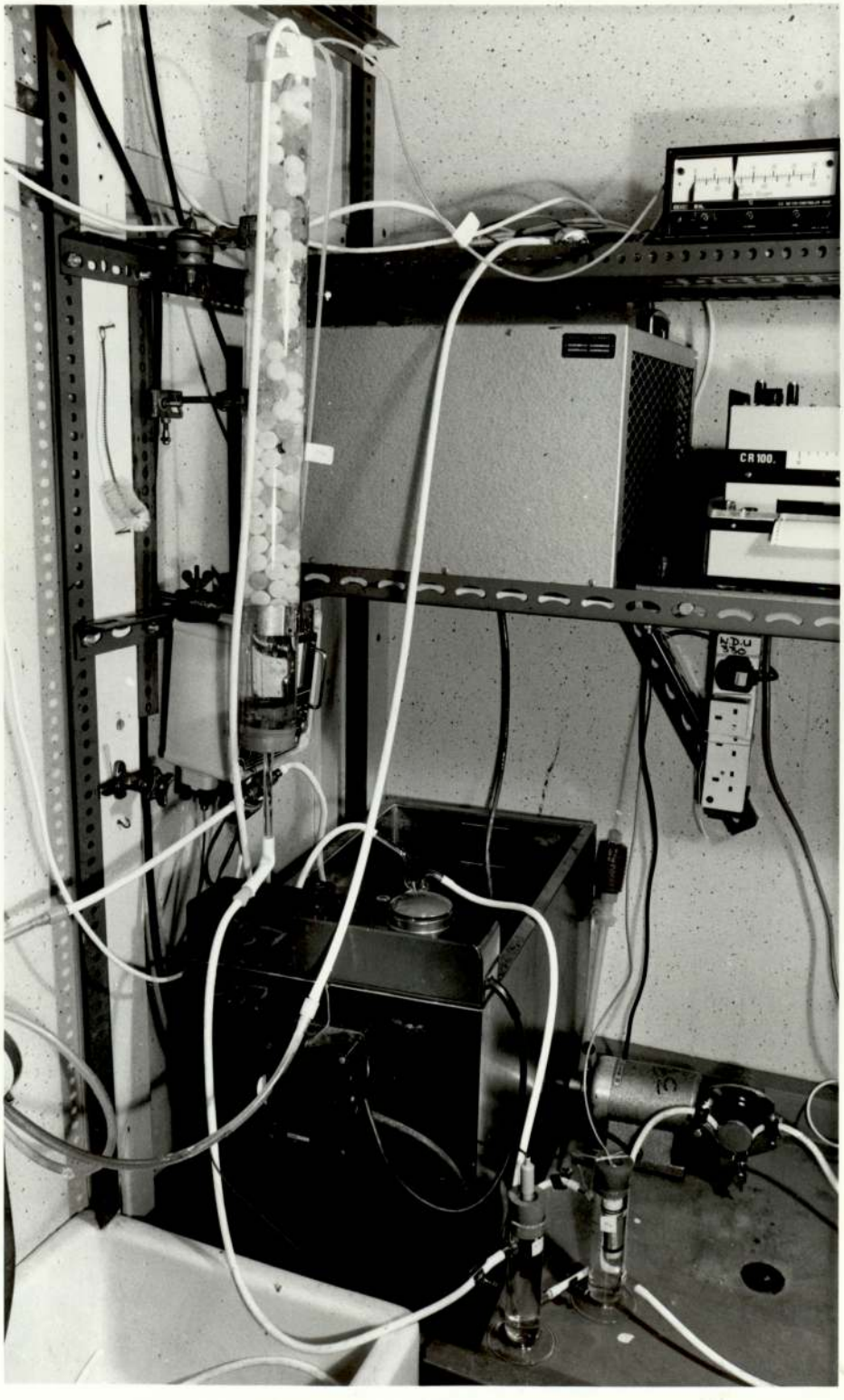
Fig.5.40. Diagrammatic Representation of Respirometric Apparatus



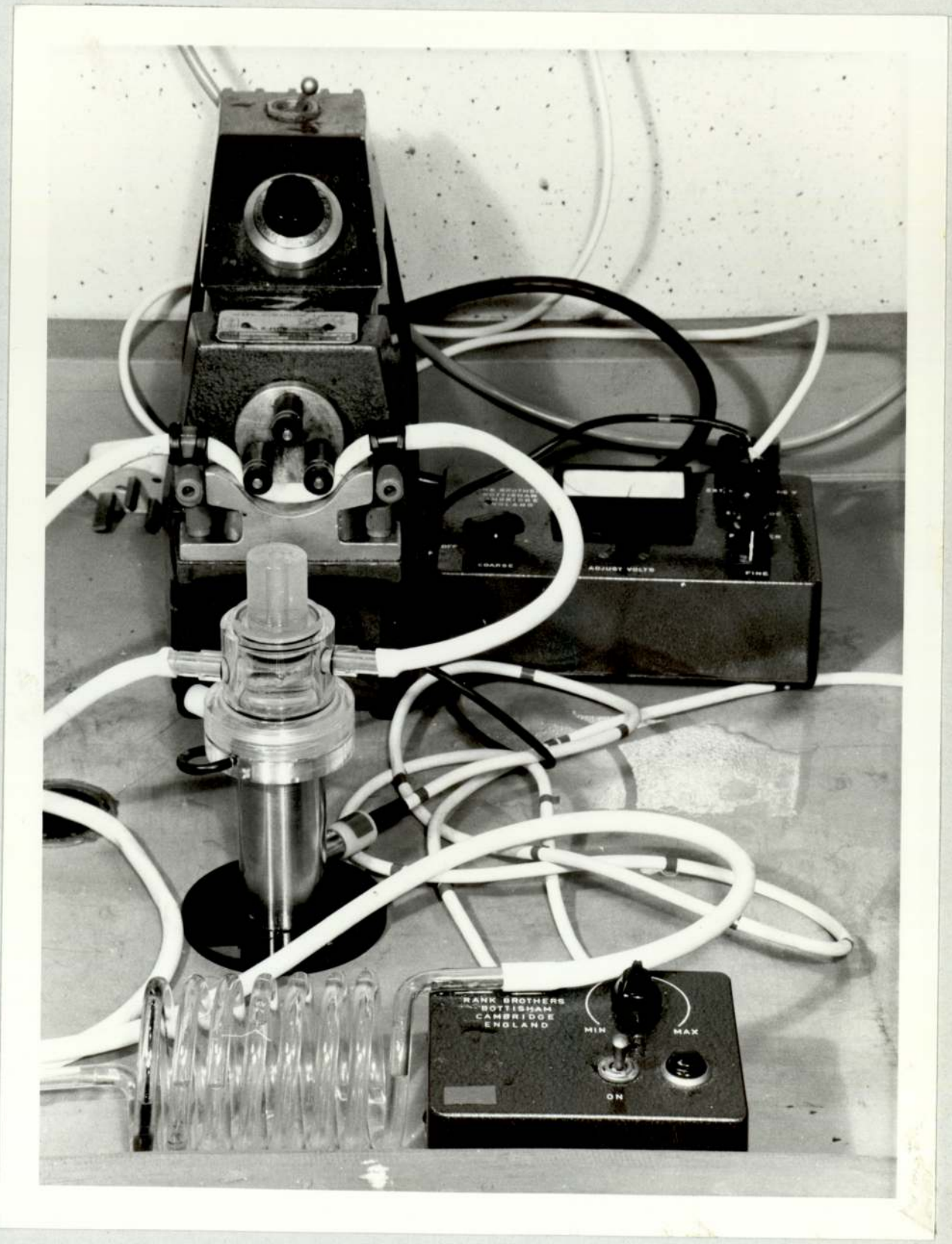
Photograph 6. Rank electrode with 15 ml respirometric cell in position on magnetic stirrer



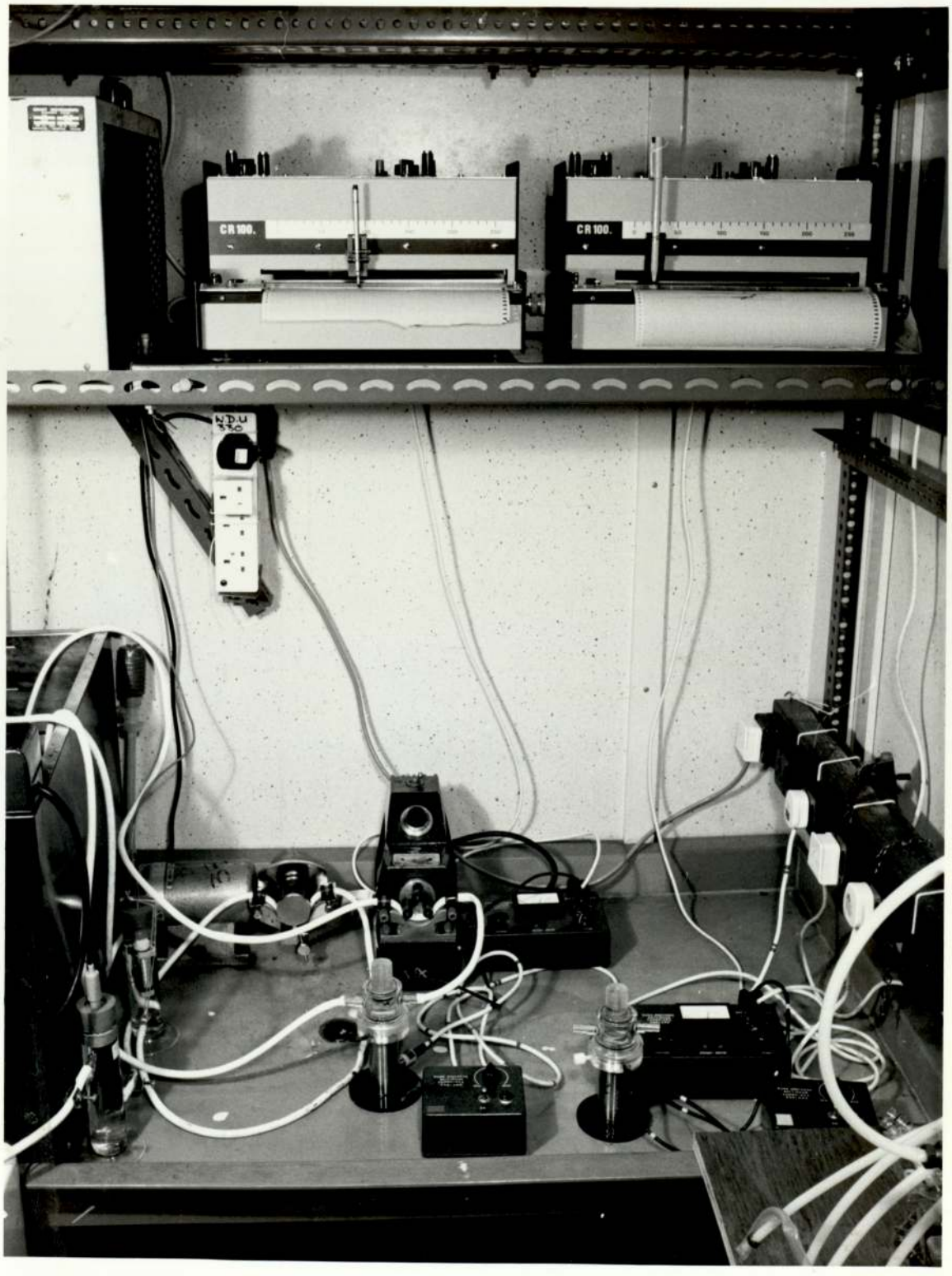
Photograph 7. Water Preparation Apparatus



Photograph 8. Apparatus to maintain temperature in outer sleeve of respirometric chamber (cooling coil would be immersed in water bath under experimental conditions)



Photograph 9. Complete Respirometry Apparatus housed in temperature controlled cabinet



acclimated at the experimental temperature for at least 24 hours.

The experimental regimes included runs at temperatures of 10°C, 15°C, 20°C and 25°C at saturated, 5.0 mgl^{-1} and 2.0 mgl^{-1} O_2.

Preliminary Investigations into the effects of Autoclaving of water used in tests.

Initially water directly from Langley Brook was used, but concern over respiration by algae/bacteria introducing error into the system was suspected. To test this, Langley water was filtered and autoclaved, the chemical parameters monitored and then any effect in the respirometry cell measured.

Table 5.44 demonstrates the changes in water quality which occurred after the filtering and autoclaving process.

Table 5.44 Comparison of raw v autoclaved Langley Brook Water

Site ->	Langley Brook Raw	Langley Brook Autoclaved
Determinand	18.3.81	20.3.81
Temperature °C	8.5	
D.O. mgl^{-1}	11.3	
Total Hardness $mgl^{-1} CaCO_3$	468	360
Ca Hardness mgl^{-1} $CaCO_3$	294	202
Mg Hardness mgl^{-1} $CaCO_3$	174	158
Phenolthalein Alkalinity mgl^{-1} $CaCO_3$	0	5
pH	<8.3	>8.3
Total Alkalinity $mgl^{-1} CaCO_3$	235	140
Chloride mgl^{-1}	52.5	53.9
Nitrate $mgNl^{-1}$	9.0	11.55
Ammonia $MgNl^{-1}$	1.4	0.7
Phosphate mgl^{-1}	2.4	2.8
Copper mgl^{-1}	0.1	

Through technical problems with the pH meter, the pH levels in the above table can only be given as < or > 8.3.

The most noticable effects of autoclaving the water were the decrease in hardness and total alkalinity values, as would be expected.

Following this water preparation, one of the Rank Electrodes was used to test the difference between the two types of water. Firstly, raw Langley Brook water was placed in the respirometry chamber, the top put in place and all parameters set as for a complete experimental run. Oxygen consumption from the cell was measured and recorded on the chart recorder for 4 hours. Secondly, the filtered and autoclaved water was placed in the same cell, and the same electrode system used to record a 4 hour period. Both recordings were made at $15^{o}C$.

Results

No oxygen consumption was recorded over 4 hours when the respirometric cell contained autoclaved water, and only 0.026 mgl^{-1} of oxygen was used over 4 hours when it contained raw water. This meant that the respiration by algae/bacteria would amount at maximum to 0.0006 mgl^{-1} hr^{-1} at $15^{o}C$. Balancing the effort required to filter and autoclave all water for experimentation, and in the light of modified hardness and alkalinity, it was decided that the above level of oxygen consumption would be negligible. Thus, all the experimentation carried out was with raw Langley Brook water, prepared in the aforementioned water preparation system as in Photograph 7.

5.9.2.1. Experimental Programme

Replicated experiments on individual larvae, acclimated to the experimental temperature, were repeatedly carried out to investigate oxygen consumption. After transfer from the holding vessel, animals were acclimated in the respirometry chamber for a short time before the readings on oxygen consumption began. A 2-hr reading was recorded on a calibrated chart recorder. At the end of the experiment the animal was dried to constant weight at $105^{o}C$ enabling calculations of oxygen consumption per gram of dry weight per hour to be made.

Summary of Experiments

✓= one experimental run

1. 3 x 3 Temperature x Dissolved Oxygen

Temp. °C	D.O. mgl^{-1}		
	Saturated	5.0	2.0
25	✓✓	✓✓	✓✓
15	✓✓	✓✓	✓✓
10	✓✓	✓✓	✓✓

✓= one experimental run

2. 3 x 4 Temperature x Dissolved Oxygen

Temp. °C	D.O. mgl^{-1}		
	Saturated	5.0	2.0
25	✓✓	✓✓	✓✓
20	✓✓	✓✓	✓✓
15	✓✓	✓✓	✓✓
10	✓✓	✓✓	✓✓

3. 3 x 2 Dissolved Oxygen x Ammonia 15°C, 500 ppm Total Ammonia

NH_3	D.O. mgl^{-1}		
	3.8	6.0	9.6
+	✓✓	✓✓	✓✓
-	✓✓	✓✓	✓✓

4. 5 levels of pH, all at 15°C and oxygen saturation

pH	5.0	7.2	7.9	9.0	12.0
	✓✓	✓✓	✓✓	✓✓	✓✓

Raw data was recorded in terms of oxygen consumption per g dry wt. per hour, and graphs were plotted to assess the trend in respiratory rates.

Statistical analyses were then performed either manually or on the computer to assess any significant changes and interactions of the

parameters under investigation. Manual statistical analyses were performed on data from experimental programme 1 (Anovar). For the factorial design in experimental programme 2 the computer was employed to assess any interactions. Experiments 3 and 4 were straightforward analyses.

Further runs of H. angustipennis were made, in addition to those listed in the programmes above, for the purpose of calculating the incipient limiting point for this species at 10° and 15°C.

5.9.3. Results

Experiment 1. Effects of temperature and dissolved oxygen

The results of replicate tests at different temperatures and oxygen concentrations are given in Table 5.45.

TABLE 5.45. Respiratory Rates of H. angustipennis at three different levels of temperature and dissolved oxygen

Temp.	mg/l O_2/g dry wt./hr					
	Sat.	mean	5.0 mgl^{-1}	mean	2.0 mgl^{-1}	mean
25°C	2.9068	3.1094	2.7976	2.6253	3.5020	4.1151
	3.3120		2.4531		4.7282	
15°C	1.6809	1.9492	1.3100	1.0668	0.9968	0.8491
	2.2175		0.8236		0.7015	
10°C	1.6070	1.7242	0.7659	0.6061	1.2260	0.7301
	1.8414		0.4464		0.2343	

The analysis of variance summary calculated on the above data is shown in Table 5.46.

TABLE 5.46. Analysis of variance summary table to show the effect of three different levels of dissolved oxygen and temperature on the respiratory rate of H. angustipennis

Variable	df.	s.s.	m.s.	F ratio	From tables 5%	1%
Temperature	2	18.35	9.17	45.85	4.26	8.02
D.O.	2	2.07	1.03	5.15	4.26	8.02
Temp.x D.O.	4	3.1	0.77	3.85	3.63	6.42
Error	9	1.76	0.2			
Total	17	25.28				

The anovar summary in Table 5.46 demonstrates that increasing temperature has a highly significant effect on the oxygen consumption of H. angustipennis. The effect of changing dissolved oxygen levels also has a significant effect on oxygen consumption, as does the combined effect of dissolved oxygen with temperature.

This analyses was followed by a calculation of the least significant difference, in the following manner

$$\text{Least significant Difference} = \frac{2 \times \text{Error m.s.}}{2}$$

$$= 0.2$$

$$= 0.4472$$

$$\text{L.S.D.} = t \quad \text{error m.s} \quad \text{at } P = 0.05,\ df = 9$$

$$= 2.26 \times 0.4472$$

$$= 1.01$$

Comparisons of the means from each experimental level (see Table 5.45) were then made. The combinations of pairs which were significant are summarised in Table 5.47.

Conclusions

In conclusion, these results show that there was a significant difference in the respiratory rate at different temperatures. This was not always so at different oxygen levels at the same temperature. There was some evidence to show that at the lowest dissolved oxygen, there was an increase in respiratory rate, probably due to the increased activity of the organism under stress.

TABLE 5.47. SUMMARY OF SIGNIFICANT VALUES WHERE PAIRS EXCEED THE L.S.D. LEVEL

Mean ->	3.1094	2.6253	4.1151	1.9492	1.0668	0.8491	1.7242	0.6061	0.7301
Value	1	2	3	4	5	6	7	8	9
3.1094	1			sig	sig	sig	sig	sig	sig
2.6253	2		sig		sig	sig		sig	sig
4.1151	3			sig	sig	sig	sig	sig	sig
1.9492	4					sig		sig	sig
1.0668	5								
0.8491	6								
1.7242	7							sig	
0.6061	8								
0.7301	9								

sig = combinations of values where the difference is above the value 1.01.

Experiment 2 Effects of temperature and dissolved oxygen 3 x 4

TABLE 5.48 Respiratory Rates H. angustipennis

Temp.	mgl^{-1} O_2/g dry wt/ hr. D.O.		
	9.0	5.0	2.0
25°C	2.9068 3.3120	2.7976 2.4531	3.5020 4.7282
20°C	1.9700 3.2580	0.9463 1.0241	2.3470 1.2240
15°C	1.6809 2.2175	1.3100 0.8236	0.9968 0.7015
10°C	1.6070 1.8414	0.7659 0.4464	1.2260 0.2347

Analysis of Variance, Table 5.49 is set out overleaf, This shows that the effect of temperature upon the respiratory rate of H. angustipennis is linear, whilst the respiratory rate at constant temperature, increases in a quadratic fashion. These trends are substantiated by Figs.5.41 and 5.42.

Fig.5.41 shows the relationship of oxygen consumption with increasing temperature in H. angustipennis at three different oxygen tensions. Table 5.50 compares other studies on similar species and shows a similar trend over a temperature range of 2-28°C for all Hydropsychids. Fig.5.42 shows the respiratory rates plotted against oxygen tensions at four temperatures.

Further experiments to make increased numbers of readings were carried out at 10°C and 15°C. An alternative method of calculation of oxygen consumption was used here by measuring the gradient off the chart recorder trace and converting the drop in rate to mg/g dry wt/hr. Figs 5.43 and 5.44 depict the results by this method.

TABLE 5.49. Analysis of variance summary table to show the effect of three different levels of dissolved oxygen and four levels of temperature on the respiratory rate of H. angustipennis

(where Variable A = O_2; Variable B = Temperature)

VARIABLE	Degrees of Freedom	Sum of Squares	Mean Square	F-Ratio	Sig. level	1%
A	2	4.23631	2.11816	7.86442	H.S.	**
A linear	1	1.22066	1.22066	4.53213	N.S.	
A quadratic	1	3.01567	3.01567	11.1967	H.S.	**
B	3	18.3673	6.12244	22.7318	V.H.S	***
B linear	1	15.968	15.968	59.2871	V.H.S.	***
B quadratic	1	2.23345	2.23345	8.2925	Sig	*
B cubic	1	.165837	.165837	.615731	N.S.	
A*B	6	3.58653	.597755	2.21938	N.S.	
AL*BL	1	1.83684	1.83684	6.81994	Sig.	*
AL*BG	1	.92933	.92933	3.45047	N.S.	
AL*BC	1	9.76308E-02	9.7630BE-02	.36249	N.S.	
AG*BL	1	.390153	.390153	1.44858	N.S.	
AG*BG	1	1.32034E-02	1.32034E-02	4.90224E-02	N.S.	
AG*BC	1	.319355	.319355	1.18572	N.S.	
Error	12	3.23201	.269334			
Total	23	29.4222				

Fig.5.41. Oxygen uptake (respiratory rate) of H. angustipennis at three different oxygen levels and increasing temperature

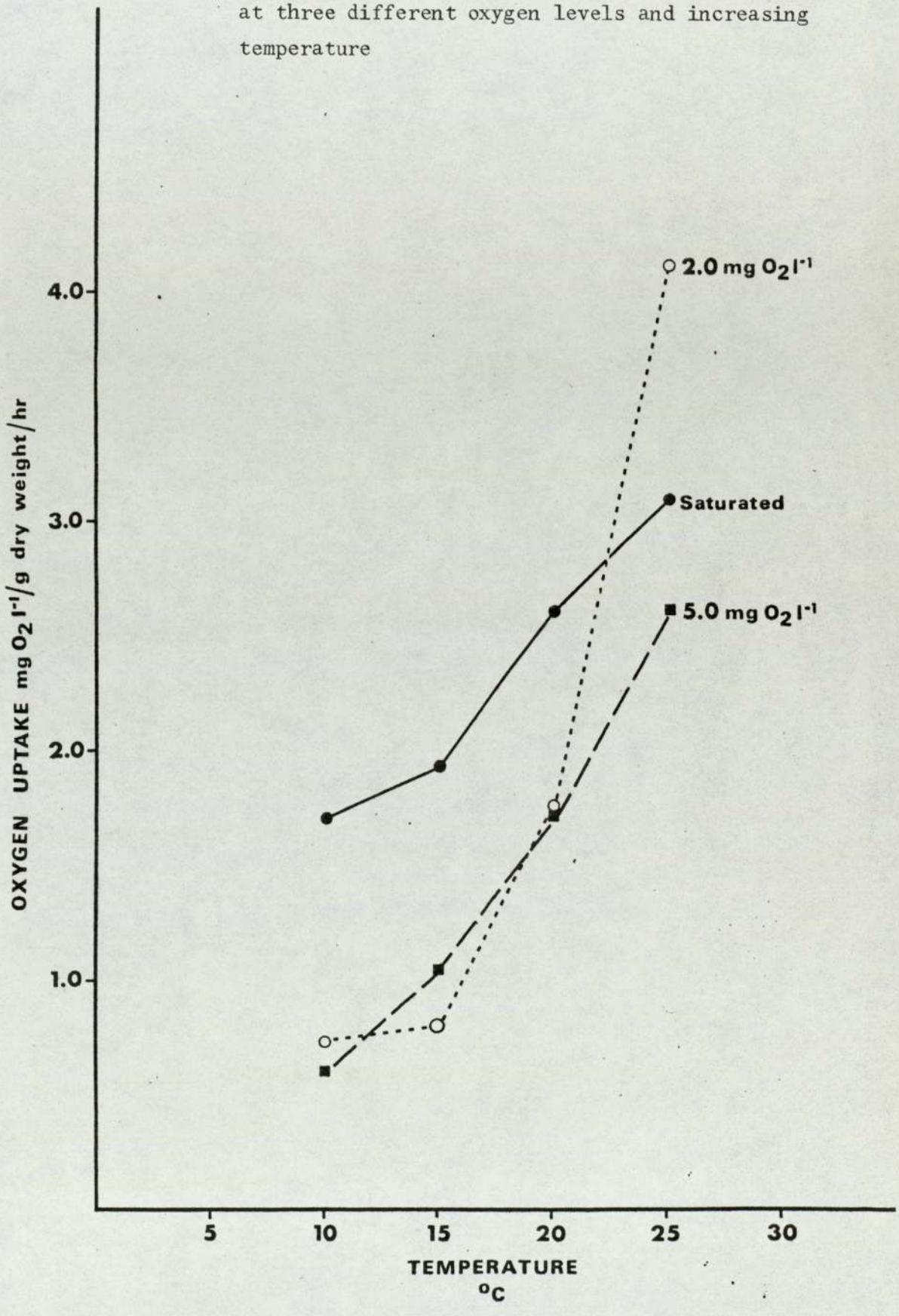


Fig.5.42 Oxygen uptake (respiratory rate) of H. angustipennis at three different temperatures

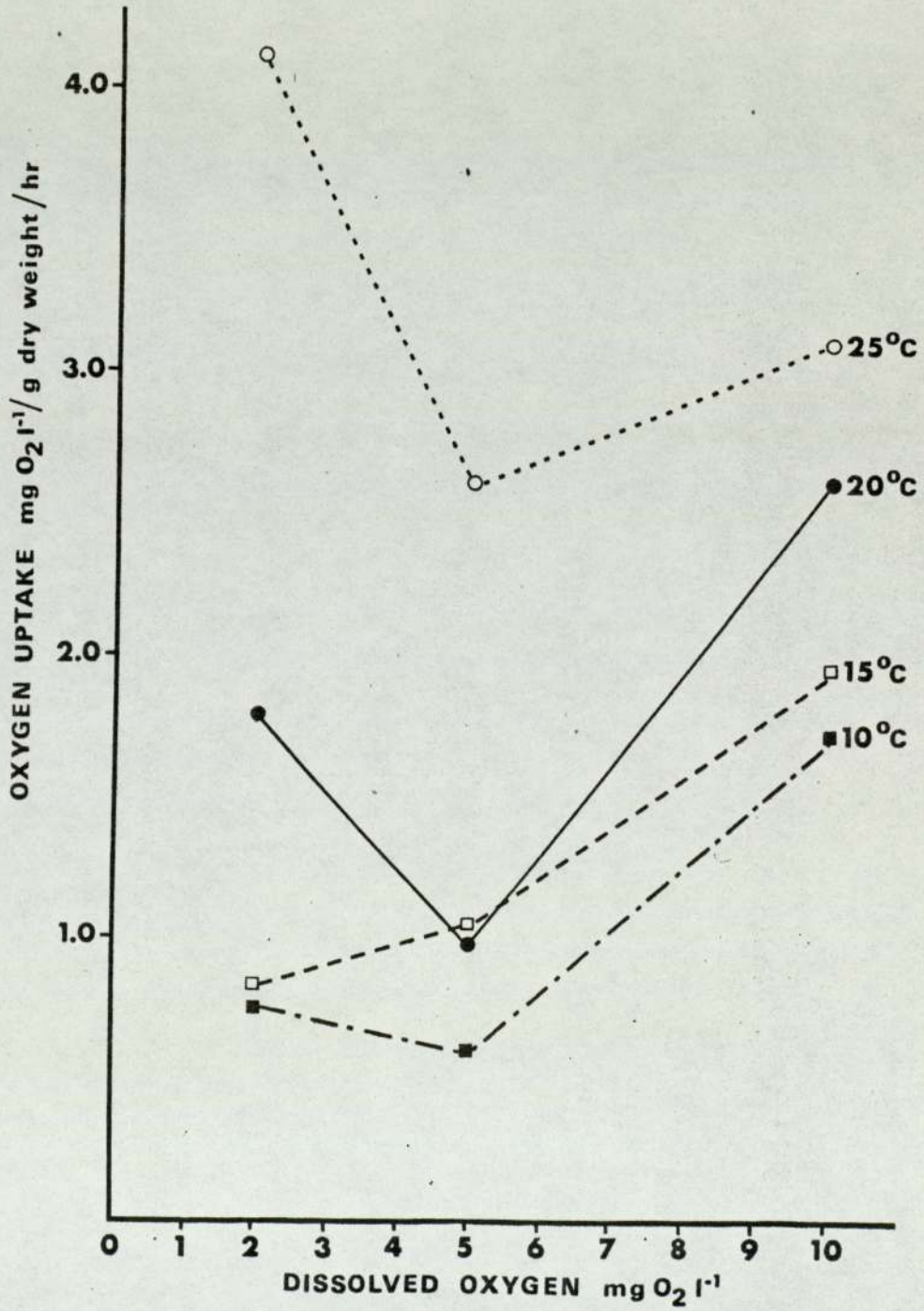


TABLE 5.50 Oxygen uptake by Caddis Fly Larvae (Values = mean uptake mgl^{-1} O_2 dry wt/hr)

Temperature °C -> / Species ↓	2	5	10	12	15	17.5	20	23	25	28	Reference
H. fluvipes		0.49	0.74		0.87		1.98		2.06		Edington & Hildrew 1973
D. felix		0.43	0.51		1.06		1.83		2.42		
D. felix		0.5	0.6		1.1		1.8		2.4		Hildrew & Edington 1979
H. instabilis		0.6	0.8		0.9		1.95		2.05		
H. pellucidula		0.35	0.45		0.65		0.9		1.5		
P. conspersa	0.1	0.17	0.2		0.5		0.6	0.64	0.6	0.6	
P.flavomaculatus	0.2	0.26	0.27		0.38		0.55	0.62	0.78	0.5	Philipson & Moorhouse 1976
C. flavidus		0.3	0.34		0.38		0.42		0.65	0.6	
H. picicornis	0.15	0.17	0.22		0.3		0.35	0.46	0.68	0.64	
Polycentropids		0.1						>	3.0		Greenwood (unpub.)
H. angustipennis		1.16	1.72	1.55	1.59		2.61		3.11		Hirst 1981 (unpub.)

Fig.5.43. Oxygen consumption by H. angustipennis at 10°C

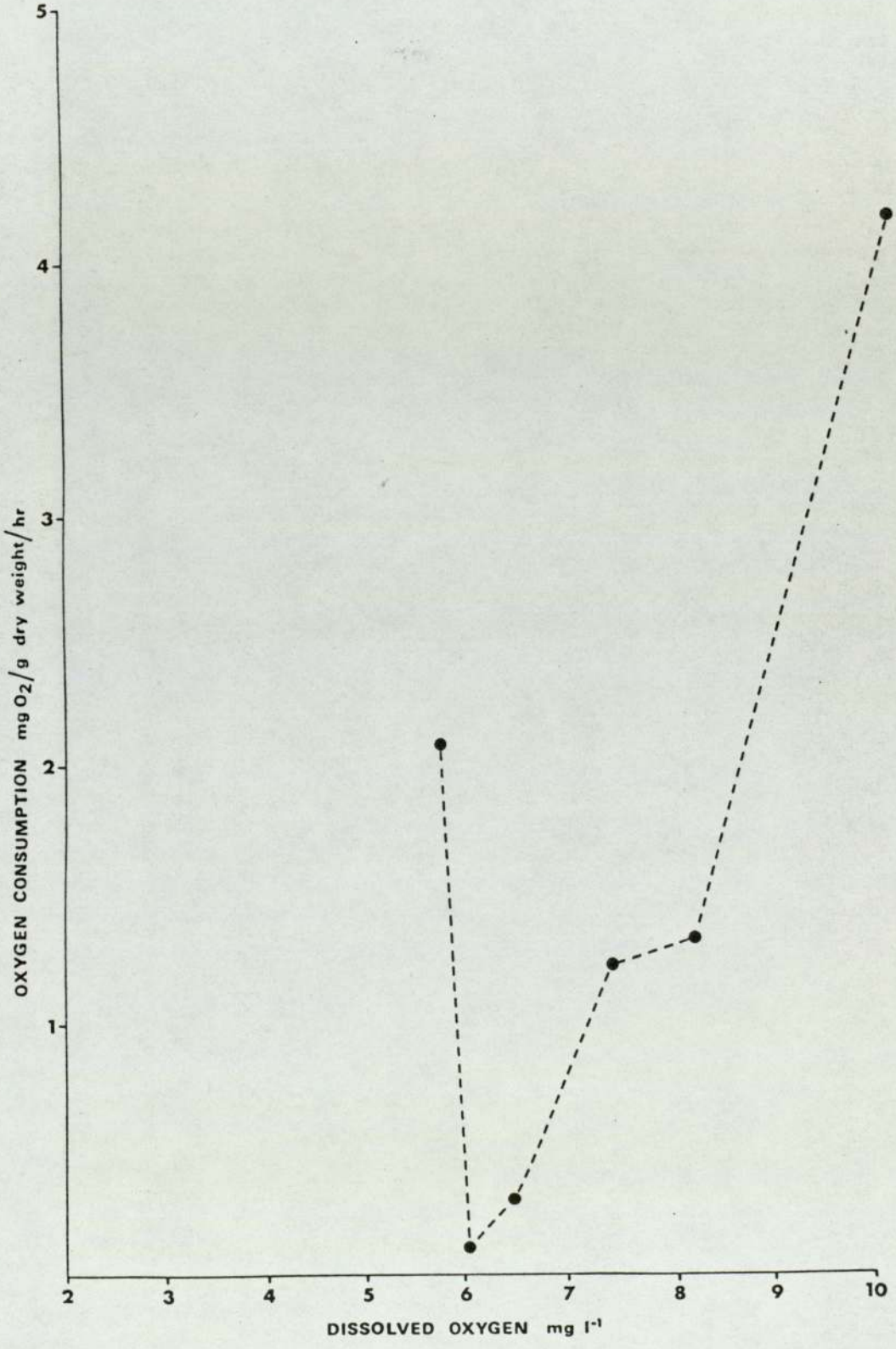
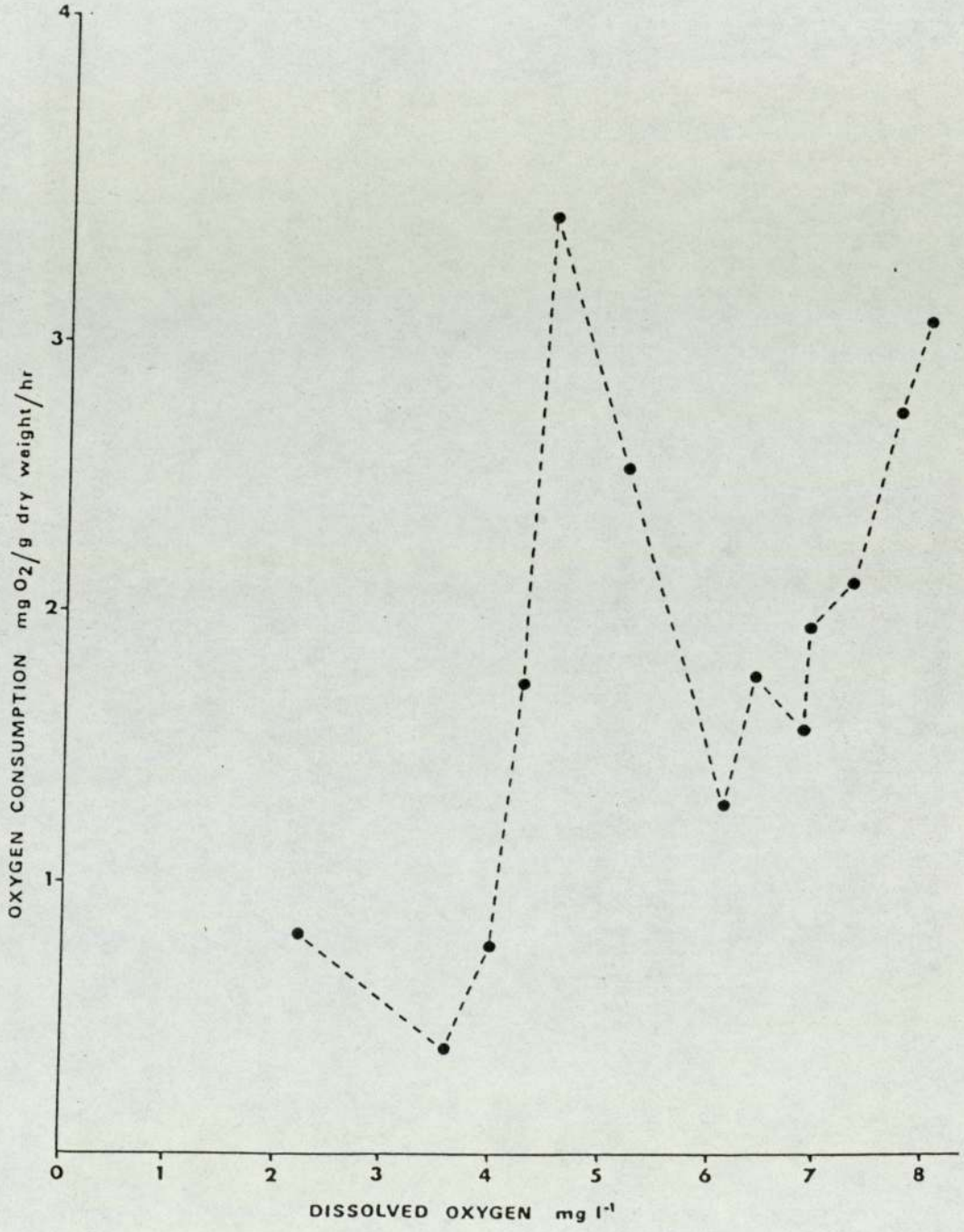


Fig.5.44. Oxygen consumption by H. angustipennis at 15°C



Experiment 3 Effect of ammonia toxicity at 15°C

Tables 5.51 and 5.52 below give the results of raw readings of respirometric rate at three levels of dissolved oxygen with and without the presence of ammonia, followed by the analysis of variance of these results. Factorial design is an improvement on the one-way analysis as it picks up any significant interactions.

TABLE 5.51 Respiratory rates for H. angustipennis at three levels of dissolved oxygen in the presence and absence of ammonia

(variable A = ammonia; variable B = dissolved oxygen)

	B		
DO mgl^{-1}	3.8	6.0	9.6
+ NH_3	1.4184 1.2980	0.995 0.948	1.886 1.862
- NH_3	0.657 0.778	2.1222 1.3270	2.6600 2.5900

TABLE 5.52 Analysis of variance summary table to show the effect of temperature and ammonia on the respriatory rate of H. angustipennis (Variable A = ammonia; variable B = temperature)

Variable	S.S.	df	m.s	F. ratio	
A	0.24849	1	0.2484	4.456	NS
B	3.16925	2	1.5846	28.417	0.1 ***
A & B	1.29316	2	0.6465	11.595	1.0 **
Error	0.33457	6	0.0057		
	5.04548	11			

It was found that ammonia alone had no effect but temperature and a combination of temperature and ammonia had a significant effect on respiratory rate.

Experiment 4. Effect of differing pH levels on respiratory rate at constant dissolved oxygen levels, saturated at 15°C.

Table 5.53 gives details of the replicates of respiratory rate measured at five different levels of pH followed by Table 5.54 giving the Anovar summary data.

TABLE 5.53. Respiratory Rates for H. angustipennis at 4 different levels of pH

pH	5.0	7.2	7.9	9.0	12.0
O_2 consumption mgl^{-1}	1.0622	2,1960	2.66	3.4222	2.6035
	1.7012	1.8471	2.59	3.1780	2.8750
		1.5970			
t	2.7634	5.6401	5.25	6.6002	5.4785
$\bar{x}$	1.3817	1.8800	2.625	3.3001	2.7393

TABLE 5.54. Analysis of variance table to show the effect of increasing pH on the respiratory rate of H. angustipennis

Source	s.s.	df	Mean sq. (Var.est.)	F ratio
pH	4.8325	4	1.2081	1.1386
Error (within gps.)	0.4175	6	0.0695	

From tables the significance level at df.4, 6 = 4.56 at the 5% level, therefore pH is not significant.

5.9.4. Discussion and Conclusions

(a) Effects of temperature and dissolved oxygen (Experiments 1 and 2)

We may conclude from these results that the effect of temperature upon the respiratory rate of H. angustipennis is linear, i.e. oxygen consumption increases with increasing temperature, as for all insects. This is true at whatever oxygen tensions the measurements are made. However, the respiratory rate, at the same temperature increases in a quadratic fashion with increased oxygen tensions. Although in these experiments the parameters of current and temperature were constant, in the field this would not be so. At high O_2 tensions the larva would be receiving sufficient oxygen from the water as it flowed over its body - thus respiratory undulations would be minimal but it would be in a metabolically favourable state, having sufficient energy reserves for locomotory activity if required. As the oxygen tension decreases, the gradient on the graph decreases as consumption decreases, demonstrating it to be a "conformer".

However, at between 5.0 mgl^{-1} - 6.0 mgl^{-1} O_2 there is a rapid increase in oxygen consumption. This may be explained, and confirmed by Philipson's work (1954) in which he found H. instabilis to increase its undulatory activity for respiration at decreased oxygen levels.

Additional respiratory undulations would cause an increase in oxygen consumption, as the animal attempted to maintain at least a 'standard' rate of activity, this is a similar reaction to that reported in fish (Warren, 1971). Referring to Figs.5.43 and 5.44, at 10°C the onset of this rapid undulatory activity is at 5.6 mgl^{-1} O_2 and at 15°C 6.2 mgl^{-1} O_2, slightly higher, as would be expected. It is suspected that this is not an incipient limiting point value, but rather a physiological response to the increasingly adverse oxygen tension in the environment. The stress provokes undulatory rather than locomotory activity.

The 'incipient limiting point' is anticipated to be below 2 mgl^{-1} O_2 as it has been demonstrated that it is a species which is extremely tolerant of low oxygen concentrations. In comparison Grant & Hawkes (1982) found an 'incipient limiting point' in Gammarus pulex of below 2.0 mgl^{-1} and this species is generally less tolerant of depleted oxygen than H. angustipennis as shown in comparative survival times (Davis, 1971). In field conditions, depleted oxygen may be a result of organic pollution and there may be diel patterns (Hawkes and Davies, 1971).

These results suggest that for a limited period, H. angustipennis could withstand low oxygen tensions by increasing its undulatory activity. As has been demonstrated, it can withstand several days without food too, so in the field this would not be the primary factor affecting its survival.

In a normal riffle habitat, presumably the larvae would, in times of adverse oxygen conditions, seek an alternative site which was more favourable, e.g. an area of increased current velocity in a different niche between stones.

(b) Effects of Ammonia

Analysis of variance, with replicates containing ammonia at different oxygen concentrations found that ammonia alone had no significant effect on the respiratory rate, but that oxygen had an effect, as did the combination of ammonia together with decreasing oxygen concentrations. In the field, elevated ammonia levels would rarely be observed without concomitant changes in dissolved oxygen.

Although experimental concentrations were in excess of field concentrations H. angustipennis had shown such resistance to ammonia in toxicity testing that these levels had to be used in order to get any effect in the experimental period.

(c) Effects of pH

Respiratory rates measured over a range of pH from 5.0 - 12.0 all at 15°C and oxygen saturation showed that there was no significant effect on oxygen consumption by pH effect alone.

However, pH may affect the toxicity of other parameters, such as ammonia, metal ions and have a combined effect when in conjunction with other deleterious conditions such as low oxygen tensions.

6. SYNTHESIS OF FIELD AND LABORATORY WORK

6. SYNTHESIS OF FIELD AND LABORATORY WORK

Looking in retrospect at the task proposed in the Introduction and outlined in Chapter 3, it is apparent that the project was rather ambitious! However, much of the proposed work was satisfactorily completed and the results encouraged investigations into related subject areas.

In drawing together the conclusions from this work, it is necessary to initially summarise the result of field investigations then look at laboratory findings. The aim of the field work was to identify the factors responsible for dictating the distribution of caseless caddis larvae, particularly the Hydropsychids in river systems. Intensive sampling and survey work, followed by an array of analysis techniques highlighted some parameters instrumental in shaping the distribution of certain species of Trichopteran larvae. From chi-square tests it was concluded that both Hydropsyche spp. and Rhyacophila dorsalis distribution was highly correlated with chemical water quality. R. dorsalis was restricted to sites with water quality of Chemical Class 1 or 2, being present at 11 of the 77 sites surveyed. The distribution of Hydropsyche spp. was more widespread, being present in all water qualities at 33 of the 77 sites.

Preliminary analyses of survey data showed that the distribution of Hydropsychids was generally associated with certain other species within the invertebrate community, these commonly being mayflies, oligochaetes crustacea and certain snails. Upon correlations calculated by computer the positive associations were shown to be with Leuctra sp., Baetis sp., E. ignita, R. semicolorata, A. fluvialtilis, Hydrophilidae and Oligochaeta. This biological data, generated from samples taken at sites originally chosen on a statistically random basis confirmed general observations. Certain physico-chemical parameters were also shown to be associated with the distribution of Hydropsyche larvae,

namely dissolved oxygen, BOD, pH and some metals. Once again, these findings confirmed the ideas regarding factors affecting distribution, although it is recognised that no one single parameter will independently be responsible. This aspect was shown to be true, after further sophisticated computer techniques of Cluster Analysis and Principal Component Analysis had been applied to the survey data. One single parameter does not directly dictate the presence or absence of Hydropsyche in the benthic community, as seen from Principal Component Analysis. A multitude of inter-related factors are involved, which leads to the gradation effect of the Cluster Analysis, Fig.4.3, rather than showing discrete groups . These extensive analyses are not considered to be very useful in this kind of ecological work.

Since completing this work, a number of papers, all presented at the Third International Symposium on Trichoptera have become available. Work by Kiss (1981), Ward (1981), Bueno-Soria et al.,(1981) and Tachet (1981) from areas as distant as Hungary, U.S.A., Mexico and France have all related in some way to Trichoptera distribution. Common factors linking all of these papers are the authors' observations on certain parameters affecting distribution. These are predominantly temperature, dissolved oxygen, food supply, velocity and substratum type. Essentially, therefore, this research has demonstrated quantitatively what has been assumed by many workers.

In the light of the species associations recorded and distribution in different water qualities, one may conclude that both R. dorsalis and Hydropsyche spp. are at the correct position in the Chandler Score and BMWP Score system, Where the two species are recognised to have different tolerance to organic pollution they are separately identified and receive a different score. The Trent Biotic Index considers all caseless caddis in the same group, which in view of these research findings is perhaps a little too generalised, but one recognises that it is

essentially a broad and simple biotic index which gives a reasonable indication of water quality.

It was shown from the major survey and the study of the R. Tean (Section 4.5) that organic pollution causes changes in the abundance and distribution of species of caseless caddis larvae and the associated benthic community. Differential tolerances of R. dorsalis and H. angustipennis have been demonstrated by the experimental work using three different concentrations of organically enriched effluent (Section 5.2). The relative LT 50 values were:-

H. angustipennis	River water	23 days
	25% effluent	17.5 "
	50% effluent	13.5 "
R. dorsalis	River water	20 days
	25% effluent	12.5 "
	50% effluent	8.0 "

This means that if field data are considered in context, presence/absence or change in abundance of these two species may be used as an "indicator" of pollutional status.

The role of Trichopteran larvae in assessing metal pollution has great potential. It was shown from field studies on the R. Ystwyth and R. Rheidol (Section 4.6) that Hydropsyche and Rhyacophila are extremely tolerant of heavy metals. Classically stoneflies are recognised to be very tolerant of heavy metals, which was confirmed in field sampling and metal analyses of tissues, but caseless caddis may be of even more practical use. Firstly, they are larger and easier to collect than stoneflies, providing more bulk of tissue for analyses in the laboratory. They are more robust than the Plecopterans for handling and transporting and more readily identified. In this context they are good "indicators". This work has shown, as Nehring et al. (1979) reported, that Trichopterans are useful as bioconcentrators and monitors of heavy metal in

the field situation, this has been confirmed by both field and laboratory investigations of zinc and copper. At low concentrations of heavy metal, the larvae of H. angustipennis actively bioconcentrate the metal as shown in Section 5.8. Potentially they would make excellent "in situ" biomonitors of heavy metal pollution. We know from the radioisotope studies that they rapidly bioconcentrate the metal, that they can eliminate most of it if transferred to a metal-free environment. The overall slight gain in metal, as shown in Fig.5.39 suggests that over their life cycle they may bioaccumulate it. It has been shown that earlier instars of H. angustipennis are more sensitive (cf. Zinc toxicity, Table 5.26) where relative LT50 values for final instar and third instar larvae were as follows:-

At 1,000 mgl^{-1} Zn the LT50 values for final and third instar were 8.5 and ? days respectively,

At 560 mgl^{-1} Zn, the LT50 values for final and third instar were 12.0 and 7.5 days respectively,

At 320 mgl^{-1} Zn, the LT50 values for final and third instar were 12.5 and 7.6 days respectively,

At 180 mgl^{-1} Zn, the LT50 values for final and third instar were 17.5 and 13.0 days respectively, and finally,

At 100 mgl^{-1} Zn, the LT50 values for final and third instar were 20.0 and 18.0 days respectively.

As field concentrations are usually much lower than these experimental concentrations, it follows that smaller instars of H. angustipennis may well be able to withstand the toxic effect of zinc in the field situation and may start to bioconcentrate the metal from the very early stages of development. More work is required to establish relative tolerances of different instars.

From experience gained in handling caseless caddis larvae, especially H. angustipennis, it has been demonstrated that they are

eminently suitable as a laboratory species to be used for toxicity testing. They have many of the attributes required for such a role. The field work showed that the abundance of caseless caddis larvae in the rivers, at differing water qualities means that there is a plentiful supply available for collection. They are easy to collect, transport, identify accurately and their characteristics lend themselves to those required of an organism to be used for laboratory work. As an indigenous species in our rivers, it seems more logical to base water quality criteria upon such results, rather than on those from Zebra fish and goldfish, or perhaps even from Daphnia, a lentic species used for assessing pollutional stress to a lotic environment. Within the laboratory it has been proven that H. angustipennis are easy to rear and maintain in large numbers, and thus are readily available for toxicity testing and experimentation (Section 5.1). Their ability to maintain normal activity under control conditions, i.e. net spinning, undulatory movements for respiration, means that as a test organism deaths did not occur in the control situation, thus not invalidating the test. However, in the test chambers, when the pollutant was under examination, the fact that death was easy to establish was another useful facet, making these larvae useful test species. Another aspect to consider is the differential tolerance of different instars of the same species. This is often important in assessing the potential harm a pollutant may cause. It is relatively easy, by measuring head capsule size to establish the instar of H. angustipennis.

From the ranges of toxic substances tested in this research, the larvae lent themsleves to all investigations, i.e. metals, copper and zinc, ammonia and pesticides.

Results of the 96 hr LC50 tests of H. angustipennis with copper sulphate are summarised in Table 5.19. The harder the water characteristic the more tolerant H. angustipennis, although the theoretical con-

centration of copper was the same, the toxic effect being modified by the complexing of copper and the decreasing amount of free copper ions in water of increasing hardness. Extrapolated values for 48hr LC50, as expected show increased copper concentrations. The value of using an ion specific electrode was demonstrated in this section of the work, to identify the exact concentration of Cu^{2+} ions in the system. To summarise, the following values for 95hr LC50, 48hr LC50 and the Cu^{++} concentrations for the copper toxicity tests at 19 ppm, 100 ppm and 200 ppm $CaCO_3$ were as follows:-

Water Hardness $CaCO_3$	96 hr LC50 mgl^{-1} Cu	48hr LC50 mgl^{-1} Cu	Cu^{2+} Conc^{n}. mgl^{-1} Cu
19 ppm	132	210	100
100 ppm	220	220	50
200 ppm	225	310	54

It was enlightening to demonstrate that H. angustipennis was so tolerant to copper but with the knowledge of the chemical activity of copper in natural waters, when assessing copper pollution in rivers other factors working simultaneously such as elevation of temperature, decrease in dissolved oxygen, presence of chelating agents, must all be considered when predicting the effect of an effluent upon the river. The extended experiments on zinc toxicity to H. angustipennis showed that zinc was tolerated far better than copper. At a water hardness of 19 ppm $CaCO_3$, the comparable LC50 value for zinc would be approximately 900 mgl^{-1}. The LT50 values already summarised in Table 5.26 show that in final instar larvae the effect of copper may be measured in hours, whereas the effect of zinc is measured in days. The relatively short length of time for the concentration of zinc to affect third as opposed to final instar larvae is to be expected when using an organism at an earlier stage of development.

Results from the isotope investigations (Section 5.8) substantiate the findings of the acute toxicity experiments. It was shown that more zinc is bioconcentrated in larvae immersed in soft water than those in hard water, Fig.5.38, thus supporting the fact that there is greater toxic effect in softer waters. From Fig.5.39 it may also be suggested that not only do H. angustipennis bioconcentrate metal from the environment, they may bioaccumulate it over their life cycle. Sources of metal in the diet of these larvae also contribute to their total uptake of metal, but as this is in the alimentary tract, it has not yet been established if they actually absorb metal from the food, through the gut and into the tissues. Certainly some of the metal taken in is excreted in the faeces.

Results of the acute toxicity of ammonia to H. angustipennis confirmed results by previous workers, Table 5.29. The larvae are extremely tolerant of unionised ammonia even in oxygen-poor environments. As such, they may not be such useful 'markers' or 'indicators' of heavily organically polluted sites. They are more tolerant of ammonia than fish, but further work is required to establish when other factors exert an effect on ammonia toxicity to fish species.

As aquatic macroinvertebrates appear to demonstrate a gradation of sensitivity to organic pollution, using a more sensitive species such as G. pulex may prove a more suitable indicator species for ammonia toxicity testing in the future.

One area of toxicity testing where the use of H. angustipennis is to be recommended is that of pesticide toxicity. The two algicides Diquat and Terbutryne had surprisingly different effects upon this insect larva. Diquat, even at recommended levels of application killed all larvae within 24 hrs. whereas none were killed with Terbutryne (see Table 5.30). The manufacturers of these products quote only a limited amount of toxicity data. There is enormous scope for further

investigation to be carried out here. In view of the acute sensitivity of H. angustipennis to one preparation, and extreme tolerance to another, it may prove a useful tool for accurately assessing levels of specific pesticides in a watercourse.

The effects of dissolved oxygen and temperature have been stressed throughout this work as important factors affecting distribution of Hydropsyche larvae in river systems. Respiratory results showed H. angustipennis to increase their rate of oxygen consumption as temperature increased. However, they are not strict "conformers" when oxygen tensions are compared with oxygen consumption, as between 5.0 and 6.0 mgl^{-1} O_2, there was always a rapid increase in oxygen consumption. It is proposed that this value does not represent the incipient limiting point for H. angustipennis, but rather a more genuine physiological response to an oxygen level at which increasing undulatory activity is required to maintain normal status. The incipient limiting point is anticipated to be below 2 mgl^{-1} O_2 but in field conditions this would mean certain death. At levels of 5 - 6 mgl^{-1}, although under stress, the larva could maintain itself for a short period of time, presumably it would seek an alternative position which was more favourable, or withstand the short period whilst the slug of effluent causing the deleterious conditions moved down river.

Investigations into the effect of ammonia and pH had no significant effect upon oxygen consumption and alone do not affect the survival of Hydropsyche. However, combination of factors such as dissolved oxygen, temperature, pH, food supply, still require further investigation to fully elucidate the integrated factors determining the distribution of caseless caddis larvae.

6.1 SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

The Hydropsychids and Rhyacophilids could be of use as indicators in river water quality monitoring for the following reasons:-

1. There is a relatively high proportion of these species in the macro-invertebrate benthic communities along the profile of rivers of differing water qualities.
2. They are relatively easy to locate, collect and are sufficiently robust to withstand transfer back to the laboratory with the minimum of attention.
3. Comprehensive taxonomic keys facilitate rapid and accurate identification of larval and adult forms.
4. Maintenance of large numbers under laboratory conditions has been shown to be feasible.
5. Their location in biotic indexes has been shown to be appropriate when taken in the context of the complete invertebrate assemblage found at that site.
6. Presence/absence or relative abundance of Hydropsychids and Rhyacophilids can reveal the water quality of the river. Experimental work at Checkley has proven their relative tolerances to organic pollution.
7. Regular monitoring at one site identifies changes in water quality.
8. Both groups are useful as indicators of heavy metal pollution. Interpretation of data should be made in the light of information regarding other physico-chemical parameters such as temperature, dissolved oxygen, water hardness and presence of chelating agents.
9. The toxicity of copper to Hydropsyche angustipennis is more acute than that of zinc. The effect of both toxicants can be altered by changes in water hardness.
10. The ability of the Hydropsychids to bioconcentrate metals in field and laboratory environments indicates that they are potentially

useful as "in situ" biomonitors of heavy metals.

11. Respirometric studies have shown temperature changes and oxygen tensions to affect oxygen consumption by H. angustipennis. A rapid increase in consumption at between 5-6 mgl^{-1} O_2 indicates a physiological stress to the animal. This may be an ecologically significant level, although higher than the incipient limiting point which is anticipated to be at 2 mgl^{-1} O_2.
These values may explain the distribution within river systems.
12. Further acute toxicity studies and respirometry investigations under different conditions are still required to extend the knowledge and increase the potential for using caseless caddis larvae in water quality monitoring.

APPENDICES

APPENDIX 1a

ASTON BIOLOGICAL DATA

(Numbers of individuals are from 3 x 0.05 m^2 cylinder samples)

(Biological scores calculated from one set of data only)

SITE → / FAUNA ↓	Hebden Bridge	Sowerby Bridge	R.Calder Clifton Beck	R. Calder Mirfield	R.Calder Huddersfield R.Colne	R. Calder Dewsbury	R. Calder Horbury	R. Dearne u/s Barnsley	R. Dearne d/s Barnsley	R. Dove Darfield
Platyhelminthes		3								
A. fluviatilis			1		10					
P. jenkinsi			1		1			1		
L. pereger				7	11			2		1
L. stagnalis										
Planorbis sp.										
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.								5		
Pisidium sp.										
Anodonta sp.										
Naididae	✓			✓	✓					
Tubificidae	20									
Lumbriculidae										
Oligochaeta		16	140	206	336			107		174
G. complanata		4	1		2					
E. octoculata	1	15	1	18				4		
E. testacea					3					
H. stagnalis		1								
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina	71	17	3							
Astacus sp.										
G. pulex	1		42	1						73
G. tigrinus										
C. pseudogracilis										
A. aquaticus		31	2	4	104			562		3
A. meridianus										
Baetis sp.	16	3	156		3					
Caenis sp.										
E. ignita	13		2							
E. danica										
E. venosus			1							
E. dispar										
E. torrentis										
R. semicolorata			1							
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.										
Nemoura sp.										
Leuctra sp.	13									
Perla sp.										
I. grammatica			1							
Chloroperla sp.										

SITE → / FAUNA ↓	Hebden Bridge	Sowerby Bridge	R.Calder Clifton Beck	R. Calder Mirfield	R.Calder Huddersfield R. Colne	R. Calder Dewsbury	R. Calder Horbury	R. Dearne u/s Barnsley	R.Dearne d/s Barnsley	R. Dove Darfield
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										
Gerridae										
Gyrinidae										
Haliplidae										
Elminthidae	4									
Dytiscidae								8		
Sialis lutaria										
Sialis fuluginosa										
H. angustipennis										
H. pellucidula										
H. contubernalis										
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.	2									
Rhyacophila dorsalis	1									
P. flavomaculatus	3									
P. conspersa										
Psychomyiidae										
Glossosomatidae										
Phryganidae										
Sericostomatidae	1									
Limnephilidae			1					1		
Goerinidae										
Hydroptilidae										
Molannidae										
Simulium sp.										
Dicranota sp.	2		6							
Pedicia sp.										
Tipula sp.										
Atherix sp.										
Chironomidae(Green)	55	6	36	72	16					3
C.riparius (Red)			3	3	3					
Ceratopogonidae										
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.	VIII	VI	IX	V	V			IV		IV
Diversity Index	2.17	2.78	2.48	1.72	1.59			0.99		1.22
Chandler Score	606	183	705	159	220			162		140
B.M.W.P.	63	16	78	18	22			18		17

SITE → / FAUNA ↓	R. Dearne Broomhill	R. Dearne H/s R. Don Mexborough	R. Don Kilnhurst	R. Don Sheffield	R. Loxley R. Don trib.	R. Drone R. Rother trib.	R. Whitting Whitting-ton	Blackburn Brook R. Don Trib.	Washburn	Dowles Brook. R. Severn trib.
Platyhelminthes										
A. fluviatilis										
P. jenkinsi	6									
L. pereger		8	1			3	3			
L. stagnalis										
Planorbis sp.										
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.										
Pisidium sp.										
Anodonta sp.										
Naididae										
Tubificidae										
Lumbriculidae										8
Oligochaeta	538	51	1194			192	6	1064		
G. complanata	4									
E. octoculata						1				
E. testacea							4			
H. stagnalis										
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina										
Astacus sp.										
G. pulex	80									
G. tigrinus										263
C. pseudogracilis										
A. aquaticus	13	291	42			29	141	1		
A. meridianus										
Baetis sp.										
Caenis sp.										5
E. ignita										
E. danica										7
E. venosus										5
E. dispar										
E. torrentis										
R. semicolorata										
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.										
Nemoura sp.										
Leuctra sp.										
Perla sp.										
I. grammatica										
Chloroperla sp.										3

SITE → / FAUNA ↓	R. Dearne Broomhill	R. Dearne u/s R. Don Mexborough	R. Don Kilnhurst	R. Don Sheffield	R. Loxley R. Don trib.	R. Drone R. Rother trib.	R. Whitting Whitting-ton	Blackburn Brook R. Don trib	Washburn	Dowles Brk. R. Severn trib.
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										
Gerridae										
Gyrinidae										
Haliplidae										
Elminthidae										
Dytiscidae										
Sialis lutaria										
Sialis fuluginosa										
H. angustipennis										
H. pellucidula										1
H. contubernalis										1
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.										
Rhyacophila dorsalis										
P. flavomaculatus										1
P. conspersa										1
Psychomyiidae										
Glossosomatidae										
Phryganidae										5
Sericostomatidae										
Limnephilidae										
Goerinidae										4
Hydroptilidae										
Molannidae										
Simulium sp.										6
Dicranota sp.										
Pedicia sp.										
Tipula sp.										
Atherix sp.										
Chironomidae(Green)	12	34	11				6	9		1
C.riparius (Red)										
Ceratopogonidae										
Limnophora sp.										
Hemerodroma sp.										6
Other diptera										
T.B.I.	V	III	III			III	III	II		VIII
Diversity Index	0.99	0.82	0.41			1.54	0.76			1.37
Chandler Score	143	72	52			97	101	38		626
B.M.W.P.	18	9	6			10	1	3		62

SITE → / FAUNA ↓	R. Stour Dog-kennel Lane	R. Stour d/s Coombes-wood Brook	R. Devon Hawton	R. Poulter Elkesley	R. Maun Mansfield	R. Maun Clip-stone	R. Maun Edwin-stowe	Rainworth water Rainworth	Rainworth water Ollerton	Erewash Pinxton
Platyhelminthes				2					2	
A. fluviatilis	1									
P. jenkinsi	7		13	49						
L. pereger	22				1			1		
L. stagnalis		1								
Planorbis sp.			1							
Valvata sp.			22							
Physa sp.										
Theodoxus sp.										
Bythinia sp.									7	
Zonitoides sp.										
Sphaerium sp.			48	7						
Pisidium sp.			15							
Anodonta sp.										
Naididae										
Tubificidae	5	237	50	108	26		4	10	39	2112
Lumbriculidae	300									
Oligochaeta		4						4		
G. complanata			2							
E. octoculata			2	5	20					
E. testacea										
H. stagnalis			4	4						
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina	1			14						
Astacus sp.										
G. pulex	6		1					14		
G. tigrinus										
C. pseudogracilis										
A. aquaticus		72	188	375	250		3		76	1
A. meridianus										
Baetis sp.	2	2	5					1		
Caenis sp.			42	2						
E. ignita										
E. danica										
E. venosus										
E. dispar										
E. torrentis										
R. semicolorata										
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.										
Nemoura sp.										
Leuctra sp.										
Perla sp.										
I. grammatica										
Chloroperla sp.			1							

SITE → / FAUNA ↓	R.Stour Dog-kennel Lane	R.Stour d/s Coombes-wood Brook	R. Devon Hawton	R. Poulter Elkesley	R. Maun Mansfield	R. Maun Clip-stone	R. Maun Edwin-stowe	Rainworth water Rainworth	Rainworth water Ollerton	Erewash Pinxton
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae			14	42						
Gerridae										
Gyrinidae										
Haliplidae			14	6						
Elminthidae			1	3						
Dytiscidae			15	14					4	
Sialis lutaria			17	2						
Sialis fuluginosa										
H. angustipennis										
H. pellucidula										
H. contubernalis										
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.										
Rhyacophila dorsalis										
P. flavomaculatus			19							
P. conspersa										
Psychomyiidae										
Glossosomatidae										
Phryganidae										
Sericostomatidae				15						
Limnephilidae			1	5				1		
Goerinidae										
Hydroptilidae										
Molannidae										
Simulium sp.										
Dicranota sp.								1		
Pedicia sp.										
Tipula sp.										
Atherix sp.									3	
Chironomidae(Green)	2	95	11	92	15		9	19	120	44
C.riparius (Red)	1			2						
Ceratopogonidae			2		3			3		
Limnophora sp.				6						
Hemerodroma sp.										
Other diptera										
T.B.I.	IV	IV	VII	IV	IV		III	V	V	III
Diversity Index	0.29	1.44	2.98	2.30	1.21		1.42	2.24	1.82	0.15
Chandler Score	121	95	707	243	107		65	202	227	55
B.M.W.P.	13	10	62	30	12		6	23	19	6

SITE → / FAUNA ↓	Bagthorpe Brk. Ere-wash trib.	Beauvale Brk. Nether Green	R. Churnet Upper Hul-me	R. Churnet Bridgend	R. Churnet Abbey Gn. Road	R. Churnet d/s Wardles Works	R. Churnet Cheddleton Station	R. Churnet Consall	R. Churnet Froghall	R. Churnet Oakamoor
Platyhelminthes	1000								3	
A. fluviatilis			3	4	3		1			
P. jenkinsi		41								
L. pereger		20		14		3				
L. stagnalis										
Planorbis sp.										
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.								4		
Pisidium sp.				4				2		
Anodonta sp.										
Naididae										
Tubificidae	8	65	15	46	32	8	222	8	901	
Lumbriculidae										
Oligochaeta										
G. complanata				4		4	18	9	1	
E. octoculata	4					8	28			2
E. testacea										
H. stagnalis			1							
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina				14				2		
Astacus sp.										
G. pulex	115	2816	1	18			2			
G. tigrinus	1									
C. pseudogracilis										
A. aquaticus						50	82	23		
A. meridianus										
Baetis sp.			35	5	11	2	71	31	31	26
Caenis sp.										
E. ignita			2				1			
E. danica										
E. venosus				1			2		5	5
E. dispar										3
E. torrentis										
R. semicolorata					1		17	3	36	4
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.			5							
B. risi										
Protonemura sp.							1		2	
Amphinemura sp.			2	2				5	2	
Nemoura sp.				1						
Leuctra sp.			7	1				2	1	
Perla sp.										
I. grammatica			27		6			10		4
Chloroperla sp.			58							

SITE → / FAUNA ↓	Bagthorpe Brk. Ere-wash trib.	Beauvale Brk. Nether Green	R. Churnet Upper Hul-me	R. Churnet Bridgend	R. Churnet Abbey Gn. Rd.	R. Churnet d/s Wardles Works	R. Churnet Cheddleton Station	R. Churnet Consall	R. Churnet Froghall	R. Churnet Oakamoor
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										
Gerridae										
Gyrinidae										
Haliplidae									1	
Elminthidae			6					1	24	
Dytiscidae	17				1					
Sialis lutaria										
Sialis fuluginosa										
H. angustipennis			2	1				3		1
H. pellucidula									2	3
H. contubernalis										
H. siltalai								1		
H. instabilis								2		
H. fluvipes										
Hydropsyche spp.										
Rhyacophila dorsalis			3			1	1		7	9
P. flavomaculatus			2	1						
P. conspersa			1		1					
Psychomyiidae										
Glossosomatidae										
Phryganidae										
Sericostomatidae								2		
Limnephilidae							1	2		1
Goerinidae				6						
Hydroptilidae										
Molannidae										
Simulium sp.			2	39		7				
Dicranota sp.			12	2			7			
Pedicia sp.						1				
Tipula sp.										
Atherix sp.										
Chironomidae (Green)	3	22	7	60	4	35	44	25	179	845
C. riparius (Red)	7									
Ceratopogonidae										
Limnophora sp.									5	
Hemerodroma sp.										
Other diptera										
T.B.I.	IV	IV	X	VIII		IV	VI	IX	VII	VI
Diversity Index	1.27	0.054	3.31	2.89	2.47	2.42	2.62	3.27	1.2	0.37
Chandler Score	166	88	991	553		232	391	651	495	272
B.M.W.P.	17	7	103	53		24	39	63	54	31

SITE → / FAUNA ↓	R. Churnet Alton	R. Tean Checkley -bank	R. Tean Beamhurst	R. Blythe Cheswick Green	R. Blythe Henwood Mill	R. Blythe Temple Balsall	R. Blythe u/s East-cote Brook	R. Blythe Stone-bridge	Langley Brk. u/s Middle-ton Works	R. Bourne Over Whitacre
Platyhelminthes							9			
A. fluviatilis		3	16	6	20	6		23	1	
P. jenkinsi		6	3	29	148	1			16	
L. pereger		2		191	2					2
L. stagnalis										
Planorbis sp.				5			1			
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.					1					
Sphaerium sp.				10	138	2		7		4
Pisidium sp.				4	41	4				
Anodonta sp.										
Naididae		✓	✓							
Tubificidae	19	53	71	77	101	11	34	61	4	
Lumbriculidae						1				
Oligochaeta										16
G. complanata			2	2		3		2		
E. octoculata	4		9			5		7		
E. testacea				1						
H. stagnalis										
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina		4	2						5	2
Astacus sp.									1	
G. pulex		114	140			17	141	115	9	12
G. tigrinus										
C. pseudogracilis										
A. aquaticus			66		10		72	7	2	
A. meridianus										
Baetis sp.	10	59	3	4		255	16	15	11	2
Caenis sp.										
E. ignita		165	2		25	14	7	14	25	
E. danica						1		3	1	
E. venosus	2	3								
E. dispar										
E. torrentis										
R. semicolorata	4									
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.						1				
Nemoura sp.										
Leuctra sp.										
Perla sp.	7									
I. grammatica										
Chloroperla sp.										

SITE → / FAUNA ↓	R. Churnet Alton	R. Tean Checkley-bank	R. Tean Beamhurst	R. Blythe Cheswick Green	R. Blythe Henwood Mill	R. Blythe Temple Balsall	R. Blythe u/s East-cote Brook	R. Blythe Stone-bridge	Langley Brk. u/s Middle-ton Works	R. Bourne Over Whitacre
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										
Gerridae										
Gyrinidae										
Haliplidae				1					2	
Elminthidae		1				4		3		1
Dytiscidae										
Sialis lutaria										
Sialis fuluginosa										
H. angustipennis	3					22			2	8
H. pellucidula	9					12				
H. contubernalis										
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.										
Rhyacophila dorsalis	1					2				1
P. flavomaculatus										
P. conspersa										
Psychomyiidae										
Glossosomatidae								2		
Phryganidae						4		1		
Sericostomatidae										
Limnephilidae										
Goerinidae										2
Hydroptilidae		38							5	4
Molannidae										
Simulium sp.		4	7		1	4	8	2	19	
Dicranota sp.		25					8			
Pedicia sp.										
Tipula sp.	1									
Atherix sp.	4									
Chironomidae(Green)	5	1	42	116	51	796	25	11	10	24
C.riparius (Red)		1	4	3					3	
Ceratopogonidae					1	9		4	1	
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.	VI	VII	V	VI	VI	IX	VII	VII	VI	VI
Diversity Index	2.96	2.72	1.7	2.05	2.06	1.06	2.18	2.63	2.55	2.89
Chandler Score	334	769	125	308	341	755	314	489	386	505
B.M.W.P.	32	59	15	30		34	36	56	37	49

SITE → / FAUNA ↓	R. Bourne d/s Fillongley	R. Bourne Daw Mill Bridge	R. Anker Atherstone	R. Anker Witherley	R. Anker Leathermill Bridge	R. Tame u/s Blue Billy Tip	R. Tame West Bromwich	R. Tame Gt. Bridge	R. Tame Bescot	Ford Brook Clayhanger
Platyhelminthes										
A. fluviatilis		1			8					
P. jenkinsi		1								
L. pereger			6		6					
L. stagnalis										
Planorbis sp.										
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.					3					
Pisidium sp.					1					
Anodonta sp.										
Naididae										
Tubificidae		80	1704	1820	6228	150				102
Lumbriculidae										
Oligochaeta			2							
G. complanata										
E. octoculata			23		7					
E. testacea		1								
H. stagnalis										
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina										
Astacus sp.										
G. pulex	502	76			1					
G. tigrinus										
C. pseudogracilis										
A. aquaticus			927	631	103					
A. meridianus										
Baetis sp.	12	90								
Caenis sp.										
E. ignita	9	2								
E. danica										
E. venosus										
E. dispar										
E. torrentis										
R. semicolorata										
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.										
Nemoura sp.										
Leuctra sp.										
Perla sp.										
I. grammatica										
Chloroperla sp.										

SITE → / FAUNA ↓	R. Bourne d/s Fillongley	R. Bourne Daw Mill Bridge	R. Anker Atherstone	R. Anker Witherley	R. Anker Leathermill Bridge	R. Tame u/s Blue Billy Tip	R. Tame West Bromwich	R. Tame Gt. Bridge	R. Tame Bescot	Ford Brook Clay-hanger
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										
Gerridae										
Gyrinidae										
Haliplidae		1								
Elminthidae		6								
Dytiscidae	2									
Sialis lutaria										
Sialis fuluginosa										
H. angustipennis										
H. pellucidula										
H. contubernalis										
H. siltalai										
H. instabilis		3								
H. fluvipes										
Hydropsyche spp.										
Rhyacophila dorsalis	1	6								
P. flavomaculatus										
P. conspersa										
Psychomyiidae										
Glossosomatidae										
Phryganidae										
Sericostomatidae										
Limnephilidae		2		1						
Goerinidae										
Hydroptilidae										
Molannidae										
Simulium sp.	2	1								
Dicranota sp.										
Pedicia sp.										
Tipula sp.										
Atherix sp.		1								
Chironomidae(Green)	172	133	66	732	1115					
C.riparius (Red)										
Ceratopogonidae										
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.	IV	VI	III	III	IV	I				I
Diversity Index	1.12	2.05	1.16	1.32	1.42	0				0
Chandler Score	256	501	87	37	126	9				9
B.M.W.P.	29	53	12	6	15	1				1

SITE → / FAUNA ↓	R. Ouse Fulwell Bridge	Trib. R. Tove Preston Capes	R. Ouse Passenham	R. Tove Bozenham Mill	R. Tove Cappenham Bridge	Ascott Brook B448 Rd. Br.	Clipstone Brook Hockliffe	R. Ouse Milton Ernest	Claydon Brook Winslow	Claydon Brook Padbury
Platyhelminthes										
A. fluviatilis								2		
P. jenkinsi				12	1		3		61	274
L. pereger								2		
L. stagnalis				1	1		1		1	1
Planorbis sp.										
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.										1
Pisidium sp.										
Anodonta sp.								9		
Naididae								✓		
Tubificidae									3	1
Lumbriculidae								20		10
Oligochaeta				56	411		16	18		
G. complanata				1			4	7		
E. octoculata				4	2				2	1
E. testacea										
H. stagnalis										
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina								14		
Astacus sp.										
G. pulex	2			5	2		17	39		
G. tigrinus										
C. pseudogracilis										
A. aquaticus				2				4	2	3
A. meridianus								4		
Baetis sp.				9					3	
Caenis sp.				1			1			3
E. ignita								46		
E. danica										
E. venosus										
E. dispar										
E. torrentis										
R. semicolorata										
Heptagenia sp.										
Leptophlebia sp.										2
Paraleptophlebia sp.										1
B. risi										
Protonemura sp.										
Amphinemura sp.										
Nemoura sp.										
Leuctra sp.										
Perla sp.										
I. grammatica										
Chloroperla sp.										

SITE → / FAUNA ↓	R.Ouse Fulwell Bridge	Trib. R. Tove. Preston Capes	R. Ouse Passenham	R. Tove Bozenham Mill	R. Tove Cappenham Bridge	Ascott Brook B448 Rd. Bridge	Clipstone Brook Hocklliffe	R. Ouse Milton Ernest	Claydon Brook Winslow	Claydon Brook Padbury
Other Plecoptera										
Plecoptera										
Zygoptera							1			
Notonectidae										
Aphelocheiridae				3						
Corixidae										
Gerridae										
Gyrinidae				8						
Haliplidae				3				7		
Elminthidae							4	5	1	
Dytiscidae								1		
Sialis lutaria										2
Sialis fuluginosa										
H. angustipennis				11	2			3		2
H. pellucidula								11		
H. contubernalis								2		
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.										
Rhyacophila dorsalis									1	
P. flavomaculatus										2
P. conspersa										
Psychomyiidae										
Glossosomatidae										
Phryganidae										
Sericostomatidae										
Limnephilidae				6				3		
Goerinidae										
Hydroptilidae					1			1		
Molannidae								1		
Simulium sp.				1						
Dicranota sp.										
Pedicia sp.										
Tipula sp.										
Atherix sp.										
Chironomidae (Green)	2			103	7			21	132	8
C.riparius (Red)				2	79				2	2
Ceratopogonidae										
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.	IV			VII	V		IV	VII	IV	VII
Diversity Index	0.92			2.24	1.33		1.70	3.45	1.42	0.91
Chandler Score	68			546	182		162	742	151	328
B.M.W.P.	3			54	23		18	67	16	38

SITE → / FAUNA ↓	Claydon Brook. Road Rail Bridge	R. Ouzel Stanbridge Ford	R. Ouse Olney	R. Ouse Ravenstone Mill	R. Ouzel Caldecote Mill	R. Ouzel Willen Rd. Bridge	R. Ouzel Simpson Rd. Bridge	Warm Brook u/s Chapel-en-le-Frith	Warm Brook d/s Chapel-en-le-Frith	Roych Brook u/s Black Brook
Platyhelminthes								23		
A. fluviatilis			1							
P. jenkinsi		2	2	1	3	2	145	17	5	3
L. pereger			2			1	1		1	4
L. stagnalis										
Planorbis sp.							5			
Valvata sp.			5			2				
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.		1	17			2	14			
Pisidium sp.					1		6			
Anodonta sp.										
Naididae				✓						
Tubificidae				2				38	858	167
Lumbriculidae				38				4		
Oligochaeta		19	27		1050	87	13	3		
G. complanata		2		1	3	2		1	1	1
E. octoculata			6	1		1	11		4	2
E. testacea			1							
H. stagnalis		1								
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina		4								
Astacus sp.										
G. pulex		20	4	2			1	1		
G. tigrinus										
C. pseudogracilis										
A. aquaticus		5	4	5		15	2			10
A. meridianus		1								
Baetis sp.								58	2	
Caenis sp.				4	1	4		1		
E. ignita								6		
E. danica										
E. venosus				1						
E. dispar										1
E. torrentis										
R. semicolorata								3		
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.								9		
Nemoura sp.										
Leuctra sp.								65		
Perla sp.										
I. grammatica										
Chloroperla sp.										

SITE → / FAUNA ↓	Claydon Brook. Road/ Rail Bridge	R. Ouzel Stanbridge Ford	R. Ouse Olney	R. Ouse Ravenstone Mill	R. Ouzel Caldecote Mill	R. Ouzel Willen Rd. Bridge	R. Ouzel Simpson Rd. Bridge	Warm Brook u/s Chapel-en-le-Frith	Warm Brook d/s Chapel-en-le-Frith	Roych Brook u/s Black Brook
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae			1							
Corixidae		2								
Gerridae										
Gyrinidae			3							
Haliplidae						1				
Elminthidae			2					3		
Dytiscidae		1	1			1				
Sialis lutaria	1									
Sialis fuluginosa	3									
H. angustipennis			11		1		2			
H. pellucidula										
H. contubernalis										
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.		2			4	2				
Rhyacophila dorsalis								14	1	
P. flavomaculatus			7							
P. conspersa										
Psychomyiidae										
Glossosomatidae										
Phryganidae										
Sericostomatidae										
Limnephilidae			1					1		
Goerinidae								8		
Hydroptilidae			2		1			151		
Molannidae										
Simulium sp.										
Dicranota sp.								1		
Pedicia sp.								23		1
Tipula sp.										
Atherix sp.										
Chironomidae(Green)	10	40	16	4	4	16	140	39	364	108
C.riparius (Red)										
Ceratopogonidae		2	1							
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.		VI	VIII	VI	IV	VII	V	VIII	II	IV
Diversity Index	1.09	2.66	3.67	1.78	0.063	1.93	1.53	3.06	1.05	1.28
Chandler Score		355	555	150	101	445	203	801	80	205
B.M.W.P.		40	77	17	13	46	23	62	16	18

SITE → / FAUNA ↓	R. Sett Hayfield	R. Sett u/s R. Goyt	R. Bollin Langley	R. Bollin Jarmin	R. Bollin Beech Bridge	Harrop Brk. u/s Bollington	R. Croco u/s R. Dove	R. Goyt Otterspool Bridge	R. Goyt Iron Bridge Compstall	R. Bollin Mobberley Brook
Platyhelminthes			6							
A. fluviatilis			1	1				1	1	
P. jenkinsi						2				
L. pereger			4							
L. stagnalis										
Planorbis sp.										
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.										
Pisidium sp.										
Anodonta sp.										
Naididae										
Tubificidae	12	53		49	344		232	444	89	1074
Lumbriculidae						1				3
Oligochaeta			32							
G. complanata										
E. octoculata								1		4
E. testacea										
H. stagnalis										
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina										
Astacus sp.										
G. pulex				1	2	6				
G. tigrinus					2					
C. pseudogracilis										
A. aquaticus	1					2	1	13	29	6
A. meridianus										
Baetis sp.	47	29	10	14		57		15	31	
Caenis sp.		8	4							
E. ignita	25	14		29		160				2
E. danica										
E. venosus				1		12				
E. dispar		1								
E. torrentis										
R. semicolorata	3		5					2	3	
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.	2							2		
Nemoura sp.			2							
Leuctra sp.	26	9				2				
Perla sp.										
I. grammatica	18		2	3		1		1		
Chloroperla sp.	3									

SITE → / FAUNA ↓	R.Sett Hayfield	R.Sett u/s R. Goyt	R. Bollin Langley	R. Bollin Jarmin	R. Bollin Beech Bridge	Harrop Brk. u/s Bollington	R. Croco u/s R. Dove	R.Goyt Otterspool Bridge	R.Goyt Iron Bridge, Compstall	R.Bollin Mobberley Brook
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										
Gerridae										
Gyrinidae										
Haliplidae										
Elminthidae		6	1	2						
Dytiscidae			1							
Sialis lutaria										
Sialis fuluginosa										
H. angustipennis										
H. pellucidula									1	
H. contubernalis									2	
H. siltalai	1							7		
H. instabilis								1		
H. fluvipes										
Hydropsyche spp.			4					19		
Rhyacophila dorsalis	4			1						
P. flavomaculatus						3				
P. conspersa										
Psychomyiidae										
Glossosomatidae										
Phryganidae										
Sericostomatidae										
Limnephilidae	1			1						2
Goerinidae										
Hydroptilidae				9						
Molannidae										
Simulium sp.			2			143		366	129	244
Dicranota sp.	11	5		4		3				2
Pedicia sp.										
Tipula sp.										
Atherix sp.										
Chironomidae(Green)		32	18	123	104	36	3		74	44
C.riparius (Red)										
Ceratopogonidae							1			
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
P. irroratus		1								
T.B.I.	VIII	VI	IX	VII	II	VII	II			
Diversity Index	2.77	2.64	2.99	2.46	0.64	2.62	0.28	1.17	2.14	1.07
Chandler Score	534	420	699	544	33	636	65			
B.M.W.P.	43	37	65	55	3	60	6			

SITE → / FAUNA ↓	R. Dane Biddulph Brk.d/sETW										
Platyhelminthes											
A. fluviatilis											
P. jenkinsi											
L. pereger											
L. stagnalis											
Planorbis sp.											
Valvata sp.											
Physa sp.											
Theodoxus sp.											
Bythinia sp.											
Zonitoides sp.											
Sphaerium sp.											
Pisidium sp.											
Anodonta sp.											
Naididae											
Tubificidae	335										
Lumbriculidae											
Oligochaeta											
G. complanata											
E. octoculata											
E. testacea											
H. stagnalis											
P. geometrica											
T. tessulatum											
H. sanguisuga											
Hydracarina											
Astacus sp.											
G. pulex											
G. tigrinus											
C. pseudogracilis											
A. aquaticus	73										
A. meridianus											
Baetis sp.											
Caenis sp.	1										
E. ignita											
E. danica											
E. venosus											
E. dispar											
E. torrentis											
R. semicolorata											
Heptagenia sp.											
Leptophlebia sp.											
Paraleptophlebia sp.											
B. risi											
Protonemura sp.											
Amphinemura sp.											
Nemoura sp.											
Leuctra sp.											
Perla sp.											
I. grammatica											
Chloroperla sp.											

SITE → / FAUNA ↓	R. Dane Biddulph Brook d/s ETW										
Other Plecoptera											
Plecoptera											
Zygoptera											
Notonectidae											
Aphelocheiridae											
Corixidae											
Gerridae											
Gyrinidae											
Haliplidae											
Elminthidae											
Dytiscidae											
Sialis lutaria											
Sialis fuluginosa											
H. angustipennis											
H. pellucidula											
H. contubernalis											
H. siltalai											
H. instabilis											
H. fluvipes											
Hydropsyche spp.											
Rhyacophila dorsalis											
P. flavomaculatus											
P. conspersa											
Psychomyiidae											
Glossosomatidae											
Phryganidae											
Sericostomatidae											
Limnephilidae											
Goerinidae											
Hydroptilidae											
Molannidae											
Simulium sp.	117										
Dicranota sp.											
Pedicia sp.											
Tipula sp.											
Atherix sp.											
Chironomidae (Green)	124										
C. riparius (Red)											
Ceratopogonidae											
Limnophora sp.											
Hemerodroma sp.											
Other diptera											
T.B.I.	III										
Diversity Index	1.77										
Chandler Score	114										
B.M.W.P.	11										

APPENDIX 1b

WATER AUTHORITY BIOLOGICAL DATA

SITE → / FAUNA ↓	Hebden Bridge	Sowerby Bridge	Clifton Beck	R. Calder Mirfield	R. Colne Hudders-field	R. Calder Dewsbury	R. Calder Harbury	R. Dearne u/s Barnsley	R. Dearne d/s Barnsley	R. Dove Darfield
Platyhelminthes	1	2								
A. fluviatilis	56					1				
P. jenkinsi			15						1	3
L. pereger	1	1			6			7		6
L. stagnalis								18		
Planorbis sp.										
Valvata sp.					3					
Physa sp.				1	1	23	48			
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.					1			2	3	
Pisidium sp.										
Anodonta sp.										
Naididae										
Tubificidae										
Lumbriculidae										
Oligochaeta	208	46	43	351	33	1508	1650	9	274	8
G. complanata	13	10		6				3		6
E. octoculata	18		24	7	71	1		7	1	3
E. testacea										
H. stagnalis		1								2
P. geometrica					1					
T. tessulatum										
H. sanguisuga										
Hydracarina	2									
Astacus sp.										
G. pulex		1	9					8		360
G. tigrinus										
C. pseudogracilis										
A. aquaticus	74	55	4	277	8	5	78	328	864	88
A. meridianus										
Baetis sp.	1	52	404							640
Caenis sp.										
E. ignita	21									
E. danica										
E. venosus										
E. dispar										
E. torrentis										
R. semicolorata										
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.	1									
Amphinemura sp.										
Nemoura sp.										
Leuctra sp.										
Perla sp.										
I. grammatica										
Chloroperla sp.										

SITE → / FAUNA ↓	Hebden Bridge	Sowerby Bridge	Clifton Beck	R. Calder Mirfield	R. Colne Hudders-field	R. Calder Dewsbury	R. Calder Harbury	R. Dearne u/s Barnsley	R. Dearne d/s Barnsley	R. Dove Darfield
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										1
Gerridae										
Gyrinidae										
Haliplidae								5		
Elminthidae	2									
Dytiscidae								2		2
Sialis lutaria										1
Sialis fuluginosa										
H. angustipennis										
H. pellucidula										
H. contubernalis										
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.	1									54
Rhyacophila dorsalis	2									
P. flavomaculatus										
P. conspersa										
Psychomyiidae										
Glossosomatidae										
Phryganidae								64		
Sericostomatidae										
Limnephilidae										
Goerinidae										
Hydroptilidae										2
Molannidae										
Simulium sp.	1								373	2020
Dicranota sp.										
Pedicia sp.										
Tipula sp.	2									
Atherix sp.										
Chironomidae(Green)	34	76	5	95	22	151	544	21		1233
C.riparius (Red)										
Ceratopogonidae										
Limnophora sp.								3		13
Hemerodroma sp.										
Other diptera										
T.B.I.										
Diversity Index										
Chandler Score										
B.M.W.P.										

SITE → / FAUNA ↓	R. Dearne Broomhill	R. Don Kilnhurst	R. Loxley	R. Drone	R. Don Oughtibridge	Ewden Beck	R. Seven	R. Wharfe Tadcaster	R. Wharfe Boston Spa	R. Wharfe Harewood
Platyhelminthes					3	1				
A. fluviatilis	36				68	10	6		12	7
P. jenkinsi	9		2	6	2	7		20	64	
L. pereger	4	111		30		2			1	3
L. stagnalis										
Planorbis sp.					3					1
Valvata sp.										
Physa sp.		123								
Theodoxus sp.									26	
Bythinia sp.								11		1
Zonitoides sp.										
Sphaerium sp.	12		6	1			15	147	290	6
Pisidium sp.										
Anodonta sp.										
Naididae										
Tubificidae										
Lumbriculidae										
Oligochaeta	354	345	60	30		24	112	500	40	4
G. complanata	1				14			1		1
E. octoculata	4			3	2	1	32	4	13	4
E. testacea										
H. stagnalis		2								
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina										
Astacus sp.								21	7	
G. pulex	1902		80			6	3		13	6
G. tigrinus										
C. pseudogracilis										
A. aquaticus	2	90	2		681			3	1	1
A. meridianus					23					
Baetis sp.			36	112	34	56	192	16	29	62
Caenis sp.								20	14	
E. ignita			64				136	97	480	
E. danica										
E. venosus							16	6		
E. dispar										
E. torrentis										
R. semicolorata										
Heptagenia sp.									72	
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.						8				
Nemoura sp.										
Leuctra sp.			5			1	24	17	37	
Perla sp.										11
I. grammatica						2				
Chloroperla sp.						1				

SITE → / FAUNA ↓	R. Dearne Broomhill	R. Don Kilnhurst	R. Loxley	R. Dene	R. Don Oughtibridge	Ewden Beck	R. Seven	R. Wharfe Tadcaster	R. Wharfe Boston Spa	R. Wharfe Harewood
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										
Gerridae										
Gyrinidae										
Haliplidae										
Elminthidae							40		43	9
Dytiscidae										
Sialis lutaria			3							
Sialis fuluginosa										
H. angustipennis										
H. pellucidula										
H. contubernalis										
H. siltalai						24				
H. instabilis										
H. fluvipes										
Hydropsyche spp.	4					6	64	2	58	16
Rhyacophila dorsalis			4				24	2	17	12
P. flavomaculatus			5							
P. conspersa										
Psychomyiidae									2	
Glossosomatidae									39	2
Phryganidae										
Sericostomatidae			1				8			
Limnephilidae			1					1		
Goerinidae										
Hydroptilidae	1			1				5	11	1
Molannidae										
Simulium sp.	8		4	4			68	19	267	
Dicranota sp.			2			1	17			
Pedicia sp.										
Tipula sp.								3	1	
Atherix sp.				1			5			
Chironomidae(Green)	11	30	124	444	237	13	3168	125	93	12
C.riparius (Red)										
Ceratopogonidae			1				3		1	4
Limnophora sp.										
Hemerodroma sp.			2							
Other diptera										
T.B.I.										
Diversity Index										
Chandler Score										
B.M.W.P.										

SITE → / FAUNA ↓	R. Wharfe Bolton Bridge	R. Wharfe Burnsall	R. Devon Hawton	R. Poulter Elkesley	R. Maun Clipstone	R. Maun Edwinstowe	Rainworth Water Ollerton	R. Erewash Pinxton	Bagthorpe Brook	Beauvale Brook
Platyhelminthes			6							
A. fluviatilis	1	5							2	16
P. jenkinsi	12		19					5	5	5
L. pereger		1		3				4	3	
L. stagnalis										
Planorbis sp.			17							
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.							15			
Zonitoides sp.										
Sphaerium sp.	3	3	36					1		
Pisidium sp.			20	9					1	
Anodonta sp.										
Naididae										
Tubificidae			16	36	20	180	18	61	48	33
Lumbriculidae										
Oligochaeta	17	92								
G. complanata			8							1
E. octoculata		1	4						1	
E. testacea						58				
H. stagnalis	1									
P. geometrica			1							
T. tessulatum										
H. sanguisuga										
Hydracarina	25	9	39	31				4	38	
Astacus sp.										
G. pulex	2	1		3					127	812
G. tigrinus										
C. pseudogracilis										
A. aquaticus				650	3	700	17	126	40	
A. meridianus			43							
Baetis sp.	104	412								
Caenis sp.	25	2	22							
E. ignita	67									
E. danica			5							
E. venosus										
E. dispar	13									
E. torrentis										
R. semicolorata	27	46								
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.		41								
Nemoura sp.										
Leuctra sp.	47	10								
Perla sp.		4								
I. grammatica	1	36								
Chloroperla sp.		1								

SITE → / FAUNA ↓	R. Wharfe Bolton Bridge	R. Wharfe Burnsall	R. Devon Hawton	R. Poulter Elkesley	R. Maun Clipstone	R. Maun Edwinstowe	Rainworth Water Ollerton	R. Erewash Pinxton	Bagthorpe Brook	Beauvale Brook
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae			16	47						
Gerridae										
Gyrinidae		2								
Haliplidae				15			5		14	
Elminthidae		36	3						4	
Dytiscidae				2			6	43	2	
Sialis lutaria			11	19						
Sialis fuluginosa										
H. angustipennis									1	
H. pellucidula										
H. contubernalis										
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.	5	38					29			
Rhyacophila dorsalis	5	23	74							
P. flavomaculatus	1	1								
P. conspersa									3	
Psychomyiidae	24									
Glossosomatidae	16	25								
Phryganidae										
Sericostomatidae	3								14	
Limnephilidae										
Goerinidae									6	
Hydroptilidae	9	3								
Molannidae										
Simulium sp.	10	26			96					
Dicranota sp.	4	4		2		89				
Pedicia sp.										
Tipula sp.		1								
Atherix sp.		5								
Chironomidae(Green)	68	230	41	59	91	1800	75	36	55	16
C.riparius (Red)				4			5		1	
Ceratopogonidae	5	2								
Limnophora sp.				6						
Hemerodroma sp.										
Other diptera										
T.B.I.										
Diversity Index										
Chandler Score										
B.M.W.P.										

SITE → / FAUNA ↓	R. Churnet Upper Hulme	R. Churnet Bridgend	R. Churnet Abbey Green Rd	R. Churnet d/s Wardles	R. Churnet Cheddleton Station	R. Churnet Consall	R. Churnet Froghall	R. Churnet Oakamoor	R. Churnet Alton	R. Blythe Cheswick Green
Platyhelminthes							2	4	1	
A. fluviatilis		2	5		10		2	16		1
P. jenkinsi								4		14
L. pereger			15				1			3
L. stagnalis										
Planorbis sp.		2				1				2
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.						1				5
Pisidium sp.						1	4			3
Anodonta sp.										
Naididae										
Tubificidae	3	45	25	200	190	6	7		10	48
Lumbriculidae								2		
Oligochaeta										
G. complanata			3	4	28	1	3			6
E. octoculata				28	33	1	11	3		2
E. testacea						12		1		
H. stagnalis					1	1	1			
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina	44	18	28	4		1	8	2	2	85
Astacus sp.										
G. pulex		5	1	9	1	1	40	12	3	42
G. tigrinus										
C. pseudogracilis								5	1	
A. aquaticus			1	54	544	30	63	12	22	18
A. meridianus										
Baetis sp.	1	9	36	23		10	9	44	42	2
Caenis sp.						3				1
E. ignita							16	39	36	1
E. danica						1				
E. venosus	3	9	14	6						
E. dispar										
E. torrentis										
R. semicolorata		7	4			3		2		
Heptagenia sp.	1					4				
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.	6									
Amphinemura sp.	21		25			36				
Nemoura sp.		15	3	1						
Leuctra sp.	19	12	9			3		7	9	
Perla sp.										
I. grammatica	14	22	40			15				
Chloroperla sp.	7					2				

SITE → / FAUNA ↓	R.Chumet Upper Hulme	R. Chumet Bridgend	R. Chumet Abbey Green Rd.	R. Chumet d/s Wardles	R. Chumet Cheddle-ton Station	R.Chumet Consall	R.Chumet Froghall	R. Chumet Oakamoor	R. Chumet Alton	R. Blythe Cheswick Green
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										
Gerridae										
Gyrinidae										
Haliplidae			2			2	2	5	5	4
Elminthidae		1						1		
Dytiscidae			11							2
Sialis lutaria		1								
Sialis fuluginosa			3							
H. angustipennis						2		26		
H. pellucidula										
H. contubernalis										
H. siltala						1				1
H. instabilis			5	1		1				
H. fluvipes										
Hydropsyche spp.	7	1					1	4		
Rhyacophila dorsalis				4			7	6	17	
P. flavomaculatus	2	7	8							
P. conspersa										
Psychomyiidae										
Glossosomatidae									2	
Phryganidae										
Sericostomatidae		2				1				
Limnephilidae		3	9			1	4			6
Goerinidae										
Hydroptilidae								3	1	
Molannidae										
Simulium sp.	2			12				4		7
Dicranota sp.	1								3	
Pedicia sp.			2							
Tipula sp.		1								
Atherix sp.										
Chironomidae(Green)	4	7		35	16	32	25	58	17	64
C.riparius (Red)							2			
Ceratopogonidae							1			
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.										
Diversity Index										
Chandler Score										
B.M.W.P.										

SITE → / FAUNA ↓	R. Blythe Henwood Mill	R. Blythe u/s East-cote Brook	R. Blythe Stone-bridge	Langley Brook Middleton	R. Bourne Over Whitacre	R. Anker Witherley	R. Anker Leather-mill Bridge	R. Tame u/s Blue Billy Tip	R. Tame W. Bromwich	R. Tame Bescot
Platyhelminthes		8					5			
A. fluviatilis							14			
P. jenkinsi			9	3			7			
L. pereger	22						41			
L. stagnalis										
Planorbis sp.	2			1			1			
Valvata sp.										
Physa sp.	8						3			
Theodoxus sp.										
Bythinia sp.	7									
Zonitoides sp.										
Sphaerium sp.	21		5				30			
Pisidium sp.	13		4	2			25			
Anodonta sp.										
Naididae										
Tubificidae	12	32	28	46	39	60	35	29	750	100
Lumbriculidae						3				
Oligochaeta										
G. complanata	2	4	1	4	4	3	1			
E. octoculata	8	11	2			14	1			
E. testacea										
H. stagnalis			5			4				
P. geometrica										
T. tessulatum										
H. sanguisuga						1				
Hydracarina				12	69		6			
Astacus sp.										
G. pulex	5	36	44	12	57	1	32			
G. tigrinus										
C. pseudogracilis			2			5	5			
A. aquaticus	36		26	9	3	5000	172			1
A. meridianus										
Baetis sp.		18	3		27					
Caenis sp.		45	9							
E. ignita			3							
E. danica				3						
E. venosus										
E. dispar										
E. torrentis										
R. semicolorata										
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.										
Nemoura sp.										
Leuctra sp.										
Perla sp.										
I. grammatica										
Chloroperla sp.										

SITE → / FAUNA ↓	R.Blythe Henwood Mill	R. Blythe u/s Eastcote Brook	R. Blythe Stonebridge	Langley Brook Middleton	R. Bourne Over Whitacre	R. Anker Witherley	R. Anker Leathermill Bridge	R. Tame u/s Blue Billy Tip	R. Tame W. Bromwich	R. Tame Bescot
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae		18								
Gerridae										
Gyrinidae										
Haliplidae	12	10		3			28			
Elminthidae				3						
Dytiscidae					6					
Sialis lutaria		3		1	5					
Sialis fuluginosa										
H. angustipennis			4	12			250			
H. pellucidula										
H. contubernalis										
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.					8					
Rhyacophila dorsalis			1		6					
P. flavomaculatus			5							
P. conspersa		3								
Psychomyiidae										
Glossosomatidae		1		12						
Phryganidae										
Sericostomatidae					5		1			
Limnephilidae		7	2		11					
Goerinidae										
Hydroptilidae					19					
Molannidae										
Simulium sp.			5	2						
Dicranota sp.										
Pedicia sp.										
Tipula sp.					2				2	
Atherix sp.										
Chironomidae(Green)	20	30	56	25	58	12	39			
C.riparius (Red)		33				10				
Ceratopogonidae		7		1						
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.										
Diversity Index										
Chandler Score										
B.M.W.P.										

P = Present

SITE → / FAUNA ↓	R. Ouse Fulwell Bridge	R. Tove Preston Capes	R. Ouse Passenham	R. Tove Bozenham Mill	R. Tove Cappenham Bridge	Ascott Brook	Clipstone Brook	R. Ouse Milton Ernest	Claydon Brook Winslow	Claydon Brook Padbury
Platyhelminthes		P								
A. fluviatilis	P			P					P	P
P. jenkinsi	P		P	P	P		P			P
L. pereger	P	P	P	P		P	P	P	P	
L. stagnalis						P				P
Planorbis sp.			P					P		P
Valvata sp.			P		P				P	
Physa sp.										
Theodoxus sp.										
Bythinia sp.		P								
Zonitoides sp.										
Sphaerium sp.	P		P	P					P	P
Pisidium sp.										
Anodonta sp.										
Naididae										
Tubificidae						P				
Lumbriculidae		P								
Oligochaeta	P		P	P	P		P		P	P
G. complanata			P	P	P				P	P
E. octoculata			P		P					
E. testacea										
H. stagnalis										
P. geometrica				P						
T. tessulatum										
H. sanguisuga										
Hydracarina	P	P	P		P	P	P		P	P
Astacus sp.										
G. pulex	P	P	P	P	P		P	P	P	
G. tigrinus										
C. pseudogracilis										
A. aquaticus			P	P				P		P
A. meridianus										
Baetis sp.	P	P	P	P	P	P	P	P	P	
Caenis sp.			P	P	P			P		P
E. ignita								P		
E. danica										
E. venosus										
E. dispar										
E. torrentis										
R. Semicolorata										
Heptagenia sp.										
Leptophlebia sp.		P	P		P	P				
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.										
Nemoura sp.	P	P							P	
Leuctra sp.										
Perla sp.										
I. grammatica										
Chloroperla sp.										

P = Present

SITE → / FAUNA ↓	R. Ouse Fulwell Bridge	R. Tove Preston Capes	R. Ouse Passenham	R. Tove Bozenham Mill	R. Tove Cappenham Bridge	Ascott Brook	Clipstone Brook	R. Ouse Milton Ernest	Claydon Brook Winslow	Claydon Brook Padbury
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae			P				P			
Aphelocheiridae										
Corixidae			P				P	P		
Gerridae										
Gyrinidae										
Haliplidae	P		P	P	P	P		P		P
Elminthidae	P		P	P	P	P	P			P
Dytiscidae	P		P	P			P	P		P
Sialis lutaria				P			P			P
Sialis fuluginosa										
H. angustipennis										
H. pellucidula										
H. contubermalis										
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.					P		P		P	P
Rhyacophila dorsalis		P					P			
P. flavomaculatus		P	P							
P. conspersa										
Psychomyiidae										
Glossosomatidae										P
Phryganidae										
Sericostomatidae										
Limnephilidae	P	P		P			P			P
Goerinidae	P									
Hydroptilidae				P						
Molannidae										
Simulium sp.		P				P			P	
Dicranota sp.										
Pedicia sp.										
Tipula sp.							P			
Atherix sp.										
Chironomidae(Green)	P	P	P	P	P	P	P	P	P	P
C.riparius (Red)										
Ceratopogonidae		P	P				P	P		
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.										
Diversity Index										
Chandler Score										
B.M.W.P.										

P = Present

SITE → / FAUNA ↓	R. Ouzel Stanbridge Ford	R. Ouse Olney	R. Ouse Ravenstone Mill	R. Ou-el Caldecote Mill	R. Ouzel Willen	R. Ouzel Simpson	Warm Brk. u/s Chapel-en-le-Frith	Warm Brk. d/s Chapel-en-le-Frith	Roych Brook	R. Sett Hayfield
Platyhelminthes						P				
A. fluviatilis		P		P			P			
P. jenkinsi		P		P	P	P				
L. pereger		P	P		P	P				
L. stagnalis										
Planorbis sp.		P		P		P				
Valvata sp.		P		P	P	P				
Physa sp.						P				
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.		P	P		P	P				
Pisidium sp.										
Anodonta sp.										
Naididae								P		
Tubificidae							P	P	P	
Lumbriculidae								P		
Oligochaeta	P	P		P	P	P				
G. complanata	P	P	P	P	P	P				
E. octoculata				P	P	P				
E. testacea										
H. stagnalis										
P. geometrica			P							
T. tessulatum										
H. sanguisuga										
Hydracarina	P	P	P	P		P	P	P		P
Astacus sp.										
G. pulex		P	P	P		P				
G. tigrinus										
C. pseudogracilis										
A. aquaticus	P			P	P	P		P		
A. meridianus										
Baetis sp.	P	P	P	P		P	P		P	
Caenis sp.		P	P	P	P	P			P	
E. ignita		P	P						P	
E. danica										
E. venosus										P
E. dispar										
E. torrentis									P	P
R. Semicolorata										
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.									P	
B. risi									P	P
Protonemura sp.							P		P	P
Amphinemura sp.							P			P
Nemoura sp.										
Leuctra sp.							P			P
Perla sp.										
I. grammatica							P			P
Chloroperla sp.										P

P = Present

SITE → / FAUNA ↓	R. Ouzel Stanbridge Ford	R. Ouse Olney	R. Ouse Ravenstone Mill	R. Ouzel Caldecote Mill	R. Ouzel Willen	R. Ouzel Simpson	Warm Brk. u/s Chapel-en-le-Frith	Warm Brk. d/s Chapel-en-le-Frith	Roych Brook	R. Sett. Hayfield
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae		P								
Corixidae			P							
Gerridae										
Gyrinidae										
Haliplidae	P		P		P	P				
Elminthidae		P								
Dytiscidae	P	P	P		P					
Sialis lutaria	P									
Sialis fuluginosa										
H. angustipennis										
H. pellucidula										
H. contubernalis										
H. siltala										
H. instabilis										
H. fluvipes										
Hydropsyche spp.	P	P		P		P			P	P
Rhyacophila dorsalis									P	
P. flavomaculatus		P								P
P. conspersa										
Psychomyiidae		P								
Glossosomatidae										
Phryganidae										
Sericostomatidae										
Limnephilidae										P
Goerinidae										
Hydroptilidae	P									
Molannidae										
Simulium sp.	P			P		P			P	P
Dicranota sp.										
Pedicia sp.										
Tipula sp.										
Atherix sp.										
Chironomidae (Green)	P	P	P	P	P	P	P	P	P	P
C. riparius (Red)										
Ceratopogonidae		P	P							
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.										
Diversity Index										
Chandler Score										
B.M.W.P.										

P = Present

SITE → / FAUNA ↓	R. Sett u/s R. Goyt	R. Bollin Langley	R. Bollin Jarmin	R. Bollin Beech Bridge	Ri Croco	R. Goyt Otterspool Bridge	R. Goyt Compstall	Mobberley Brook	R. Dane Biddulph	
Platyhelminthes		P	P			P				
A. fluviatilis		P								
P. jenkinsi										
L. pereger		P								
L. stagnalis										
Planorbis sp.										
Valvata sp.										
Physa sp.										
Theodoxus sp.										
Bythinia sp.										
Zonitoides sp.										
Sphaerium sp.										
Pisidium sp.										
Anodonta sp.										
Naididae		P			P					
Tubificidae	P	P	P	P	P	P	P	P	P	
Lumbriculidae		P		P					P	
Oligochaeta										
G. complanata			P							
E. octoculata				P		P	P			
E. testacea										
H. stagnalis			P							
P. geometrica										
T. tessulatum										
H. sanguisuga										
Hydracarina										
Astacus sp.										
G. pulex										
G. tigrinus			P	P	P	P		P		
C. pseudogracilis										
A. aquaticus										
A. meridianus						P	P	P		
Baetis sp.	P	P	P			P	P		P	
Caenis sp.		P								
E. ignita	P					P	P			
E. danica										
E. venosus	P						P			
E. dispar										
E. torrentis										
R. Semicolorata	P	P	P							
Heptagenia sp.										
Leptophlebia sp.										
Paraleptophlebia sp.										
B. risi										
Protonemura sp.										
Amphinemura sp.										
Nemoura sp.		P								
Leuctra sp.	P									
Perla sp.										
I. grammatica			P							
Chloroperla sp.										

P = Present

SITE → / FAUNA ↓	R. Sett u/s R. Goyt	R. Bollin Langley	R. Bollin Jarmin	R. Bollin Beech Bridge	R. Croco	R. Goyt Otterspool Bridge	R. Goyt Compstall	Mobberley Brook	R. Dane Biddulph	
Other Plecoptera										
Plecoptera										
Zygoptera										
Notonectidae										
Aphelocheiridae										
Corixidae										
Gerridae										
Gyrinidae										
Haliplidae										
Elminthidae		P								
Dytiscidae		P					P			
Sialis lutaria	P									
Sialis fuluginosa										
H. angustipennis										
H. pellucidula										
H. contubernalis										
H. siltalai										
H. instabilis										
H. fluvipes										
Hydropsyche spp.	P	P	P			P				
Rhyacophila dorsalis	P		P			P				
P. flavomaculatus										
P. conspersa										
Psychomyiidae										
Glossosomatidae										
Phryganidae										
Sericostomatidae										
Limnephilidae										
Goerinidae										
Hydroptilidae										
Molannidae										
Simulium sp.	P	P				P	P	P		
Dicranota sp.								P		
Pedicia sp.										
Tipula sp.										
Atherix sp.										
Chironomidae(Green)	P	P	P	P		P	P	P	P	
C.riparius (Red)										
Ceratopogonidae										
Limnophora sp.										
Hemerodroma sp.										
Other diptera										
T.B.I.										
Diversity Index										
Chandler Score										
B.M.W.P.										

APPENDIX 2a

(Chemical Data)

(Aston)

SITE →	CALDER HEBDEN BRIDGE	CALDER SOWERBY BRIDGE	CLIFTON BECK	CALDER MIRFIELD	COLNE HUDDERS-FIELD
DETERMINAND	1	2	3	4	5
Temperature ^{o}C	14.0	14.0	14.0	18.0	16.0
pH.	6.5	6.6	7.4	6.8	6.7
D.O. mgl^{-1}	10.1	10.1	8.5	9.3	8.8
B.O.D mgl^{-1}	1.1	1.8	6.7	2.1	3.0
Total Hardness mgl^{-1} ($CaCO^{3}$)	39.0	69.0	188.0	82.0	112.0
Ca Hardness mgl^{-1} ($CaCO^{3}$)	23.0	43.0	119.0	57.0	80.0
Mg Hardness mgl^{-1} ($CaCO_{3}$)	16.0	26.0	69.0	25.0	32.0
Phenolthalein Alkalinity mgl^{-1} ($CaCO_{3}$)	0.0	0.0	0.0	0.0	0.0
Total Alkalinjty mgl^{-1} ($CaCO_{3}$)	10.0	20.0	120.0	30.0	20.0
Chloride mgl^{-1}	16.3	32.6	53.2	44.0	48.9
Nitrate $mgNl^{-1}$	0.6	2.0	2.55	3.75	2.6
Ammonia $mgNl^{-1}$	0.1	0.1	1.7	0.2	0.5
Phosphate mgl^{-1}	0.3	0.3	1.3	0.6	1.0
Suspended solids (105^{o}C) mgl^{-1}	4.0	7.1	9.5	2.0	7.0
Copper mgl^{-1}	0.05	0.05	0.06	0.69	0.08
Cadmium mgl^{-1}	0.0	0.0	0.0	0.0	0.0
Lead mgl^{-1}	0.1	0.1	0.2	0.15	0.1
Chromium mgl^{-1}	0.01	0.01	0.02	0.08	0.02
Nickel mgl^{-1}	0.0	0.05	0.07	0.1	0.05
Iron mgl^{-1}	3.3	0.8	3.75	5.3	3.05
Zinc mgl^{-1}	0.01	0.45	0.02	0.03	0.02

SITE →	CALDER DEWSBURY	CALDER HORBURY	DEARNE U/S BARNSLEY	DEARNE D/S BARNSLEY	DOVE DARFIELD
DETERMINAND	6	7	8	9	10
Temperature ^{o}C	16.0	15.9	14.0	14.0	15.0
pH.	7.0	7.0	6.9	6.8	7.2
D.O. mgl^{-1}	7.5	5.7	6.2	6.75	8.25
B.O.D mgl^{-1}	7.0	5.0	4.6	6.25	5.3
Total Hardness mgl^{-1} ($CaCO^3$)	136.0	147.0	255.0	282.0	391.0
Ca Hardness mgl^{-1} ($CaCO^3$)			156.0	170.0	190.0
Mg Hardness mgl^{-1} ($CaCO_3$)			99.0	112.0	200.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)			0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_3$)	67.0	75.6	120.0	130.0	180.0
Chloride mgl^{-1}	97.4	95.7	94.3	134.0	148.0
Nitrate $mgNl^{-1}$	3.9	3.77	4.55	4.95	3.75
Ammonia $mgNl^{-1}$	2.8	2.8	0.6	0.6	0.6
Phosphate mgl^{-1}	0.4	0.5	1.1	1.5	1.5
Suspended solids (105^{o}C) mgl^{-1}	28.0				
Copper mgl^{-1}			0.05	0.11	0.06
Cadmium mgl^{-1}			0.02	0.005	0.015
Lead mgl^{-1}			0.25	0.3	0.35
Chromium mg_l^{-1}			0.06	0.04	0.04
Nickel mgl^{-1}			0.15	0.04	0.18
Iron mgl^{-1}			17.1	21.0	5.8
Zinc mgl^{-1}			0.02	0.05	0.06

SITE →	DEARNE BROOM HILL	DEARNE U/S DON	DON KILN HURST	DON SHEFFIELD	LOXLEY
DETERMINAND	11	12	13	14	15
Temperature ^{o}C	15.0	15.0	16.0	15.0	14.0
pH.	6.75	6.9	6.8	6.53	6.9
D.O. mgl^{-1}	8.55	6.85	8.05	9.1	10.0
B.O.D mgl^{-1}	7.4	6.25	5.8	2.3	3.2
Total Hardness mgl^{-1} ($CaCO^{3}$)	312.0	402.0	232.0	121.0	110.0
Ca Hardness mgl^{-1} ($CaCO^{3}$)	177.0	232.0	153.0	84.0	64.0
Mg Hardness mgl^{-1} ($CaCO_{3}$)	135.0	170.0	79.0	37.0	56.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_{3}$)	0.0	0.0	0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_{3}$)	140.0	140.0	90.0	22.5	20.0
Chloride mgl^{-1}	156.0	334.0	105.6	51.8	28.4
Nitrate $mgNl^{-1}$	5.85	5.4	3.55	2.4	2.0
Ammonia $mgNl^{-1}$	2.2	2.6	4.3	0.2	0.1
Phosphate mgl^{-1}	1.4	1.8	2.7	0.2	0.5
Suspended solids (105^{o}C) mgl^{-1}					
Copper mgl^{-1}	0.87	0.10	0.05	0.06	0.07
Cadmium mgl^{-1}	0.02	0.01	0.003	0.01	0.01
Lead mgl^{-1}	0.3	0.1	0.4	0.3	0.7
Chromium mgl^{-1}	0.06	0.06	0.11	0.04	0.02
Nickel mgl^{-1}	0.23	0.04	0.06	0.02	0.02
Iron mgl^{-1}	22.0	11.95	21.0	21.1	15.2
Zinc mgl^{-1}	0.04	0.13	0.16	0.09	0.05

SITE →	DRONE	WHITTING	BLACKBURN BROOK	DON OUGHTI-BRIDGE	EWDEN BECK
DETERMINAND	16	17	18	19	20
Temperature ^{o}C	13.0	13.0	17.0	10.4	9.0
pH.	6.7	6.72	6. 75	7.04	7.0
D.O. mgl^{-1}		9.55	8.8	9.4	10.4
B.O.D mgl^{-1}	5.3	2.75	8.4	9.1	3.7
Total Hardness mgl^{-1} ($CaCO^{3}$)	240.0	162.0	222.0		
Ca Hardness mgl^{-1} ($CaCO^{3}$)	161.0	115.0	132.0		
Mg Hardness mgl^{-1} ($CaCO_{3}$)	89.0	47.0	90.0		
Phenolthalein Alkalinity mgl^{-1}($CaCO_{3}$)	0.0	0.0	0.0		
Total Alkalinity mgl^{-1} ($CaCO_{3}$)	95.0	60.0	65.0		
Chloride mgl^{-1}	53.0	25.5	55.0	49.6	20.3
Nitrate $mgNl^{-1}$	5.95	3.0	3.25	3.5	1.6
Ammonia $mgNl^{-1}$	1.0	0.4	0.8	0.8	0.85
Phosphate mgl^{-1}	1.8	0.8	1.2		
Suspended solids (105^{o}C) mgl^{-1}				32.0	9.0
Copper mgl^{-1}	0.06	0.13	0.08		
Cadmium mgl^{-1}	0.005	0.005	0.01		
Lead mgl^{-1}	0.35	0.4	0.15		
Chromium mgl^{-1}	0.04	0.06	0.04		
Nickel mgl^{-1}	0.02	0.03	0.1		
Iron mgl^{-1}	21.0	18.0			
Zinc mgl^{-1}	0.12	0.05	0.02		

SITE →	R. SEVERN	R. WHARFE TADCASTER	R. WHARFE BOSTON SPA	WHARFE HAREWOOD BRIDGE	WHARFE BOLTON BRIDGE
DETERMINAND	21	22	23	24	25
Temperature ^{o}C	8.4	10.95	10.0	9.5	9.2
pH.	7.5	7.8	7.95	7.8	7.9
D.O. mgl^{-1}	9.83	10.83	11.67	11.2	11.5
B.O.D mgl^{-1}	1.7	2.85	2.1	1.8	1.3
Total Hardness mgl^{-1} ($CaCO^{3}$)	88.9	169.6	159.0	141.7	129.0
Ca Hardness mgl^{-1} ($CaCO^{3}$)					
Mg Hardness mgl^{-1} ($CaCO_{3}$)					
Phenolthalein Alkalinity mgl^{-1}($CaCO_{3}$)					
Total Alkalinity mgl^{-1} ($CaCO_{3}$)	49.8	111.4	117.0	106.0	110.4
Chloride mgl^{-1}	16.5	18.0	15.4	14.6	11.29
Nitrate $mgNl^{-1}$	1.2	2.0	0.02	1.6	1.0
Ammonia $mgNl^{-1}$	0.1	0.1	0.2	0.15	0.05
Phosphate mgl^{-1}	0.04	0.08	0.08	0.08	0.02
Suspended solids (105^{o}C) mgl^{-1}	15.0	13.5	19.0	15.0	8.6
Copper mgl^{-1}					
Cadmium mgl^{-1}					
Lead mgl^{-1}					
Chromium mgl^{-1}					
Nickel mgl^{-1}					
Iron mgl^{-1}					
Zinc mgl^{-1}					

SITE →	WHARFE BURNSALL BRIDGE	WASH BURN LEATHLEY BRIDGE	DOWLES BROOK	STOUR DOG-KENNEL LANE	STOUR COMBESWOOD BROOK
DETERMINAND	26	27	28	29	30
Temperature ^{o}C	8.6	7.9	9.5	12.0	12.5
pH.	7.8	7.5	7.56	7.3	7.3
D.O. mgl^{-1}	11.6	11.6	10.9	9.1	8.3
B.O.D mgl^{-1}	0.5	1.6	1.3	4.7	3.9
Total Hardness mgl^{-1} ($CaCO^{3}$)	144.8	76.5	200.0	275.0	316.0
Ca Hardness mgl^{-1} ($CaCO^{3}$)			130.0	246.0	264.0
Mg Hardness mgl^{-1} ($CaCO_{3}$)			70.0	29.0	52.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_{3}$)			0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_{3}$)	129.3	33.1	140.0	195.0	160.0
Chloride mgl^{-1}	11.5	18.4	34.0	34.0	11.4
Nitrate $mgNl^{-1}$	1.03	1.5	2.6	5.2	5.15
Ammonia $mgNl^{-1}$	0.07	0.16	0.1	0.1	0.8
Phosphate mgl^{-1}	0.02	0.04	0.0	1.2	1.4
Suspended solids (105^{o}C) mgl^{-1}	5.6	10.2	0.5		
Copper mgl^{-1}			0.05	0.08	0.06
Cadmium mgl^{-1}			0.005	0.02	0.02
Lead mgl^{-1}			0.15	0.2	0.3
Chromium mgl^{-1}			0.0	0.02	0.04
Nickel mgl^{-1}			0.05	0.07	0.075
Iron mgl^{-1}			1.7	5.3	0.28
Zinc mgl^{-1}			0.01	0.01	0.12

SITE →	DEVON HAWTON	POULTER ELKESLEY	MAUN MANS-FIELD	MAUN CLIP-STONE	MAUN EDWIN-STONE
DETERMINAND	31	32	33	34	35
Temperature ^{o}C	16.0	10.0	9.0	9.5	9.5
pH.	8.25	8.15	7.7	7.0	7.1
D.O. mgl^{-1}	10.5	12.45	10.6	6.8	8.45
B.O.D mgl^{-1}	6.95	3.75	8.8	6.6	7.9
Total Hardness mgl^{-1} ($CaCO^{3}$)	1000.0	278.0	169.0	136.o	171.0
Ca Hardness mgl^{-1} ($CaCO^{3}$)	800.0	141.0	92.0	75.0	97.0
Mg Hardness mgl^{-1} ($CaCO_{3}$)	200.0	137.0	77.0	61.0	74.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_{3}$)	0.3	0.3	0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_{3}$)	200.0	205.0	160.0	170.0	180.0
Chloride mgl^{-1}	54.59	358.5	95.0	91.0	128.5
Nitrate $mgNl^{-1}$	5.95	10.4	7.2	6.6	7.8
Ammonia $mgNl^{-1}$	0.1	0.2	0.5	7.6	5.0
Phosphate mgl^{-1}	1.0	0.0	1.8	2.7	2.4
Suspended solids (105^{o}C) mgl^{-1}	13.0	4.5	6.0	6.0	24.5
Copper mgl^{-1}	0.08	0.06	0.22	0.34	0.41
Cadmium mgl^{-1}	0.25	0.015	0.015	0.01	0.155
Lead mgl^{-1}	0.3	0.25	0.25	0.6	0.6
Chromium mg_{l}^{-1}	0.06	0.04	0.06	0.06	0.16
Nickel mgl^{-1}	0.15	0.1	0.07	0.15	0.3
Iron mgl^{-1}	3.7	0.7	5.5	13.1	11.0
Zinc mgl^{-1}	0.01	0.01	0.04	0.1	0.11

SITE →	RAIN WORTH WATER	R.WATER OLLERTON	EREWASH PINXTON	BAGTHORPE BROOK	BEAUVALE BROOK
DETERMINAND	36	37	38	39	40
Temperature ^{o}C	10.0	10.0	14.0	13.0	13.0
pH.	6.8	7.9	7.05	7.5	7.25
D.O. mgl^{-1}	10.85	11.0	5.3	9.15	8.4
B.O.D mgl^{-1}	3.45	6.9	0.7	4.45	2.83
Total Hardness mgl^{-1} ($CaCO^3$)	95.0	224.0	690.0	270.0	600.0
Ca Hardness mgl^{-1} ($CaCO^3$)	45.0	132.0	360.0	151.0	300.0
Mg Hardness mgl^{-1} ($CaCO_3$)	50.0	92.0	330.0	119.0	300.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)	0.0	0.0	0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_3$)	40.0	165.0	235.0	160.0	195.0
Chloride mgl^{-1}	4.9	48.0	256.6	34.0	443.2
Nitrate $mgNl^{-1}$	7.8	12.2	9.4	2.0	1.4
Ammonia $mgNl^{-1}$	0.2	0.4	0.9	0.1	0.5
Phosphate mgl^{-1}	0.0	5.5	4.6	0.5	0.4
Suspended solids (105^{o}C) mgl^{-1}	13.5	11.0	20.0	12.5	28.5
Copper mgl^{-1}	0.04	0.11	0.14	0.07	0.09
Cadmium mgl^{-1}	0.0	0.15	0.04	0.01	0.04
Lead mgl^{-1}	0.2	0.35	0.35	0.2	0.4
Chromium mg_l^{-1}	0.02	0.04	0.07	0.03	0.06
Nickel mgl^{-1}	0.05	0.15	0.25	0.07	0.2
Iron mgl^{-1}	4.05	2.75	4.8	2.4	5.4
Zinc mgl^{-1}	0.02	0.03	0.02	0.01	0.01

SITE →	CHURNET UPPER HULME	CHURNET BRIDGEND	CHURNET ABBEY GREEN RD.	CHURNET d/s WARDLES	CHURNET CHEDDLETON STATION
DETERMINAND	41	42	43	44	45
Temperature ^{o}C	7.2	8.0	8.5	7.0	11.0
pH.	6.8	7.0	6.8	7.0	6.6
D.O. mgl^{-1}	12.1	11.8		9.8	8.95
B.O.D mgl^{-1}	1.9	2.2	2.0	8.0	2.6
Total Hardness mgl^{-1} ($CaCO^{3}$)	49.0	74.0	71.0	133.0	125.0
Ca Hardness mgl^{-1} ($CaCO^{3}$)	38.0		55.0		96.0
Mg Hardness mgl^{-1} ($CaCO_{3}$)	11.0		16.0		29.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_{3}$)	0.0		0.0		0.0
Total Alkalinity mgl^{-1} ($CaCO_{3}$)	175.0	41.0	0.0	59.0	100.0
Chloride mgl^{-1}	15.5	29.0	20.0	66.0	49.0
Nitrate $mgNl^{-1}$	2.9	2.0	0.9	2.3	3.6
Ammonia $mgNl^{-1}$	0.4	0.9	0.4	1.2	1.0
Phosphate mgl^{-1}			1.0		1.0
Suspended solids (105^{o}C) mgl^{-1}		15.0	1.0	13.0	5.5
Copper mgl^{-1}					
Cadmium mgl^{-1}					
Lead mgl^{-1}					
Chromium mgl^{-1}					
Nickel mgl^{-1}					
Iron mgl^{-1}					
Zinc mgl^{-1}					

SITE →	CHURNET CONSALL	CHURNET FROGHALL	CHURNET OAKAMOOR	CHURNET ALTON	TEAN CHECKLEY
DETERMINAND	46	47	48	49	50
Temperature ^{o}C	13.0	14.0	12.0	11.8	14.0
pH.	7.2	6.95	6.8	7.1	7.2
D.O. mgl^{-1}	9.0	9.3	10.0	12.55	8.95
B.O.D mgl^{-1}	2.3	4.0	3.6	2.15	1.95
Total Hardness mgl^{-1} ($CaCO^{3}$)	123.0	158.0	172.0	159.0	267.0
Ca Hardness mgl^{-1} ($CaCO^{3}$)		118.0	124.0	128.0	184.0
Mg Hardness mgl^{-1} ($CaCO_{3}$)		40.0	48.0	31.0	83.0
Phenolthalein Alkalinity mgl^{-1} ($CaCO_{3}$)	0.0	0.0	0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_{3}$)	80.0	70.0	60.0	115.0	165.0
Chloride mgl^{-1}	23.0	24.8	22.7	43.0	58.8
Nitrate $mgNl^{-1}$	4.0	4.0	3.2	34.0	3.6
Ammonia $mgNl^{-1}$	0.2	0.4	0.1	0.5	0.1
Phosphate mgl^{-1}		0.6	0.0	0.8	0.4
Suspended solids (105^{o}C) mgl^{-1}	9.0	12.5	13.0	7.5	11.0
Copper mgl^{-1}		0.19	0.15		0.14
Cadmium mgl^{-1}		0.01	0.01		0.03
Lead mgl^{-1}		0.01	0.15		0.35
Chromium mgl^{-1}		0.07	0.06		0.32
Nickel mgl^{-1}		0.07	0.1		0.13
Iron mgl^{-1}		5.65	4.7		2.95
Zinc mgl^{-1}		0.02	0.02		0.08

SITE →	TEAN BEAM-HURST	BLYTHE CHESWICK GREEN	BLYTHE HENWOOD MILL	BLYTHE TEMPLE BALSALL	BLYTHE u/s EASTCOTE BROOK
DETERMINAND	51	52	53	54	55
Temperature ^{o}C	13.0	12.5	13.0	16.0	13.0
pH.	7.2	7.25	7.25	8.41	7.3
D.O. mgl^{-1}	9.0	8.65	7.9	14.7	8.1
B.O.D mgl^{-1}	2.3	2.85	2.3	4.2	3.0
Total Hardness mgl^{-1} ($CaCO^3$)	123.0	237.0	235.0	306.0	284.0
Ca Hardness mgl^{-1} ($CaCO^3$)		133.0	135.0	156.0	161.0
Mg Hardness mgl^{-1} ($CaCO_3$)		104.0	100.0	150.0	123.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)	0.0	0.0	0.0	0.4	0.0
Total Alkalinity mgl^{-1} ($CaCO_3$)	80.0	160.0	150.0	185.0	170.0
Chloride mgl^{-1}	23.0	36.2	29.0	40.4	39.0
Nitrate $mgNl^{-1}$	4.0	5.6	2.6	3.2	4.35
Ammonia $mgNl^{-1}$	0.2	0.2	0.2	0.3	0.2
Phosphate mgl^{-1}		3.6	2.5	0.5	2.8
Suspended solids (105^{o}C) mgl^{-1}	9.0	7.0		7.0	7.3
Copper mgl^{-1}	0.04	0.09	0.07	0.1	0.08
Cadmium mgl^{-1}	0.01	0.01	0.015	0.015	0.01
Lead mgl^{-1}	0.2	0.15	0.1	0.2	0.15
Chromium mgl^{-1}	0.02	0.04	0.02	0.03	0.02
Nickel mgl^{-1}	0.05	0.07	0.07	0.1	0.07
Iron mgl^{-1}	2.55	2.0	1.5	1.5	1.35
Zinc mgl^{-1}	0.11	0.15	0.01	0.01	0.01

SITE →	BLYTHE STONE-BRIDGE	LANGLEY BROOK	BOURNE OVER WHITACRE	BOURNE d/s FILLONGLEY	BOURNE DAW MILL BRIDGE
DETERMINAND	56	57	58	59	60
Temperature ^{o}C	13.5	12.0	14.0	12.5	12.5
pH.	7.0	7.3	7.9	7.5	7.55
D.O. mgl^{-1}	5.8	9.6	10.01	10.8	12.4
B.O.D mgl^{-1}	4.8	0.9	2.6	3.3	2.8
Total Hardness mgl^{-1} ($CaCO^3$)	244.0	349.0	251.0	362.0	368.0
Ca Hardness mgl^{-1} ($CaCO^3$)	140.0	215.0	204.0	296.0	296.0
Mg Hardness mgl^{-1} ($CaCO_3$)	104.0	134.0	47.0	66.0	72.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)	0.0	0.0	0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_3$)	125.0	190.0	211.0	210.0	210.0
Chloride mgl^{-1}	46.8	41.8		44.7	41.8
Nitrate $mgNl^{-1}$	9.0	3.55	11.2	12.4	11.75
Ammonia $mgNl^{-1}$	0.8	0.2		0.4	0.1
Phosphate mgl^{-1}	4.8	2.8		0.8	1.1
Suspended solids (105^{o}C) mgl^{-1}		6.5	25.0	8.0	5.5
Copper mgl^{-1}	0.11	0.07	0.02	0.08	0.07
Cadmium mgl^{-1}	0.01	0.01	0.003	0.01	0.02
Lead mgl^{-1}	0.1	0.2	0.03	0.25	0.25
Chromium mgl^{-1}	0.03	0.04		0.03	0.03
Nickel mgl^{-1}	0.05	0.05	0.03	0.1	0.1
Iron mgl^{-1}	1.0	1.05		2.2	4.9
Zinc mgl^{-1}	0.02	0.01	0.01	0.01	0.01

SITE →	ANKER ATHER-STONE	ANKER WITHERLEY	ANKER LEATHER-MILL BRDG.	TAME BLUE BILLY	TAME WEST BROMWICH
DETERMINAND	61	62	63	64	65
Temperature ^{o}C	14.5	15.5	16.0	13.0	13.0
pH.	7.39	7.51	7.42	7.26	6.7
D.O. mgl^{-1}	10.2	10.2	10.3	8.65	7.9
B.O.D mgl^{-1}	9.7	6.6	5.65	8.1	5.0
Total Hardness mgl^{-1} ($CaCO^3$)	350.0	342.0	323.0	391.0	
Ca Hardness mgl^{-1} ($CaCO^3$)	208.0	222.0	216.0	307.0	329.0
Mg Hardness mgl^{-1} ($CaCO_3$)	142.0	120.0	107.0	84.0	
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)	0.0	0.0	0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_3$)	240.0	240.0	250.0	245.0	192.0
Chloride mgl^{-1}	295.6	268.7	320.0	113.4	152.4
Nitrate $mgNl^{-1}$	6.3	5.2	5.8	2.6	0.6
Ammonia $mgNl^{-1}$	13.0	9.3	13.8	1.9	1.9
Phosphate mgl^{-1}	6.4	5.2	6.3	2.4	3.4
Suspended solids (105^{o}C) mgl^{-1}	11.5	10.5	15.0	9.5	18.0
Copper mgl^{-1}	0.1	0.09	0.09	0.11	0.38
Cadmium mgl^{-1}	0.025	0.02	0.04	0.02	0.35
Lead mgl^{-1}	0.3	0.25	0.3	0.25	0.6
Chromium mgl^{-1}	0.12	0.12	0.11	0.04	0.09
Nickel mgl^{-1}	0.27	0.25	0.27	0.12	0.35
Iron mgl^{-1}	1.15	1.8	0.9	2.95	21.25
Zinc mgl^{-1}	0.05	0.04	0.04	0.035	14.0

SITE →	TAME GREAT BRIDGE	TAME BESCOT	FORD BROOK	OUSE FULWELL BRIDGE	R. TOVE ROAD BRIDGE PRESTON CAPES
DETERMINAND	66	67	68	69	70
Temperature ^{o}C		13.5	14.0	9.0	
pH.		7.05	6.7	8.0	
D.O. mgl^{-1}		8.13	2.0	17.7	
B.O.D mgl^{-1}		7.53	1.7	2.1	
Total Hardness mgl^{-1} ($CaCO^3$)			361.0	184.0	
Ca Hardness mgl^{-1} ($CaCO^3$)		235.0	233.0	171.0	
Mg Hardness mgl^{-1} ($CaCO_3$)			128.0	13.0	
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)		0.0	0.0	0.0	
Total Alkalinity mgl^{-1} ($CaCO_3$)		155.0	195.0	290.0	
Chloride mgl^{-1}		843.0	89.3	33.0	
Nitrate $mgNl^{-1}$		2.0	2.35	8.6	
Ammonia $mgNl^{-1}$		1.1	6.2	0.2	
Phosphate mgl^{-1}		4.0	1.5	0.2	
Suspended solids (105^{o}C) mgl^{-1}		24.0	20.5	4.0	
Copper mgl^{-1}				0.18	
Cadmium mgl^{-1}		0.035	0.03	< 0.01	
Lead mgl^{-1}		0.3	0.4	< 0.01	
Chromium mg_l^{-1}		0.06	0.06	< 0.01	
Nickel mgl^{-1}		4.7	0.55	< 0.01	
Iron mgl^{-1}		9.7	21.25		
Zinc mgl^{-1}		0.1	0.16	0.1	

SITE →	OUSE PASSEN-HAM	TOVE BOZENHAM MILL	TOVE CAPPENHAM BRIDGE	ASCOTT BROOK	CLIPSTONE BROOK
DETERMINAND	71	72	73	74	75
Temperature ^{o}C		9.5	9.5	9.5	9.0
pH.		7.9	8.0	7.8	7.8
D.O. mg_l^{-1}		16.25	13.2	12.2	13.3
B.O.D mg_l^{-1}		0.45	0.1	4.0	0.9
Total Hardness mg_l^{-1} ($CaCO^3$)		357.0	342.0	183.0	197.0
Ca Hardness mg_l^{-1} ($CaCO^3$)		305.0	316.0	167.0	175.0
Mg Hardness mg_l^{-1} ($CaCO_3$)		52.0	26.0	16.0	22.0
Phenolthalein Alkalinity mg_l^{-1} ($CaCO_3$)		0.0	0.1	0.0	0.0
Total Alkalinity mg_l^{-1} ($CaCO_3$)		220.0	250.0	237.5	225.0
Chloride mg_l^{-1}		34.2	31.0	43.0	40.0
Nitrate $mgNl^{-1}$		7.0	7.0		8.0
Ammonia $mgNl^{-1}$		0.5	0.4	0.3	0.3
Phosphate mg_l^{-1}		0.0	1.0	0.8	0.8
Suspended solids (105^{o}C) mg_l^{-1}		27.0	4.0	4.5	7.0
Copper mg_l^{-1}		0.12	0.06	0.16	0.06
Cadmium mg_l^{-1}		< 0.01	< 0.01		
Lead mg_l^{-1}		< 0.01	0.015		
Chromium mg_l^{-1}		< 0.01	0.01		
Nickel mg_l^{-1}		< 0.01	< 0.01		
Iron mg_l^{-1}					
Zinc mg_l^{-1}		0.01	0.01		

SITE →	OUSE MILTON ERNEST	CLAYDON BROOK WINSLOW	CLAYDON BROOK PADBURY	CLAYDON BROOK RD./RAIL BRIDGE	OUZEL STANBRIDGE FORD
DETERMINAND	76	77	78	79	80
Temperature ^{o}C	9.8	10.0	11.0	10.5	10.5
pH.	7.85	7.8	8.2	7.85	7.65
D.O. mgl^{-1}	10.5	11.2	15.8	13.6	13.5
B.O.D mgl^{-1}	2.25	0.6	2.7	2.0	4.3
Total Hardness mgl^{-1} ($CaCO^3$)	178.0	188.0	196.0	186.0	176.0
Ca Hardness mgl^{-1} ($CaCO^3$)	166.0	173.0	181.0	164.0	167.0
Mg Hardness mgl^{-1} ($CaCO_3$)	12.0	15.0	15.0	22.0	9.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)	0.0	0.0	0.2	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_3$)	210.0	290.0	235.0	225.0	290.0
Chloride mgl^{-1}	48.0	41.0	45.0	51.0	82.5
Nitrate $mgNl^{-1}$	8.6	9.0	7.8	10.2	12.3
Ammonia $mgNl^{-1}$	0.4	0.3	0.1	0.2	1.6
Phosphate mgl^{-1}	0.2	0.4	0.2	1.6	4.4
Suspended solids (105^{o}C) mgl^{-1}	5.0	5.0	1.0	9.0	15.0
Copper mgl^{-1}	0.1	0.06	0.14	0.1	0.12
Cadmium mgl^{-1}			<0.01		0.0
Lead mgl^{-1}			0.02		< 0.01
Chromium mgl^{-1}			0.01		0.01
Nickel mgl^{-1}			0.02		0.006
Iron mgl^{-1}					
Zinc mgl^{-1}			0.05		0.02

SITE →	OUSE OLNEY	OUSE RAVENSTONE MILL	OUZEL CALDER-COTE MILL	OUZEL WILLEN	OUZEL SIMPSON
DETERMINAND	81	82	83	84	85
Temperature ^{o}C	10.0	9.5	8.0	12.0	9.0
pH.	7.6	7.8	7.5		8.0
D.O. mgl^{-1}	10.9	10.65	10.15	8.12	14.1
B.O.D mgl^{-1}	1.75	2.5	1.95	9.53	1.95
Total Hardness mgl^{-1} ($CaCO^3$)	348.0	346.0	334.0		
Ca Hardness mgl^{-1} ($CaCO^3$)	318.0	322.0	298.0		296.0
Mg Hardness mgl^{-1} ($CaCO_3$)	30.0	24.0	36.0		
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)	0.0	0.0	0.0		0.1
Total Alkalinity mgl^{-1} ($CaCO_3$)	215.0	230.0	165.0		225.0
Chloride mgl^{-1}	47.5	46.5	63.5	58.7	5.6
Nitrate $mgNl^{-1}$	7.7	8.2	9.8	9.0	9.6
Ammonia $mgNl^{-1}$	0.4	0.6	1.9	0.32	0.1
Phosphate mgl^{-1}	1.3	1.3	3.0	1.56	2.6
Suspended solids (105^{o}C) mgl^{-1}	33.5	3.0	24.0		7.5
Copper mgl^{-1}	0.06	0.05	0.08	0.01	0.16
Cadmium mgl^{-1}	0.0			< 0.01	< 0.01
Lead mgl^{-1}	0.2			0.01	0.02
Chromium mgl^{-1}	0.01			< 0.01	< 0.01
Nickel mgl^{-1}	0.006			< 0.01	0.01
Iron mgl^{-1}					
Zinc mgl^{-1}	0.02			0.01	0

SITE →	WARM BROOK u/s CHAPEL EN-LE-FRITH	WARM BROOK d/s CHAPEL EN-LE-FRITH	ROYCH BROOK	SETT HAYFIELD	SETT NEWMILLS
DETERMINAND	86	87	88	89	90
Temperature ^{o}C	10.0	11.0	12.0	12.0	12.5
pH.	7.85	7.5	7.4	6.65	6.7
D.O. mgl^{-1}	10.23	10.35	10.1	10.3	9.98
B.O.D mgl^{-1}	1.83	2.35	3.7	1.6	2.43
Total Hardness mgl^{-1} ($CaCO^3$)	143.0	152.0	143.0	70.0	79.0
Ca Hardness mgl^{-1} ($CaCO^3$)	118.0	129.0	120.0	52.0	55.0
Mg Hardness mgl^{-1} ($CaCO_3$)	25.0	23.0	23.0	18.0	24.0
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)	0.0	0.0	0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_3$)	77.5	90.0	85.0	22.5	30.0
Chloride mgl^{-1}	26.23	26.23	53.17	13.47	13.5
Nitrate $mgNl^{-1}$	1.6	2.0	1.4	0.6	1.0
Ammonia $mgNl^{-1}$	0.1	0.1	0.1	0.2	0.2
Phosphate mgl^{-1}	0.8	0.7	1.2	0.7	1.0
Suspended solids (105°C) mgl^{-1}	18.0	14.5	17.0	15.0	12.0
Copper mgl^{-1}	0.08	0.51	0.04	0.05	0.05
Cadmium mgl^{-1}	0.01	0.01	0.055	0.005	0.01
Lead mgl^{-1}	0.15	0.2	0.25	0.4	0.1
Chromium mgl^{-1}	0.04	0.04	0.04	0.03	0.03
Nickel mgl^{-1}	0.07	0.07	0.1	0.05	0.07
Iron mgl^{-1}	7.7	10.2	6.2	4.0	6.95
Zinc mgl^{-1}	0.01	0.01	0.025	0.02	0.01

SITE →	BOLLIN LANGLEY	BOLLIN JARMIN	BOLLIN BEECH BROOK	HARROP BROOK	CROCO
DETERMINAND	91	92	93	94	95
Temperature ^{o}C	10.5	16.5	15.5	13.0	14.0
pH.	7.5	7.4	7.0	6.9	7.55
D.O. mgl^{-1}	11.4	8.75	9.4	10.1	8.4
B.O.D mgl^{-1}	1.6	2.05	3.9	3.55	3.55
Total Hardness mgl^{-1} ($CaCO^3$)		135.0	183.0	96.0	583.0
Ca Hardness mgl^{-1} ($CaCO^3$)		100.0	135.0	72.0	540.0
Mg Hardness mgl^{-1} ($CaCO_3$)		35.0	48.0	24.0	43.0
Phenolthalein Alkalinity mgl^{-1}($CaCO_3$)		0.0	0.0	0.0	0.0
Total Alkalinity mgl^{-1} ($CaCO_3$)	57.6	85.0	110.0	37.5	110.0
Chloride mgl^{-1}	20.0	22.0	34.7	14.9	7300.0
Nitrate $mgNl^{-1}$	0.92	1.2	1.95	0.8	8.5
Ammonia $mgNl^{-1}$	0.18	0.2	0.4	0.2	1.4
Phosphate mgl^{-1}	0.13	0.2	0.6	0.8	1.6
Suspended solids (105^{o}C) mgl^{-1}	7.0	12.5	2.5	18.0	5.0
Copper mgl^{-1}	0.04	0.04	0.06	0.1	0.27
Cadmium mgl^{-1}	0.025	0.01	0.015	0.01	0.09
Lead mgl^{-1}	0.0	0.1	0.15	0.1	0.9
Chromium mgl^{-1}	0.02	0.02	0.02	0.02	0.01
Nickel mgl^{-1}	0.05	0.07	0.07	0.07	0.06
Iron mgl^{-1}	0.4	1.6	1.35	5.4	3.7
Zinc mgl^{-1}	0.01	0.005	0.005	0.02	0.03

SITE →	GOYT OTTERS-POOL BRIDGE	GOYT IRON BRIDGE	MOBBERLEY BROOK	BIDDULPH BROOK	
DETERMINAND	96	97	98	99	
Temperature ^{o}C	13.0	14.0	13.0	16.5	
pH.	6.95	6.8	7.0	6.5	
D.O. mgl^{-1}	9.25	7.7	4.85	7.35	
B.O.D mgl^{-1}	7.65	7.3	4.45	5.5	
Total Hardness mgl^{-1} ($CaCO^{3}$)	110.0	126.0	209.0	186.0	
Ca Hardness mgl^{-1} ($CaCO^{3}$)	75.0	83.0	143.0	106.0	
Mg Hardness mgl^{-1} ($CaCO_{3}$)	35.0	43.0	66.0	80.0	
Phenolthalein Alkalinity mgl^{-1} ($CaCO_{3}$)	0.0	0.0	0.0	0.0	
Total Alkalinity mgl^{-1} ($CaCO_{3}$)	60.0	120.0	220.0	70.0	
Chloride mgl^{-1}	37.6	52.5	45.4	58.8	
Nitrate $mgNl^{-1}$	4.35	2.8	1.2	17.05	
Ammonia $mgNl^{-1}$	0.8	2.1	9.2	2.5	
Phosphate mgl^{-1}	1.6	1.5	2.9	7.2	
Suspended solids (105^{o}C) mgl^{-1}	10.5	9.0	17.0	6.5	
Copper mgl^{-1}	0.37	0.12	0.14	0.	—
Cadmium mgl^{-1}	0.01	0.01	0.02	0.01	
Lead mgl^{-1}	0.1	0.15	0.25	0.2	
Chromium mgl^{-1}	0.11	0.06	0.02	0.02	
Nickel mgl^{-1}	0.15	0.15	0.1	0.1	
Iron mgl^{-1}	4.4	2.7	7.15	5.15	
Zinc mgl^{-1}	0.05	0.01	0.02	0.04	

APPENDIX 2b

(Chemical data)

(Water Authority)

SITE	.Calder Hebden Bridge	Calder Sower-by Bridge	Calder Mir-field	Calder Hudders-field/ R.Colne	Calder Dews-bury	Calder Hor-bury
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	8.90	9.63	12.90	12.27	16.68	15.90
pH.	6.52	6.76	7.01	7.19	7.04	7.04
D.O. mgl^{-1}	11.49	10.82	7.98	10.60	7.53	5.75
B.O.D. mgl^{-1}	1.19	1.91	5.57	3.34	7.08	7.51
Total Hardness mgl^{-1} ($CaCO_3$)	34.00	53.00	142.78	112.73	135.71	146.72
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCO_3$)	10.30	15.46	57.95	43.27	67.23	75.66
Chloride mgl^{-1}	21.15	25.85	116.96	64.20	97.41	95.72
Nitrate $mgNl^{-1}$	0.93	1.74	4.39	3.21	3.98	3.77
Nitrite $mgNl^{-1}$	0.01	0.0369	0.34	0.07	0.39	0.51
Total ox N $mgNl^{-1}$						
Ammonia $mgNl^{-1}$	0.1462	0.22	3.25	0.33	2.81	2.86
Phosphate mgl^{-1}	0.408	0.14	0.34	0.15	0.40	0.49
Suspended solids (105^{o}C) mgl^{-1}	7.53	14.07	28.55	20.07	28.65	32.44
Copper mgl^{-1}			0.0573			
Cadmium mgl^{-1}		0.0102				
Lead mgl^{-1}		0.0380				
Chromium mgl^{-1}		0.0452				
Nickel mgl^{-1}		0.0211				
Iron mgl^{-1}		1.4477				
Zinc mgl^{-1}			0.1257			

SITE	Dove Dar-field	Dearne Broom-hill	Dearne U/S R. Don	Don Loxley	Drone	Whit-ting Whit-ting-ton
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	10.68	11.44	11.44	9.21	9.9	9.96
pH.	7.79	7.56	7.55	7.29	7.20	7.46
D.O. mgl^{-1}	10.55	8.75	8.76	10.91		10.87
B.O.D. mgl^{-1}						
Total Hardness mgl^{-1} ($CaCO_3$)		424.08	424.08			
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCo_3$)		157.98	157.98			
Chloride mgl^{-1}	169.45	287.31	287.31	28.08	55.0	56.79
Nitrate $mgNl^{-1}$	5.45	5.10	8.10	2.75	12.00	4.43
Nitrite $mgNl^{-1}$	0.22		0.54	0.11	0.20	0.21
Total ox N $mgNl^{-1}$						
Ammonia $mgNl^{-1}$	0.98	2.17	2.18	0.85	2.00	1.04
Phosphate mgl^{-1}		1.14	1.14			
Suspended solids (105°C) mgl^{-1}	126.48	85.18	85.18	15.08	30.00	49.85
Copper mgl^{-1}	0.03	0.02	0.02	0.06		
Cadmium mgl^{-1}	0.01	0.76	0.76	0.01		
Lead mgl^{-1}	0.03	0.01	0.01	0.06		
Chromium mgl^{-1}	0.12	0.01	0.01	0.06		
Nickel mgl^{-1}	0.04	0.03	0.03	0.08		
Iron mgl^{-1}	3.41	0.54	3.66	1.26		3.43
Zinc mgl^{-1}	0.04	0.05	0.05	0.08		

SITE	Don Oughti-bridge	Ewden Beck	R. Seven	Wharfe Tad-caster	Wharfe Boston Spa	Wharfe Hare-wood Bridge
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	10.38	9.00	8.42	10.95	10.00	9.51
pH.	7.04	7.04	7.52	7.84	7.95	7.82
D.O. mgl^{-1}	9.44	10.44	9.83	10.83	11.68	11.2216
B.O.D. mgl^{-1}						
Total Hardness mgl^{-1} ($CaCO_3$)			8.92	169.64	158.88	141.68
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO^3$)						
Phenolthalejn Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCo_3$)			49.85	111.44	117.50	106.10
Chloride mgl^{-1}	49.67	20.25	16.54	18.02	15.41	14.62
Nitrate $mgNl^{-1}$	3.50	1.67	1.25	2.10		
Nitrite $mgNl^{-1}$	0.11		0.03	0.03	0.03	0.029
Total ox N $mgNl^{-1}$						1.555
Ammonia $mgNl^{-1}$	0.86	0.85	0.09	0.09	0.18	0.154
Phosphate mgl^{-1}			0.04	0.08	0.08	0.082
Suspended solids (105°C) mgl^{-1}	32.42	9.00	15.46	0.72	19.28	14.95
Copper mgl^{-1}	0.07	0.05		0.01		
Cadmium mgl^{-1}	0.01	0.01		0.00		
Lead mgl^{-1}	0.07	0.74		0.01		
Chromium mgl^{-1}	0.05	0.05		0.00		
Nickel mgl^{-1}	0.04	0.04		0.01		
Iron mgl^{-1}	2.00	1.05		0.48		
Zinc mgl^{-1}	0.32	0.11		0.02		

SITE	Wharfe Bolton Bridge	Wharfe Burnsall Bridge	Wash-burn Leath-ley Bridge	Dowles Brook	Stour Dog-Kennel Lane	Stour d/s Coombe-swood Brook
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	9.27	8.63	7.88	6.38	9.68	8.5
pH.	7.96	7.89	7.49	7.64	7.7	7.78
D.O. mgl^{-1}	11.47	11.62	11.57	11.78	8.75	9.10
B.O.D. mgl^{-1}		0.50		2.43		2.80
Total Hardness mgl^{-1} ($CaCO_3$)	129.48	144.88	76.56	204.00	306.65	412.00
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCO_3$)	110.42	129.33	33.15	101.00	171.54	215.50
Chloride mgl^{-1}	11.29	11.54	18.41	35.75	79.95	137.00
Nitrate $mgNl^{-1}$	1.00	1.04				
Nitrite $mgNl^{-1}$	0.049	0.05 LT	0.025			
Total ox N $mgNl^{-1}$			1.531	40.75	6.44	6.30
Ammonia $mgNl^{-1}$	0.059	0.07	0.167	0.625	0.218	0.65
Phosphate mgl^{-1}	0.024	0.027	0.045		0.137	
Suspended solids (105°C) mgl^{-1}	8.623	5.63	10.16	8.75	31.65	4.50
Copper mgl^{-1}					0.15	0.02
Cadmium mgl^{-1}					0.1	0.01
Lead mgl^{-1}					0.05	0.04
Chromium mgl^{-1}					0.284	0.03
Nickel mgl^{-1}					0.03	0.03
Iron mgl^{-1}						
Zinc mgl^{-1}					0.17	0.15

SITE	Devon Hawton	Poulter Elkesley	Maun Mansfield	Maun Clipstone	Maun Edwinstowe	Rainworth Water Rainworth
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	8.27	9.6	8.35	8.67	8.99	8.25
pH.	7.73	8.05	8.06	7.16	6.79	7.63
D.O. mgl^{-1}	10.81	11.69	11.22	10.42	10.36	11.05
B.O.D. mgl^{-1}	2.40	3.19	7.09	7.23	7.74	5.75
Total Hardness mgl^{-1} ($CaCO_3$)	779.59	622.92		368.62	373.62	440.83
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCo_3$)	198.32	214.08	197.85	206.38	201.3	122.42
Chloride mgl^{-1}	58.34	518.17	119.00	130.08	149.62	405.83
Nitrate $mgNl^{-1}$						
Nitrite $mgNl^{-1}$						
Total ox N $mgNl^{-1}$	9.40	9.571	7.89	9.95	10.10	12.19
Ammonia $mgNl^{-1}$	0.35	0.171	0.846	5.23	5.03	0.973
Phosphate mgl^{-1}	0.545	0.435				
Suspended solids (105°C) mgl^{-1}	13.00	16.63	24.23	20.23	25.92	40.33
Copper mgl^{-1}	0.017	0.0127				
Cadmium mgl^{-1}	0.01	0.1				
Lead mgl^{-1}	0.012	0.0227				
Chromium mgl^{-1}	0.0164	0.0127				
Nickel mgl^{-1}	0.01	0.01				
Iron mgl^{-1}						
Zinc mgl^{-1}	0.02	0.0291				

SITE	Rainworth Water Ollerton	Erewash Pinxton	Bagthorpe Brook	Beauvale Brook Nether Green	Churnet Upper Hulme	Churnet Bridgend
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	9.50	9.41	8.33	10.13	7.71	7.30
pH.	7.05	7.40	7.63	7.62	7.0	7.13
D.O. mgl^{-1}	10.28	10.62	11.87	10.63	10.97	11.46
B.O.D. mgl^{-1}	7.88	5.35	2.57	3.97	2.15	1.86
Total Hardness mgl^{-1} ($CaCO_3$)	365.5		270.67	574.5	48.88	70.33
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCO_3$)	99.5	198.45	142.33	173.58	22.0	41.66
Chloride mgl^{-1}	401.25	450.91	122.00	839.50	19.88	25.66
Nitrate $mgNl^{-1}$						
Nitrite $mgNl^{-1}$						
Total ox N $mgNl^{-1}$	16.73	8.818	4.6	2.97	1.17	1.83
Ammonia $mgNl^{-1}$	4.28	0.918	0.01	0.192	0.18	0.50
Phosphate mgl^{-1}						
Suspended solids (105°C) mgl^{-1}	57.00	62.00	5.0	516.92	32.38	7.66
Copper mgl^{-1}						0.01
Cadmium mgl^{-1}						0.005
Lead mgl^{-1}						0.01
Chromium mgl^{-1}						0.01
Nickel mgl^{-1}						0.01
Iron mgl^{-1}						0.48
Zinc mgl^{-1}						0.01

SITE	Churnet Abbey Green Road	Churnet d/s Wardles Outfall	Churnet Cheddle-ton Station	Churnet Con-sall	Churnet Frog-hall	Chur-net Oaka-moor
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	8.5	10.55	10.40	9.54	9.1	9.35
pH.	7.25	6.95	7.03	6.97	7.3	7.1
D.O. mgl^{-1}	10.49	9.82	9.29	10.04	10.39	10.55
B.O.D. mgl^{-1}	2.29	6.47	4.09	3.59	3.66	3.46
Total Hardness mgl^{-1} ($CaCO_3$)	81.04	107.27	120.52	114.55	121.66	140.50
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCO_3$)	43.69	62.72	71.40	64.64	66.50	64.75
Chloride mgl^{-1}	28.78	71.00	52.23	38.81	44.75	40.41
Nitrate $mgNl^{-1}$						
Nitrite $mgNl^{-1}$						
Total ox N $mgNl^{-1}$	1.54	2.73	3.17	3.2	3.25	3.23
Ammonia $mgNl^{-1}$	0.55	0.85	0.73	0.45	0.48	0.42
Phosphate mgl^{-1}	0.08		0.19			
Suspended solids (105°C) mgl^{-1}	24.0	41.36	24.82	37.0	47.58	50.58
Copper mgl^{-1}	0.012		0.015			
Cadmium mgl^{-1}	0.01		0.01			
Lead mgl^{-1}	0.01		0.01			
Chromium mgl^{-1}	0.01		0.01			
Nickel mgl^{-1}	0.01		0.01			
Iron mgl^{-1}	0.01					
Zinc mgl^{-1}	0.03		0.033			

SITE	Churnet Alton	Tean Checkl-eybank	Tean Beam-hurst	Blythe Ches-wick Green	Blythe Hen-wood Mill	Blythe Temple Balsall
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	9.18	8.98	10.52	9.61	9.86	10.33
pH.	7.14	7.76	7.4	7.70	7.62	8.03
D.O. mgl^{-1}	10.64	10.75	9.24	10.64	9.10	11.05
B.O.D. mgl^{-1}	3.18	2.13	7.05	3.36	2.58	3.00
Total Hardness mgl^{-1} ($CaCO_3$)	137.50	225.73	265.07	204.58	209.58	273.75
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCO_3$)	66.25	147.63	185.07	115.83	133.08	163.75
Chloride mgl^{-1}	38.50	40.36	71.14	41.75	39.83	46.00
Nitrate $mgNl^{-1}$						
Nitrite $mgNl^{-1}$						
Total ox N $mgNl^{-1}$	3.28	4.29	7.92	7.93		5.13
Ammonia $mgNl^{-1}$	0.44	0.28	0.571	0.33	0.33	0.18
Phosphate mgl^{-1}						
Suspended solids (105°C) mgl^{-1}	54.0	7.9	14.14	10.92	16.50	9.25
Copper mgl^{-1}						
Cadmium mgl^{-1}						
Lead mgl^{-1}						
Chromium mgl^{-1}						
Nickel mgl^{-1}						
Iron mgl^{-1}						
Zinc mgl^{-1}						

SITE	Blythe u/s East-cote Brook	Blythe Stone-bridge	Bourne Over Whitacre	Bourne Fillong-ley	Anker Ather-stone	Anker Wither-ley
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	9.92	10.24	9.62	10.5	11.01	10.59
pH.	7.61	7.67	7.69	7.7	7.27	7.35
D.O. mgl^{-1}	9.48	9.24	9.85		9.00	8.65
B.O.D. mgl^{-1}	3.54	3.08	1.56	4.0	4.68	4.52
Total Hardness mgl^{-1} ($CaCO_3$)	236.15	242.72	322.08	282.5	327.57	332.80
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCO_3$)	140.77	142.75	203.96	150.0	195.00	206.92
Chloride mgl^{-1}	48.15	50.83	67.54	64.0	187.21	192.03
Nitrate $mgNl^{-1}$						
Nitrite $mgNl^{-1}$						
Total ox N $mgNl^{-1}$	8.09	8.17	11.433		9.26	6.98
Ammonia $mgNl^{-1}$	1.07	0.90	0.15	0.65	7.41	7.69
Phosphate mgl^{-1}						3.89
Suspended solids (105°C) mgl^{-1}	14.38	12.58	18.75	22.0	13.5	17.12
Copper mgl^{-1}	0.03		0.02			0.0007
Cadmium mgl^{-1}	< 0.01					0.01
Lead mgl^{-1}	0.03		0.026			
Chromium mgl^{-1}	0.01					0.03
Nickel mgl^{-1}	0.01		0.01			0.01
Iron mgl^{-1}			0.026			
Zinc mgl^{-1}	0.05					0.037

SITE	Anker Leather-mill Bridge	Tame U/S Blue Billy Tip	Tame West Brom-wich	Tame Bescot	Ford Brook Clay-hanger	Ouse Ful-well Bridge
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	10.0	6.8	6.4	11.14	8.5	9.7
pH.	7.6	8.07	7.18	7.20	7.45	8.13
D.O. mgl^{-1}	10.41		9.58	6.22	3.3	10.17
B.O.D. mgl^{-1}	3.35	4.25	6.50	10. 057	11.25	2.42
Total Hardness mgl^{-1} ($CaCO_3$)	340.0	327.50	334.0	417.50	293.75	
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCO_3$)	205.3	206.25	138.40	243.34	169.25	
Chloride mgl^{-1}	145.4	111.25		197.19	78.75	39.75
Nitrate $mgNl^{-1}$						9.1
Nitrite $mgNl^{-1}$						
Total ox N $mgNl^{-1}$	7.21	4.27	1.24	8.423	1.55	
Ammonia $mgNl^{-1}$	1.05	2.25	1.24	4.938	4.90	0.11
Phosphate mgl^{-1}	2.54			4.96		
Suspended solids (105°C) mgl^{-1}	17.53	7.75	107.80	44.74	17.00	
Copper mgl^{-1}	0.013	0.127	0.05	0.07	0.04	0.01
Cadmium mgl^{-1}	0.01	0.015	0.01	0.012	0.01	0.01
Lead mgl^{-1}	0.01	0.02	0.1	0.04	0.025	0.01
Chromium mgl^{-1}	0.02	0.01	0.012	0.022	0.01	0.01
Nickel mgl^{-1}	0.022	0.0125	0.03	0.14	0.12	0.01
Iron mgl^{-1}				2.74	2.457	
Zinc mgl^{-1}	0.03	0.003	1.84	0.40	0.075	0.01

SITE	Tove Bozenham Mill	Tove Cappen-ham Bridge	Ascot Brook	Clay-don. Brook	Clay-don Brook	Ouse Stan-bridge Ford
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	11.37	11.33	8.66	9.77	8.62	10.68
pH.	8.17	8.11	8.04	8.08	7.94	8.01
D.O. mgl^{-1}	10.27	10.74	8.93	10.29	8.77	8.26
B.O.D. mgl^{-1}	2.78	3.07	2.93	2.8	3.27	5.15
Total Hardness mgl^{-1} ($CaCO_3$)		127.3		142.9		144.79
Ca Hardness mgl^{-1} ($CaCO_3$)		119.5		133.0		139.60
Mg Hardness mgl^{-1} ($CaCO_3$)		78		9.93		5.19
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCo_3$)		197.72		198.84		224.33
Chloride mgl^{-1}	38.63	40.25	38.00	50.50	55.0	78.08
Nitrate $mgNl^{-1}$	8.38	7.83	9.7	9.56	10.42	11.75
Nitrite $mgNl^{-1}$						
Total ox N $mgNl^{-1}$						
Ammonia $mgNl^{-1}$	0.11	0.14	0.50	0.20	0.37	1.628
Phosphate mgl^{-1}		0.95		3.52		
Suspended solids (105°C) mgl^{-1}	0.01					
Copper mgl^{-1}	0.01			0.01		0.01
Cadmium mgl^{-1}	0.01			0.01		0.01
Lead mgl^{-1}	0.01			0.01		0.01
Chromium mgl^{-1}	0.01			0.01		
Nickel mgl^{-1}				0.01		0.01
Iron mgl^{-1}	0.01			0.26		0.14
Zinc mgl^{-1}				0.01		0.04

SITE	Ouse Olney	Ouse Ravenstone Mill	Ouzel Willen	Ouzel Simpson	Sett Hayfield	Sett New Mills
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	12.35	12.37	12.04	9.25	15.00	9.67
pH.	8.16	7.06	8.12	8.17	7.8	7.34
D.O. mgl^{-1}	10.45	9.8	9.53	8.95	9.9	10.9
B.O.D. mgl^{-1}	2.55	3.12		3.23	0.9	2.08
Total Hardness mgl^{-1} ($CaCO_3$)	146.5					
Ca Hardness mgl^{-1} ($CaCO_3$)	139.0					
Mg Hardness mgl^{-1} ($CaCO_3$)	5.55					
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCO_3$)	211.16				25.0	42.0
Chloride mgl^{-1}	49.25	40.75	58.72	50.0	24.0	20.0
Nitrate $mgNl^{-1}$	8.78	7.82	9.07		1.1	1.41
Nitrite $mgNl^{-1}$					0.02	0.02
Total ox N $mgNl^{-1}$						
Ammonia $mgNl^{-1}$	0.15	0.22	0.32	9.93	0.05	0.09
Phosphate mgl^{-1}	1.05		1.56		0.1	0.20
Suspended solids (105°C) mgl^{-1}					5.0	12.4
Copper mgl^{-1}			0.01			
Cadmium mgl^{-1}			0.01			
Lead mgl^{-1}			0.01			
Chromium mgl^{-1}	0.01		0.01			
Nickel mgl^{-1}			0.01			
Iron mgl^{-1}						
Zinc mgl^{-1}			0.01			

SITE	Bollin Langley	Bollin Beech Bridge	Croco	Goyt Others-pool Bridge	Goyt Marple Bridge	Mobber-ley Bridge
DETERMINAND	MEAN	MEAN	MEAN	MEAN	MEAN	MEAN
Temperature ^{o}C	10.5	9.88	12.1	10.0	11.33	10.5
pH.	7.53	7.738	7.86	7.22	7.30	7.3
D.O. mgl^{-1}	11.37	11.23	9.6	9.633	9.93	7.10
B.O.D. mgl^{-1}	1.60	3.9	2.5	7.60	7.475	3.50
Total Hardness mgl^{-1} ($CaCO_3$)						
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCo_3$)	57.57	106.75	130.0	75.0	98.75	195.0
Chloride mgl^{-1}	20.00	43.00	4905.2	36.0	40.00	42.0
Nitrate $mgNl^{-1}$	0.92	2.30	7.2	2.63	1.89	2.25
Nitrite $mgNl^{-1}$	0.053	0.105	0.264	0.16	0.19	0.18
Total ox N $mgNl^{-1}$						
Ammonia $mgNl^{-1}$	0.18	0.40	1.06	0.90	1.96	5.05
Phosphate mgl^{-1}	0.13	0.21	1.18	0.40		1.10
Suspended solids (105°C) mgl^{-1}	&.00	15.75	29.20	20.67	24.50	19.0
Copper mgl^{-1}						
Cadmium mgl^{-1}						
Lead mgl^{-1}						
Chromium mgl^{-1}						
Nickel mgl^{-1}						
Iron mgl^{-1}						
Zinc mgl^{-1}						

SITE	Bidd-ulph Brook					
DETERMINAND	MEAN					
Temperature ^{o}C	15.2					
pH.	7.3					
D.O. mgl^{-1}	6.8					
B.O.D. mgl^{-1}	6.2					
Total Hardness mgl^{-1} ($CaCO_3$)						
Ca Hardness mgl^{-1} ($CaCO_3$)						
Mg Hardness mgl^{-1} ($CaCO_3$)						
Phenolthalein Alkalinity mgl^{-1} ($CaCO_3$)						
Total Alkalinity mgl^{-1} ($CaCO_3$)	89.0					
Chloride mgl^{-1}	70.0					
Nitrate $mgNl^{-1}$	18.65					
Nitrite $mgNl^{-1}$	0.44					
Total ox N $mgNl^{-1}$						
Ammonia $mgNl^{-1}$	8.9					
Phosphate mgl^{-1}	10.7					
Suspended solids (105°C) mgl^{-1}	10.0					
Copper mgl^{-1}						
Cadmium mgl^{-1}						
Lead mgl^{-1}						
Chromium mgl^{-1}						
Nickel mgl^{-1}						
Iron mgl^{-1}						
Zinc mgl^{-1}						

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