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The impact of copper in a contaminated stream with particular reference to Plectrocnemia conspersa (Curtis) (Trichoptera)

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The impact of copper in a contaminated stream with particular reference to *Plectrocnemia conspersa* (Curtis) (Trichoptera)

by

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CONTENTS

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ABSTRACT

An investigation of a copper contaminated mine drainage stream, the Darley Brook, revealed that the water quality was relatively constant over time, with an exceptionally high copper concentration (mean 0.89 mg l^{-1}) and low pH (mean 5.2) at its source. Virtually all of the copper was in a 'soluble' form with upto 29% as the free cupric ion. Overall mean leachable copper concentrations of 2139 $\mu g\ g^{-1}$ and 101 $\mu g\ g^{-1}$ were recorded in the uppermost reaches of the Darley Brook and a control stream respectively, with little temporal variation.

Biotic communities in the uppermost sampling stations were of reduced diversity. Excessive growth and high copper concentrations (maximum plant tissue concentration of 3664 $\mu g\ g^{-1}$) were recorded in Jungermannia atrovirens and Microspora sp. in the riffles and the pool-dwelling Juncus bulbosus. In these tolerant plants copper concentrations were higher in the summer than in the preceding winter. Macroinvertebrates were reduced to chironomid larvae, Coleoptera and the net-spinning caddis Plectrocnemia conspersa. This species was univoltine and larvae were more aggregated and occurred at higher densities (upto a mean of 126 m^{-2}) in the contaminated stream than in the control stream.

Each larval instar of P. conspersa from the Darley Brook contained significantly more copper than the same instar in the control stream. There was an exponential decrease in larval copper concentration with increasing weight for both populations, and maximum concentrations were always recorded in the summer.

An histological and ultrastructural investigation of P. conspersa larvae revealed the presence of copper containing granules in the cells of the malpighian tubules and in the subcuticular region. These may be primarily pigment granules, which serve to immobilise excess copper within the larva.

The metal tolerance of P. conspersa was confirmed by transfer experiments and discussed in relation to the occurrence of this species in metal rich waters.

CHAPTER 1

GENERAL INTRODUCTION

Copper is an essential element for living organisms and is a component of many metalloenzymes and respiratory pigments. One of the main sources of copper to aquatic animals and plants is the water they live in, and average concentrations of copper in U.K. rivers are generally low, less than 0.02 mg l^{-1} (Mance et al., 1984). Elevated copper concentrations have been recorded in some rivers draining urban industrial areas and metalliferous regions, and these high levels in the water are often reflected by elevated concentrations in the biota. However because copper is only moderately toxic to mammals and is not accumulated to very high levels by freshwater fish which man consumes, copper in freshwater biota has received less attention than, for example, lead or mercury in aquatic organisms (Stokes, 1979).

Most of the investigations in the U.K. on the effects of metalliferous mine drainage waters on aquatic organisms have been carried out in Wales (Jones & Howells, 1975; Mclean & Jones, 1975; Burton & Peterson, 1979; Brooker & Morris, 1980; Jones et al., 1985) and Northern England (Welsh & Denny, 1977; Harding & Whitton, 1978; Armitage, 1980; Abel & Green, 1981; Patterson & Whitton, 1981; Burrows & Whitton, 1983; Armitage & Blackburn, 1985), where lead and zinc are the main contaminants. By contrast there have been relatively few studies dealing with copper rich mine waters in the U.K. (Brown, 1977a; Foster, 1982a; Bryan & Gibbs, 1983), and many of the investigations in other countries have only considered chronic effects of copper on fish (e.g. Wilson et al., 1981).

In 1969 the Applied Geochemistry Research Group of Imperial College, London, collected nearly 50,000 stream sediment samples from tributary drainage at stream-road intersections throughout England and Wales, with the aim of identifying areas with anomalously high metal concentrations. The results showed that in South West England, both waters and sediments from tributaries draining mineralised areas (and affected by past mining activity) contained anomalously high concentrations of a number of metals, including copper, compared to tributaries draining unmineralised areas in the same river systems (Aston et al., 1974; Aston & Thornton, 1977; Thornton & Webb, 1977).

Mine drainage streams such as those in Cornwall are usually characterised by continuous flow and hence continuous contamination. The appearance of such streams is deceptive, in that the water is exceptionally clear as turbidity is very low due to the groundwater derivation of the streams. In fact such waters present a biologically hostile environment which is highly toxic to fish, and also toxic to most aquatic plants and invertebrates. Clearly the aquatic communities in these waters are composed of organisms tolerant of the changed conditions.

Another interesting feature of many of these metal contaminated streams in Cornwall is that they have been in existence for one hundred years or more, and yet during this time there have probably been very few changes in their physical characteristics; furthermore subsequent human influences superimposed on these watercourses have been comparatively few.

Although the metal enriched waters of South West England thus offer an opportunity to study the effect of long term metal contamination on aquatic plants and animals, to the author's knowledge only three such investigations have previously been undertaken. Brown (1977a) measured

the concentrations of copper, zinc and iron in the water, sediments and macroinvertebrate fauna of the River Hayle (which receives mine drainage from a number of adits), and Foster (1982a) investigated metal concentrations in the water and algal communities in this same river and in the River Gannel (which contains lead but is free of copper). The River Carnon drains an area with extensive tin and copper workings before entering the Fal Estuary, and Bryan and Gibbs (1983) have studied metal concentrations in the water, sediments and aquatic organisms in this contaminated estuary.

There is currently much interest in surface water acidification, and although coal mines are the single most important cause of this form of pollution, metalliferous mining is another source of low pH. The high acidity will bring metals into solution, which is clearly significant in metal rich areas. Acid mine drainage streams also represent important sources of trace metals to other watercourses, estuaries and coastal waters.

In addition to affecting metal solubility, pH also affects speciation and hence the toxicity of a metal. In the case of copper, Campbell and Stokes (1985) note that in several studies on algae, invertebrates and fish, copper toxicity was found to decrease with decreasing pH. Although there are relatively few accounts on the toxicity of copper to freshwater invertebrates (some of these studies are cited by Murphy, 1979; Mance et al., 1984) the toxicity of copper to fish has received much more attention. However the toxicity values for fish often range widely and Howarth and Sprague (1978) have suggested that this is no doubt partly because of the interaction with pH.

Although a number of studies on metal contaminated freshwater ecosystems are recorded in the literature, very few workers have measured metal concentrations in the various compartments of an ecosystem (Namminga et al., 1974; Eyres & Pugh-Thomas, 1978). This is important since metals can exist in soluble, colloidal and particulate phases, and consequently they can reach every part of a watercourse. Since in mine drainage streams water is the source of the metals, and the sediments act as a sink for these metals, it is necessary to analyse these two compartments. Furthermore analysis of the biotic compartment is important in view of metal uptake and possible modification by aquatic organisms; for example Krantzberg and Stokes (1981) demonstrated that freshwater benthic macroinvertebrates can affect the transfer of organically-bound copper to more weakly bound forms, with the simultaneous liberation of copper to the overlying waters.

In most investigations on metal contaminated waters samples are collected from selected compartments of the ecosystem, and are then analysed for their metal concentration by flame atomic absorption spectroscopy. Whilst this technique yields useful information, there are a number of other techniques which are also of value, but have to date not been commonly employed in pollution studies. For example, the free cupric ion (Cu^{2+}) is known to be toxic to fish and macroinvertebrates (Andrew et al., 1977; Howarth & Sprague, 1978; Dodge & Theis, 1979), and the use of a cupric ion electrode allows measurement of the concentration of free cupric ions.

Another technique that now has considerable potential use is graphite furnace atomic absorption spectroscopy. This can be used to measure both very low metal concentrations, and concentrations in small samples. For example, this technique can be used to analyse water and acid-digested samples of sediments, plants and animals.

In mine drainage streams there is a point source of metals, and it is informative to consider how metal concentrations vary with distance downstream from this point source. Investigations into the effects of spatial variation in metal concentrations on the structure of floral and faunal communities in metal enriched waters include those of Winner et al. (1975; 1980), Brown (1977a), Armitage (1980), Foster (1982a), Burrows and Whitton (1983) and Armitage and Blackburn (1985). Sampling at different stations along a stream also allows precise determination of where metals precipitate out of the water column, which leads to a more complete understanding of the physical and chemical conditions to which organisms are exposed.

Most of the studies on metal contaminated streams and rivers have involved the collection of samples at only one particular time of the year. Whilst some workers have sampled on a number of occasions (Brown, 1977a; Patterson & Whitton, 1981; Say et al., 1981; Foster, 1982a; Burrows & Whitton, 1983; Wehr & Whitton, 1983), samples were collected either at irregularly spaced intervals or over a relatively short time period.

Variation over time in the metal concentrations of one part of an ecosystem may affect not only concentrations in other parts of the ecosystem, but it may also have important ecological consequences. For example, Södergren (1976) has related the seasonal occurrence of different species of mayfly, as well as different generations of the same mayfly species, to temporal variation in metal concentrations in the water. Thus in the present study a regular sampling programme would be desirable in order to investigate any temporal variation in the metal concentrations in the water, sediments, plants and selected macroinvertebrates.

It is well established that plants and animals living in metal enriched waters generally contain higher metal concentrations than those in the surrounding environment. The degree to which an organism accumulates a particular metal is sometimes expressed by a 'concentration factor'. It is however important to realise that the concentration of a metal in an organism is influenced not only by abiotic factors (such as the metal concentration in the water or sediment), but also by morphological, physiological and behavioural factors. This is well illustrated in aquatic vegetation where the growth form can influence metal concentrations in the plant. For example, a filamentous alga exposes a large surface area to the surrounding water for metal uptake, whilst in a rooted macrophyte the sediment may provide an important additional source of metals. However the work of Harding et al. (1981) is one of the few studies which have measured metal concentrations in different plant species with different morphological forms growing in the same watercourse.

Similarly in aquatic macroinvertebrates, biotic as well as abiotic factors can influence the whole-body metal concentration, and yet few workers have considered the effect of these biotic factors (Elwood et al., 1976; Smock, 1983a; 1983b). In a review of metal accumulation by aquatic invertebrates, Wright (1978) concludes that different feeding habits are a source of interspecific variation in metal concentrations, and that even within a single species the feeding status may have an important bearing upon the whole-body metal concentration. However it was not until recently that Smock showed that factors such as feeding habit (Smock, 1983a) and size (Smock, 1983b) have an important effect on metal concentrations in aquatic insects.

Although there are a number of accounts in the literature on metal concentrations in aquatic organisms living in metal enriched waters,

few workers have considered the impact and fate of metals in the organisms. Indeed research in this area, particularly on tolerance mechanisms in aquatic plants and animals, is very fragmented and incomplete.

Metal tolerance mechanisms in aquatic organisms have proved to be complex, with physical/chemical barriers, excretion, regulation, storage and detoxification of metals being recorded (Antonovics et al., 1971; Bryan, 1976; Brown, 1982). A few investigations have been carried out on tolerance mechanisms in freshwater macroinvertebrates, for example Fish and Morel (1983) showed that the cladoceran Daphnia magna can excrete moderately strong metal-binding organic compounds; and several workers have recorded metal containing granules in freshwater invertebrates (Ballan-Dufrancais et al., 1971; Lhonore, 1973; Simkiss, 1979; Petit et al., 1980). The work by Brown (1976; 1977b) on the isopod Asellus meridianus is of particular relevance to the present study, since this is the only investigation on metal tolerance in a freshwater macroinvertebrate occurring in metal contaminated waters in South West England.

The mine drainage streams in Cornwall have been mentioned earlier on in this introduction, and one feature of these streams (especially those affected by copper) is the prominence of the larvae of the net-spinning caddis Plectrocnemia conspersa. Whilst Hildrew and Townsend (1976; 1982) have studied larvae of this species in an iron rich stream in the headwaters of the River Medway in Sussex, metal levels were not measured in the larvae. Furthermore no detailed study has been undertaken on P. conspersa in copper enriched waters, with the exception of some very limited information on this species in the River Hayle (Brown, 1977a). One aim of the present study was therefore to carry

out a quantitative investigation into the life cycle and population characteristics of P. conspersa larvae occurring in a copper contaminated mine drainage stream in Cornwall.

In addition to this quantitative study, it was considered that measurements of copper concentrations in different instars of P. conspersa taken from the copper contaminated stream would be necessary, since there are relatively few accounts of metal concentrations in Trichoptera (Harding et al., 1981; Burrows & Whitton, 1983; Smock, 1983a) and only one specifically on copper (Brown, 1977a). If elevated copper concentrations were found in P. conspersa larvae, then clearly it would be desirable to determine the precise location of this copper and to investigate its ultimate fate using histochemical and ultrastructural techniques. Metal tolerance in these larvae could be further studied by using other techniques such as the introduction of larvae from a population living in a clean control stream into the mine drainage stream under study.

In conclusion, it is evident that most of the studies on metal contaminated freshwater ecosystems, particularly those concerned with copper, are lacking in a number of important aspects which will be investigated in the present study. For example, metal concentrations are to be measured on a regular basis at different stations to gain an insight in to some of the inter-relationships between different compartments of a copper contaminated mine drainage stream. Furthermore the effect of long term copper contamination on the floral and faunal communities in this stream will be investigated, focusing in particular on P. conspersa. In addition to the measurement of copper in individuals of P. conspersa, it is also hoped to study how metal tolerance is achieved in this species using toxicological, histological and ultrastructural techniques.

CHAPTER 2

SITE

2.1. Introduction

The Applied Geochemistry Research Group (whose work was outlined in the previous chapter) have compiled geochemical maps showing the concentrations of elements in stream sediments throughout England and Wales (Wolfson Geochemical Atlas, 1978). From the map for copper, shown in Figure 2.1, it is evident that the highest concentrations of copper ($\geq$ 120 $\mu g\ g^{-1}$) are found in the South West of England, a result of the long history of mining activity in this area.

South West England is heavily mineralised especially in the areas of the Land's End and Bodmin Moor granites and their metamorphic aureoles. Figure 2.2 shows these granitic areas and the location of the River Lynher in Cornwall. The River Lynher is one of the main tributaries of the River Tamar Estuary draining the western edge of East Moor on the east side of Bodmin Moor. The river flows in a south-easterly direction for 34 Km reaching the tidal limit at Landrake.

The River Lynher catchment is shown in Figure 2.3. The upper reaches of the river drain moorland, rough pasture and marsh, while the lower catchment is mainly of meadowland and permanent grass with areas of arable land. There are also some afforested areas, mainly coniferous but with some deciduous woods. Settlements within the catchment are small and isolated with the exception of Callington where some light industry exists.

Both the Liskeard mining district and the western edge of the Callington and Tavistock mining district are within the catchment area of the River Lynher (see Figure 2.2). In the Liskeard district there were two important groups of mines in the Caradon region, on the south

FIGURE 2.1. Geochemical map showing the concentration of copper ($\mu g\ g^{-1}$) in stream sediments taken throughout England and Wales. (From the Wolfson Geochemical Atlas, 1978).

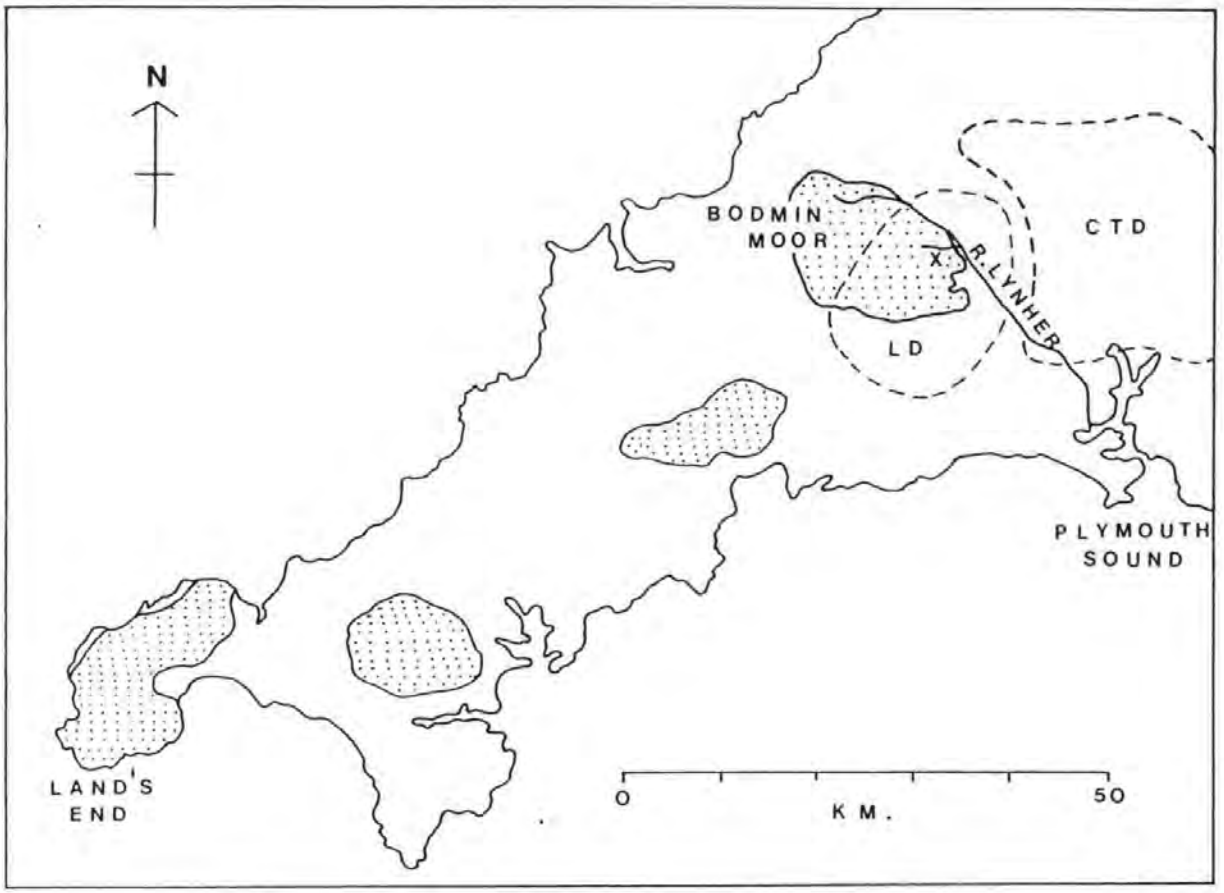


FIGURE 2.2. Location of the Darley Brook (x) and River Lynher in Cornwall, also showing the main granite areas and the Liskeard (LD) and Callington and Tavistock (CTD) mining districts.

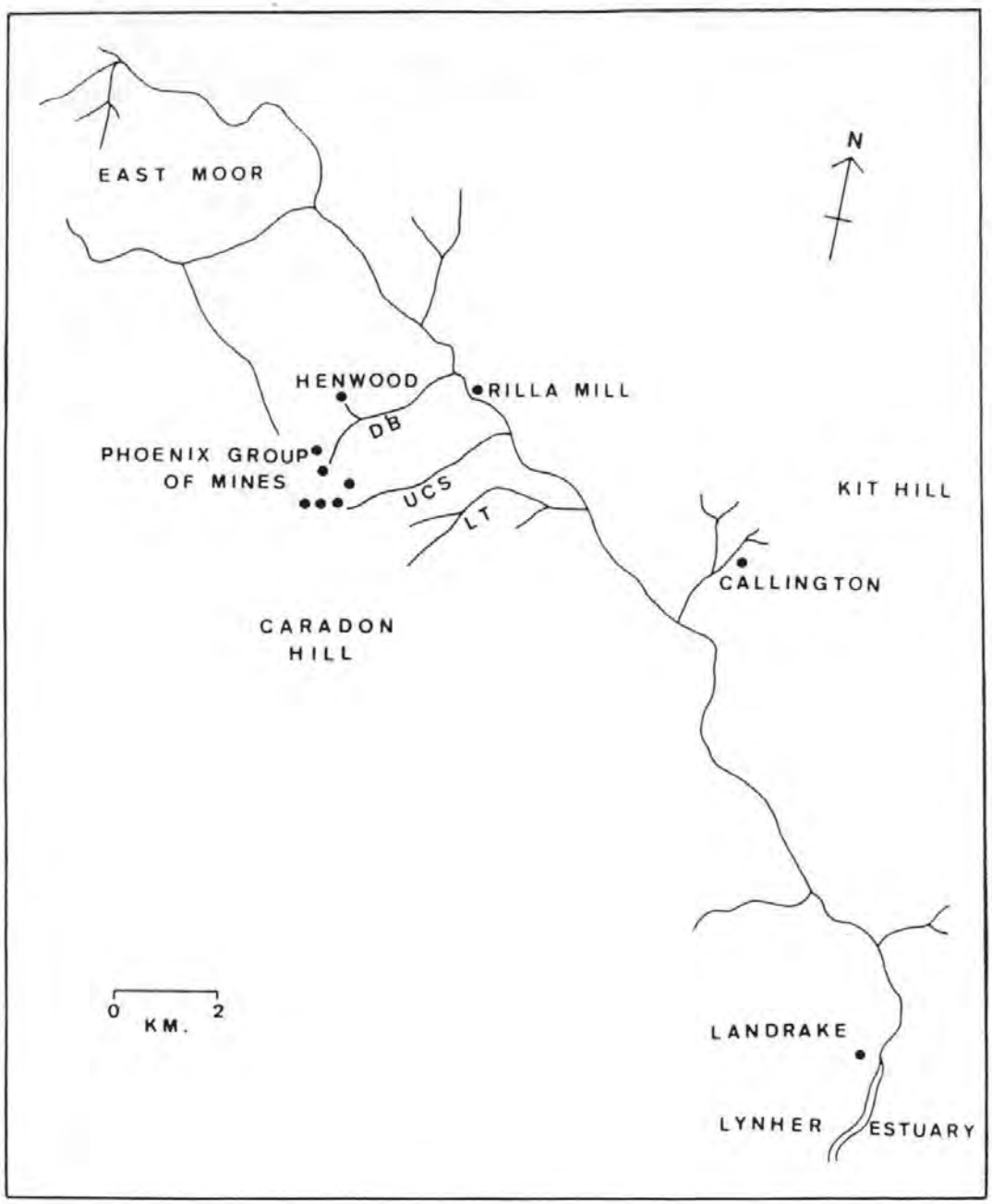


FIGURE 2.3. River Lynher catchment showing the Darley Brook (DB), Upton Cross stream (UCS), Longridge tributary (LT), the Phoenix group of mines and other features of interest.

east corner of the Bodmin Moor granite; the Phoenix group (shown in Figure 2.3) produced tin and copper, whilst the Caradon group were large copper producers but raised no tin.

Run-off from old spoil heaps, seepage and general mineralisation in the Caradon region all have some influence on the water quality of the streams draining the mining areas. In a chemical survey of the River Lynher catchment carried out by South West Water (1984), three tributaries in the Caradon region, namely Darley Brook, Upton Cross stream and Longridge tributary, were found to be affected by past mining activities, and these are shown in Figures 2.3 and 2.4. Elevated copper levels were recorded in the Darley Brook and Upton Cross stream (there was also a high zinc concentration in the latter), and borderline copper levels were measured in the Longridge tributary.

2.2. Geology and mining history

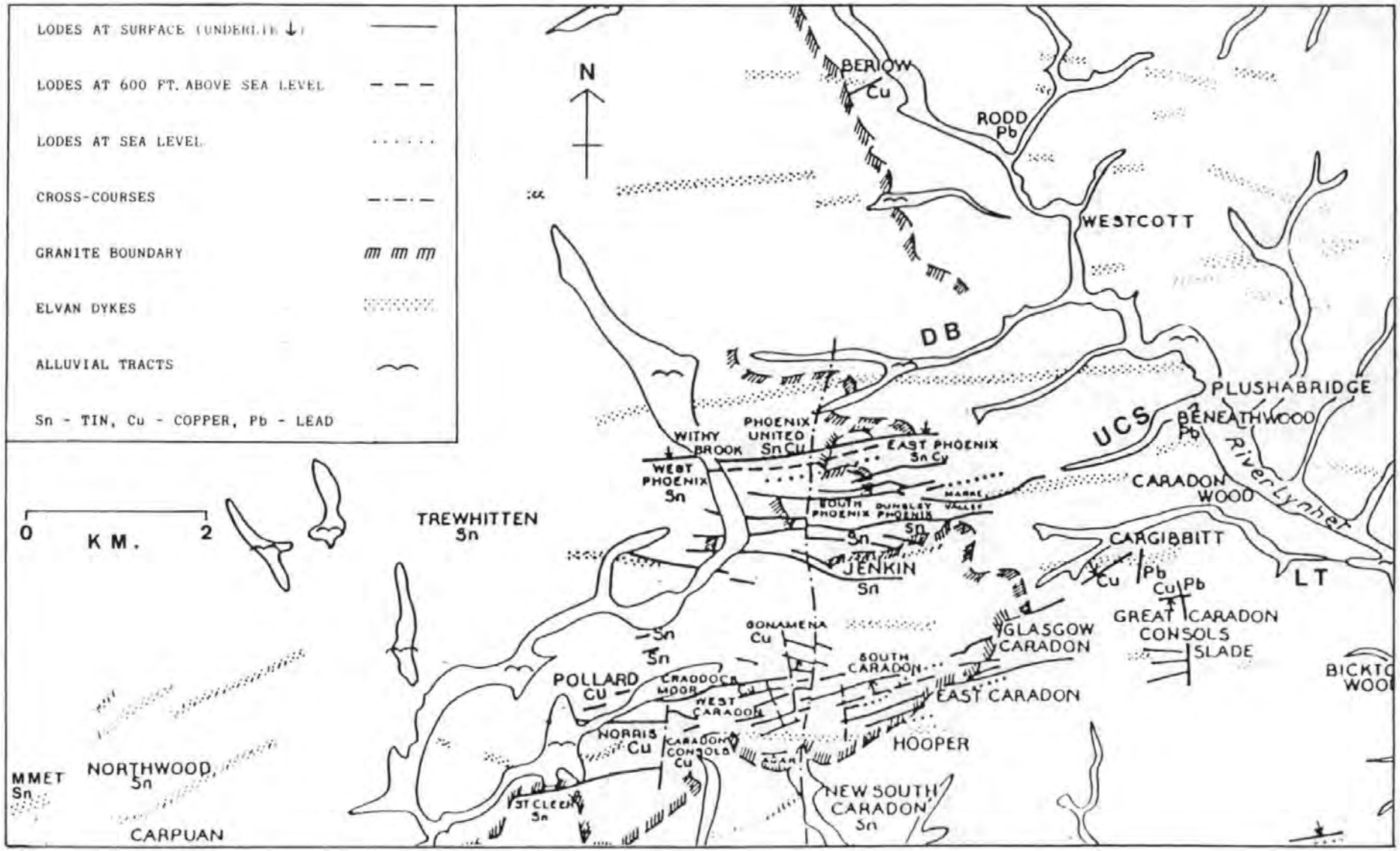
Most of South West England is composed of slaty shales and mudstones with subordinate bands of grit and conglomerate which are all embraced by the local name 'Killas' (Dines, 1956). In late Carboniferous or early Permian times granite injected into these Palaeozoic rocks formed large bosses, such as Bodmin Moor, with the adjacent killas being thermally metamorphosed.

Emanations from the magma resulted in the deposition of lode minerals, chiefly tin and copper ores, in fissures which developed along lines of weakness. Most of these fissures have an east-north-east trend, but since some run in an east-south-east direction, the lodes of the two trends are usually referred to as east-west lodes. The final stage of mineralisation was the deposition of lead, zinc and iron mainly in fissures with a north-south trend.

The sites of the major mines and lodes in the Caradon region are shown in relation to the upper catchment of the River Lynher in Figure 2.4. In both the Phoenix and Caradon groups there are several lodes in close association, most running parallel in an east-west direction. The lodes are chiefly of tin and copper, although some north-south lodes of lead occur in the Caradon group. Figure 2.4 also shows that both the Darley Brook and the Upton Cross stream drain the Phoenix group of mines, whilst the Longridge tributary receives water from the Caradon group.

Copper mining in Devon and Cornwall commenced in the late 16th century and continued only in a small way until the 18th century. The 19th century saw a rapid rise in the copper mining industry, with the peak being reached about 1860 when nearly 15,500 tons of copper were produced annually. A rapid decline then set in and by the end of the century output was negligible. Today the recovery from the whole

FIGURE 2.4. Sites of the major mines and lodes in the Caradon mining region in relation to the River Lynher; the Darley Brook (DB), Upton Cross stream (UCS) and Longridge tributary (LT) are also shown. (After Dines, 1956).


LODES AT SURFACE (UNDERLIE ↓)
LODES AT 600 FT. ABOVE SEA LEVEL
LODES AT SEA LEVEL
CROSS-COURSES
GRANITE BOUNDARY
ELVAN DYKES
ALLUVIAL TRACTS
Sn - TIN, Cu - COPPER, Pb - LEAD
N
0 KM. 2
BERIOW Cu
RODD Pb
WESTCOTT
DB
PLUSHABRIDGE
UCS
BENEATHWOOD Pb
River Lynher
WITHY BROOK
PHOENIX UNITED Sn Cu
EAST PHOENIX Sn Cu
WEST PHOENIX Sn
SOUTH PHOENIX
DUNGLEY VALLEY
MARKE
CARADON WOOD
TREWHITTEN Sn
JENKIN Sn
CARGIBBITT Cu Pb
Cu Pb
LT
GREAT CARADON CONSOLS
SLADE
BONAMENA Cu
GLASGOW CARADON
SOUTH CARADON
POLLARD Cu
CRADDOCK MOOR
WEST CARADON
EAST CARADON
NORRIS Cu
CARADON CONSOLS Cu
HOOPER
NEW SOUTH CARADON Sn
ST CLEER Sn
NORTHWOOD Sn
MMET Sn
CARPUAN

region is usually less than 50 tons of copper a year, as a by-product from the tin mines.

The Phoenix United Mine in the Caradon region (see Figure 2.4) worked two lodes in close association, one of tin and the other of copper. Mining commenced in 1836, but not far below the surface the mine was found to be unpayable for tin. About 1843 the mine was reopened, and in 1852 the main body of copper ore was encountered. Two years later the mine was in full production, producing at this time some of the richest copper ores in Cornwall.

By 1867 the copper lode appeared to have been bottomed and was approaching exhaustion. Mining turned to tin again, and throughout the 1870s the mine was one of Cornwall's major tin producers. However, by 1891 water entering the mine made it impossible to work the deepest levels continuously, and in 1897 the mine was closed due to rising costs and the low price of black tin.

In 1907 the mine was again put to work with the sinking of the Prince of Wales shaft. Six years later the shaft was finally bottomed, but over this period considerable problems were encountered with water flooding the mine. In 1914 work was stopped due both to the expense of any further development of the mine and to the high cost of pumping. Thus the Phoenix United Mine has now been idle for over 70 years (Dines, 1956; Trivett, 1981; Shambrook, 1986).

This mining activity, carried out over a period of approximately 80 years, has resulted in an extensive network of underground workings in the Phoenix United Mine. Consequently there is an increased surface area of contact between the metal ores and groundwater, and this in turn might be expected to influence the quality of the drainage water.

2.3. The Darley Brook

Darley Brook originates as groundwater flow from the Phoenix United Mine via a drainage adit (Nat. Grid Ref. SX 265723, altitude approximately 240 m) and in the wetter winter-time from an old mine shaft. It is therefore relatively unaffected by the further modifications to water quality often associated with urban, industrial and agricultural areas. The position of the adit and shaft are shown in Figure 2.5, which also shows the location of the sampling stations in the upper reaches of the Darley Brook.

Initially the stream runs through and over discarded (granite based) material from mining activity. The surrounding mine spoil is covered mainly by gorse (Ulex europoeus) reflecting the nutrient deficiency of the soil, and is reworked from time to time. The stream then flows over Upper Devonian Slates for approximately 3.5 Km through woodland and grassland before its confluence with the River Lynher (Nat. Grid Ref. SX 290737) approximately 0.5 Km upstream of Rilla Mill.

Approximately one-third of the way along the length of the Darley Brook there is a confluence with a stream which drains an area of similar geology and topography, but is relatively unaffected by mining activity. Figure 2.6 shows the position of this stream and of sampling stations along the Darley Brook.

Greer (1981) carried out a survey of the fish population in the River Lynher and its tributaries and found that the Darley Brook was the only stream totally devoid of fish, so emphasizing the need for further investigation of this stream. The Darley Brook was placed in NWC Class 2 by South West Water (1984) due to the high copper concentration recorded in the lower reaches of the stream.

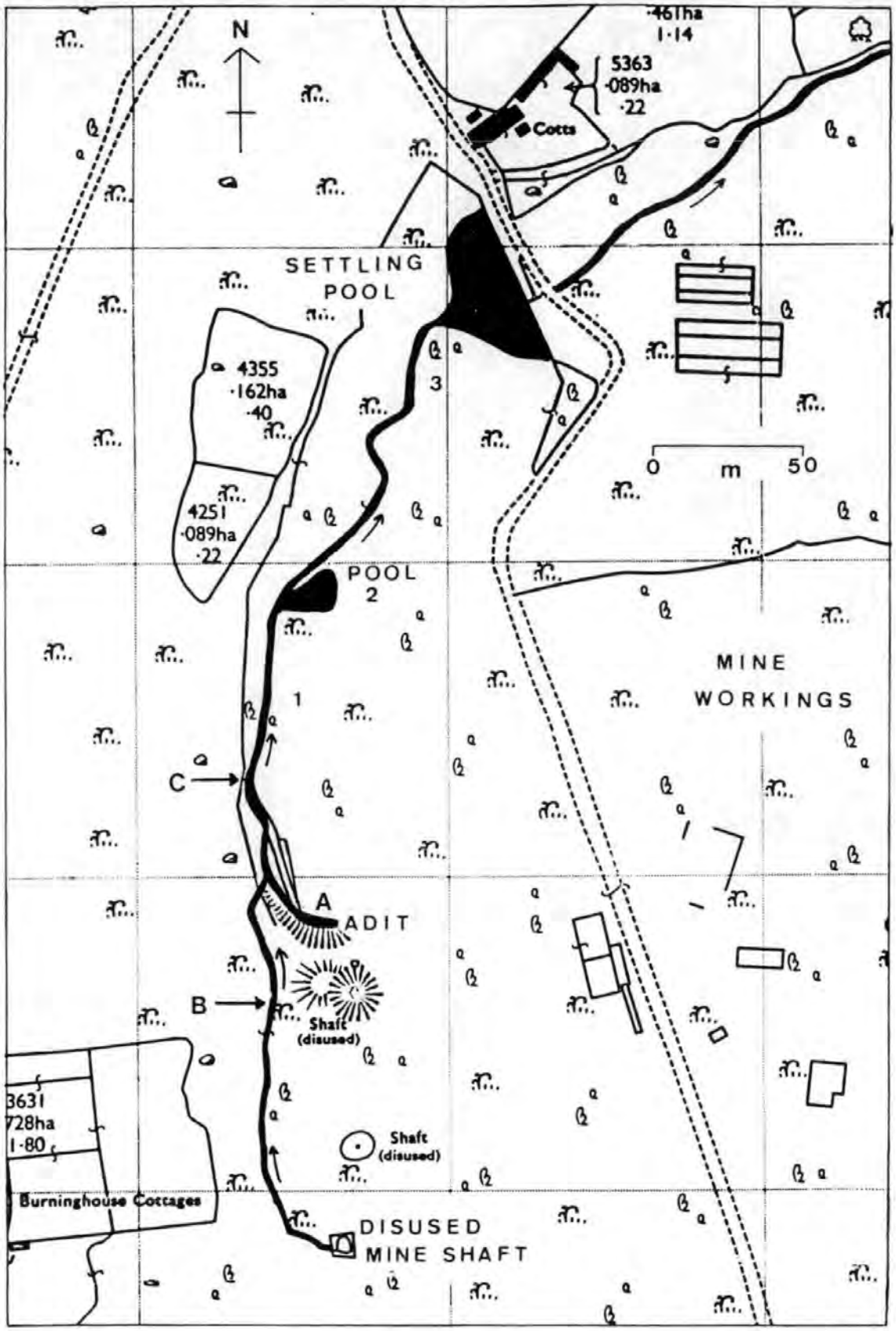
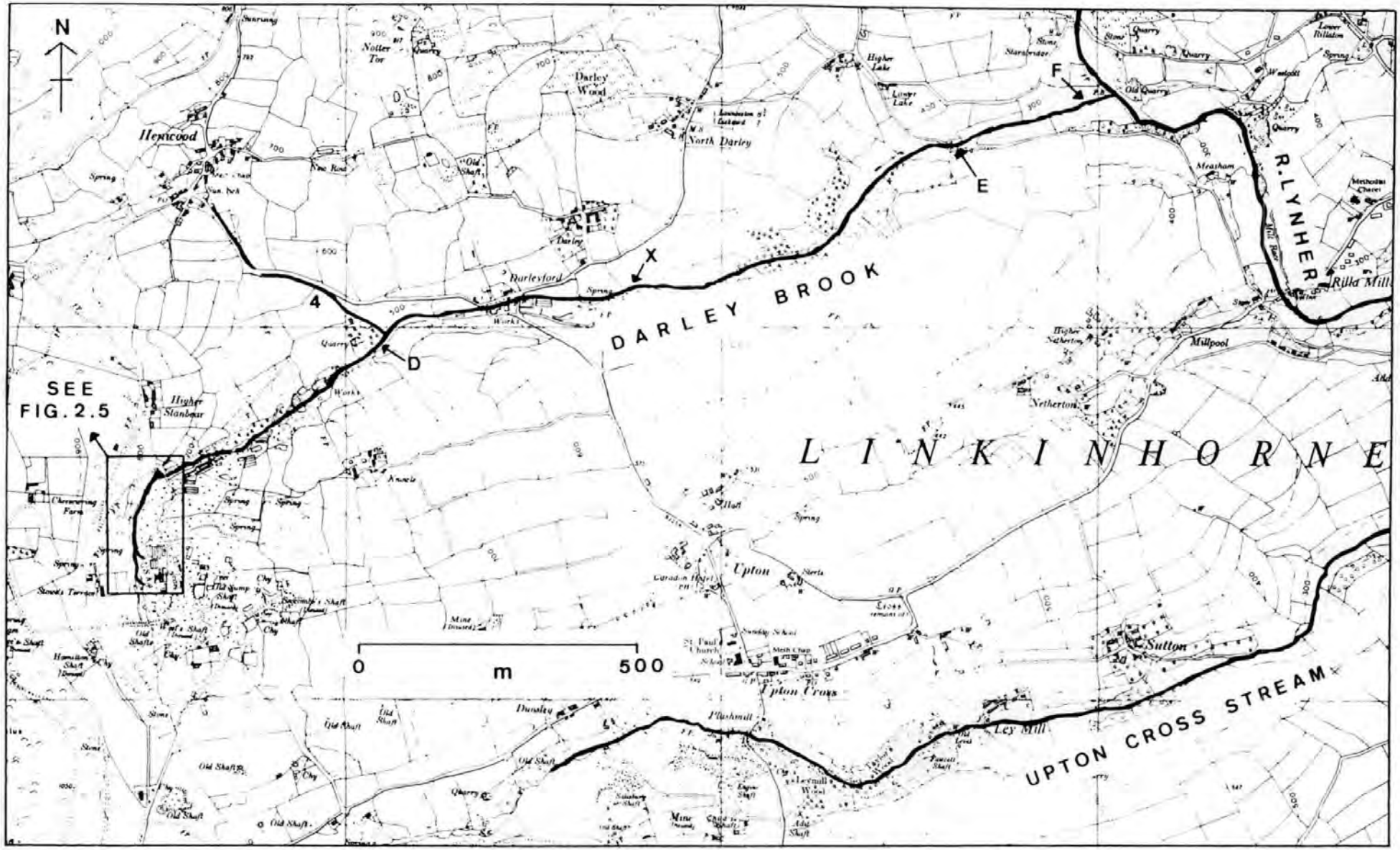


FIGURE 2.5. Upper reaches of the Darley Brook showing the adit, disused mine shaft, and location of sampling stations 1 to 3 and A to C.

FIGURE 2.6. The course of the Darley Brook from the adit source to the confluence with the River Lynher, also showing sampling stations 4, D, X, E and F, and the Upton Cross stream.


N
SEE FIG. 2.5
Hencood
Notter Tor
Darley Wood
North Darley
Higher Lake
Lower Lake
Old Quarry
Measham
R. LYNHER
Rilla Mill
Methodist Chapel
Burleyford
DARLEY BROOK
X
D
E
F
4
Higher Stanbear
Millpool
Higher Netherton
Netherton
LINKINHORNE
Upton
Sutton
Upton Cross
Dunsley
Plushmill
Ley Mill
UPTON CROSS STREAM
0
m
500

2.4. Sampling stations and sampling programme

Four main sampling stations (1 to 4 inclusive) were selected and their locations are shown in Figures 2.5 and 2.6. Stations 1, 3 and 4 are comparable riffle sections approximately 60 m in length with an average width of 1.5 m. There was a steady flow for much of the year, although there was some seasonal fluctuation in the level of the water, with the depth varying between 5 cm and 15 cm. The current velocity measured at station 1 remained below 20 cm s^{-1} for most of the year, i.e. when stream discharge was less than 18 ls^{-1} (see Figure 3.4). Station 2 is a large pool with an approximate area of 110 m^2 and a maximum depth of 1.0 m.

At station 1 (see Plate 2.1) the substratum consists predominantly of gravel produced from mining activity, together with larger stones up to 20 cm in diameter. Aquatic plants are prominent throughout the year and consist of randomly scattered clumps of the liverwort Jungermannia atrovirens (Nees) which may be accompanied by the filamentous green alga Microspora sp. The water at station 1 is crystal clear and nets of the caddis larva Plectrocnemia conspersa (Curtis) are evident.

At the lower end of station 1 the stream flows into a pool, station 2 (see Plate 2.2). There is a substratum of gravel and stones with a muddy deposit supporting the rush Juncus bulbosus (Linnaeus). The toxicity of the water to aquatic vertebrates is reflected by the presence of dead frogs which litter the upper reach of the Darley Brook in spring, especially at station 2 where dead spawn is found with the bodies.

Station 3 (see Plate 2.3) is similar to station 1, but the substratum is coated in a light grey-green precipitate and there is little or no vegetation. The nets of P. conspersa are fewer than at

PLATE 2.1. Station 1 looking downstream towards station 2 (marked with an arrow).

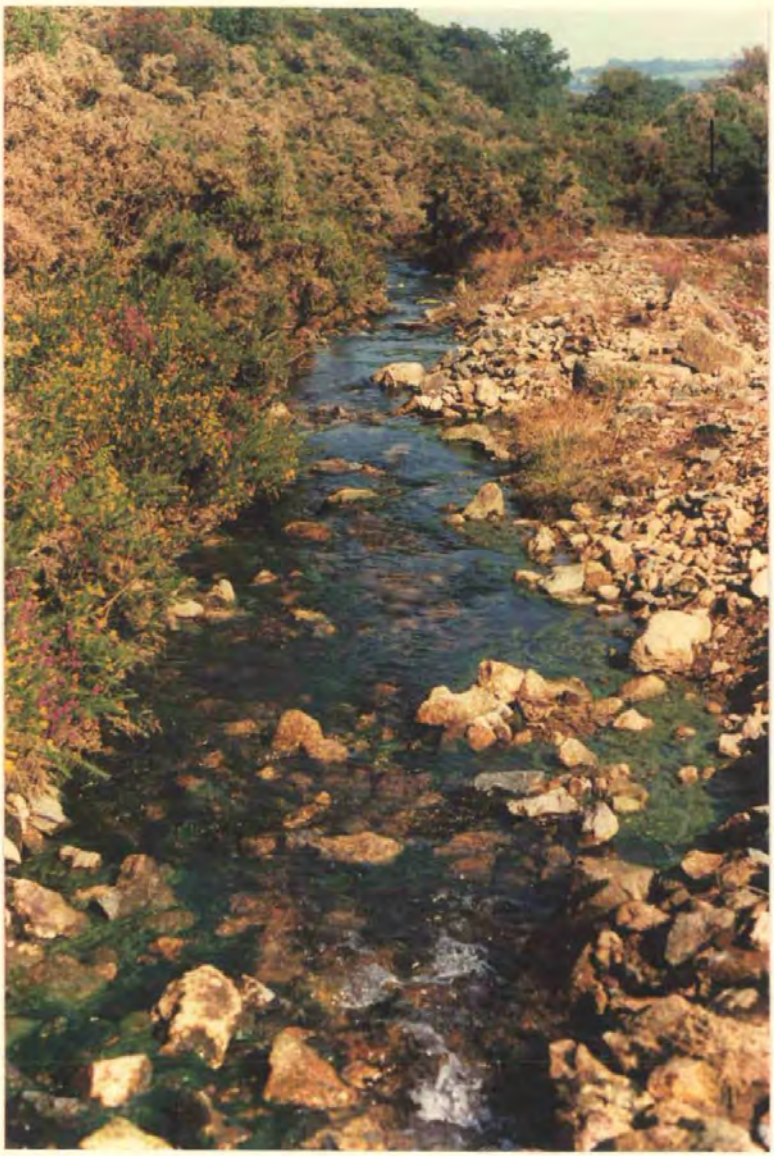
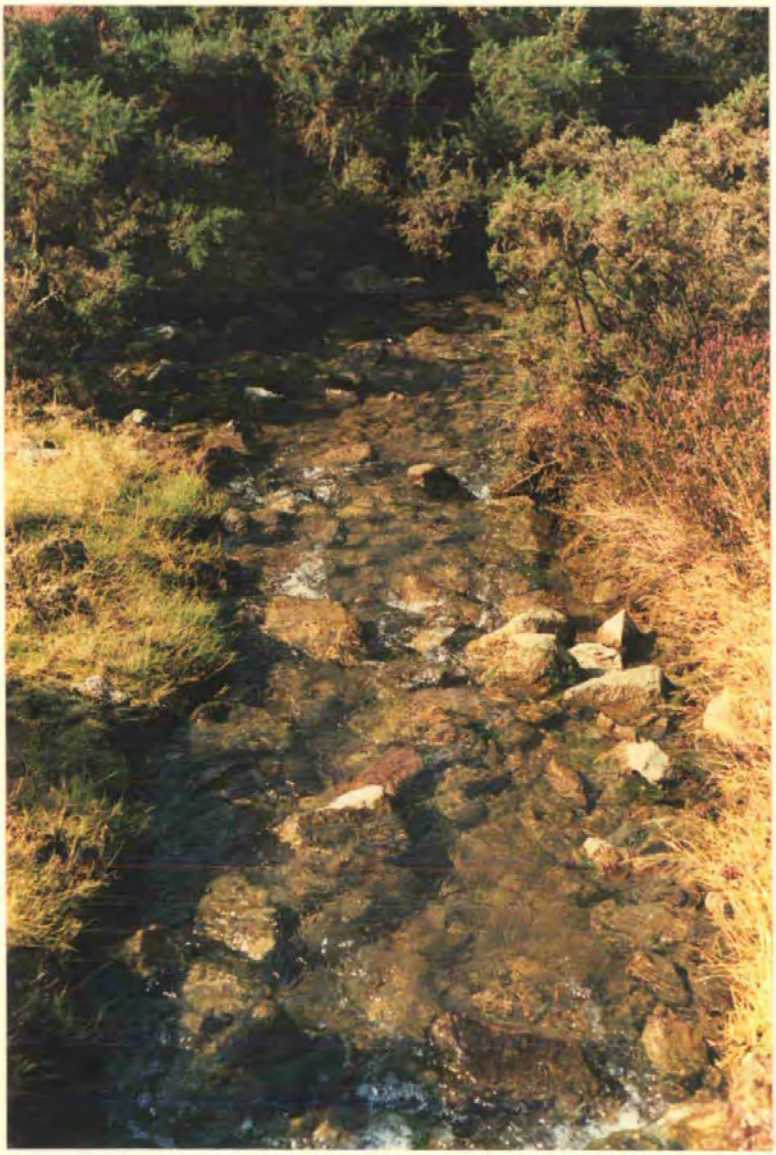


PLATE 2.2. Station 2 showing the inflow (I) on the left.
Note the emergent shoots of the rush Juncus bulbosus.

PLATE 2.3. Station 3. There is a lack of vegetation and a precipitate covers the substratum.



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stations 1 and 2 but are more prominent due to the covering of the grey-green precipitate as shown in Plate 6.3.

Station 4 (see Plate 2.4) is the stream which flows from a spring near Henwood. The stream passes through land used for rough grazing and then enters the Darley Brook approximately 1 Km along its length (Nat. Grid Ref. SX 271730). The substratum consists of stones and gravel, some of the larger stones being covered by the bryophyte Hygrohypnum luridum (Hedw.).

At these four stations several components of the aquatic ecosystem were sampled to achieve an integrated investigation into metal pollution in the Darley Brook. Water was collected monthly, sediments quarterly and plants either monthly or quarterly from January 1984 to July 1985. Invertebrates were sampled either monthly or in alternate months from December 1983 to November 1985. A complete breakdown of the sampling programme is given in Table 2.1.

Water samples were collected from six additional sampling stations (A to F inclusive), whose positions are indicated in Figures 2.5 and 2.6. Station A is at the exit from the mine drainage adit. Station B (see Plate 2.5) is the stream draining the disused mine shaft and overflows only in the winter. Station C is 10 m below the confluence of this seasonal stream and the Darley Brook, and at this station a rectangular-notch thin-plate weir was installed to measure volumetric flow (discharge). Station D is on the Darley Brook approximately 20 m above its confluence with the stream from Henwood. Station E is almost 3 Km downstream of the adit and is the sampling site for South West Water, while Station F is just prior to the confluence with the River Lynher.

Monthly water samples were collected from August 1984 to July 1985 at stations A and C, from November 1984 to February 1985 at

PLATE 2.4. Station 4, the control stream, with Sharp Tor in the background.

TABLE 2.1. Outline of the sampling programme for stations 1 to 4 from December 1983 to November 1985

SAMPLES	DATE
	1983 06/12 | 1984 23/1 20/2 20/3 25/4 21/5 18/6 18/7 20/8 24/9 19/10 21/11 13/12 | 1985 17/1 20/2 15/3 17/4 17/5 21/6 18/7 18/9 20/11
WATER AT STATIONS 1,2,3,4	— — — — — — — — — — — — — — — — — — — —
SEDIMENTS AT STATIONS 1,2,3,4	— — — — — — — — — — — — — — — — — — — —
Jungermannia atrovirens	— — — — — — — — — — — — — — — — — — — —
Microspora sp. & Juncus bulbosus	— — — — — — — — — — — — — — — — — — — —
INVERTEBRATES AT STATIONS 1 & 4	— — — — — — — — — — — — — — — — — — — —
INVERTEBRATES AT STATION 2	— — — — — — — — — — — — — — — — — — — —
INVERTEBRATES AT STATION 3	— — — — — — — — — — — — — — — — — — — —

PLATE 2.5. Confluence of the seasonal stream (station B) with the Darley Brook (A) December 1984 (B) July 1985.

B

A

station B, and from April 1985 to July 1985 at stations D, E and F.

In addition to the sampling at the four main stations, invertebrates were also collected at stations D, F and X in July 1984 and in April 1986. Station X is a riffle section of the Darley Brook with a substratum of stones and gravel, and is approximately 850 m downstream of the confluence of station 4 with the Darley Brook (see Figure 2.6).

CHAPTER 3

WATER

3.1. Introduction

The biotic community in the Darley Brook has been exposed to mine drainage for many years, and since the water represents both the source and one of the reservoirs of metals in this ecosystem it is necessary to evaluate this component. Although there is no previous information on the chemistry of the headwaters of the Darley Brook, South West Water (1984) found that the copper concentration in samples taken prior to the confluence with the River Lynher exceeded the EEC Freshwater Fish Directive G value for salmonids, so drawing attention to the need for further investigation.

There is a lack of information on seasonal variation in the quality of mine drainage waters. The concentrations of copper, zinc and iron were measured at irregular intervals in a study on the River Hayle, Cornwall (Brown, 1977a), while Patterson and Whitton (1981) investigated monthly changes in a number of physicochemical variables in a metal contaminated stream, and the water quality of a stream with elevated lead and zinc levels was studied monthly by Wehr and Whitton (1983). Thus in the investigation undertaken here, it was considered necessary to study any seasonal variation in the water quality, so that the full impact on the stream community could be assessed.

Most metals can exist in river waters in a number of different chemical and physical forms or species. Metal speciation can markedly affect the properties and behaviour of a metal in rivers, and the toxicity of heavy metals is more a function of chemical species than the total metal concentration. Several workers have shown that the

chemical species of copper most toxic to fish and aquatic invertebrates are the cupric and copper hydroxy ions (Andrew et al., 1977; Howarth & Sprague, 1978; Dodge & Theis, 1979). It was therefore considered important to measure not only the total copper concentration in the headwaters of the Darley Brook, but also the concentration of free cupric ions.

The toxic effect of metals varies with the quality of the water, hardness and pH being particularly important. In general, metals tend to be more toxic in soft waters. In a study on rainbow trout (Howarth & Sprague, 1978) a high hardness was found to decrease the toxicity of copper over a range of pH conditions. Stephenson (1983) showed that copper was 4 to 6 times more toxic in soft water to Gammarus pulex than in hard water. Thus as a prerequisite for toxicity work it is important to measure the concentrations of calcium and magnesium in the water.

Mine drainage is invariably associated with low pH due to the production of sulphuric acid during the oxidation by air, water and bacteria of sulphur (Thiobacillus thiooxidans) and iron (T. ferrooxidans and Metallogenium). The high acidity of mine waters brings metals into solution, and also affects the toxicity of these metals due to changes in speciation. Although several studies (Koryak et al., 1972; Sutcliffe & Carrick, 1973; Letterman & Mitsch, 1978) have shown that under low pH conditions benthic macroinvertebrate communities are severely restricted, it is difficult to separate the respective effects of H^+ ions and metals on the biota.

Several other water chemistry variables are of interest in this type of study. Conductivity gives an indication of the buffering capacity of water, which is particularly important to aquatic organisms exposed to high H^+ ion concentrations. In general the toxicity of

metals to organisms increases with temperature (Cairns et al., 1975), probably due to an increase in metabolic activity. Temperature has also been shown to influence the amount of zinc taken up by the aquatic larvae of Simulium ornatipes (Carter & Nicholas, 1978).

The amount of oxygen dissolved in the water is of direct importance to aquatic organisms, and the toxicity of copper to fish and aquatic invertebrates is known to increase as the oxygen concentration of the water decreases due to an increased flow of water over the respiratory surfaces. The chemistry of iron is particularly affected by redox conditions, with precipitation of iron facilitating cation exchange and adsorption (Förstner & Wittmann, 1981).

The quality of waters can also be affected by volumetric flow (discharge). Williams et al. (1973) found that metal concentrations in water increased during periods of high flow, since the greater scouring action resuspended bottom sediments. The concentrations of copper and iron were also found to increase during periods of high flow in the waters of the River Hayle (Brown, 1977a). It is therefore important to determine the relationship between metal concentrations and discharge. Information on discharge also allows an investigation of mass-flow effects, which are important when considering the transport of a metal down a stream or river.

3.2. Materials and Methods

3.2.1. Metals

Two water samples were collected at each sampling station. A filterable fraction was obtained by filtering immediately through a 0.45 μm Millipore filter (Millipore Corporation, Bedford, Massachusetts) into a 150 ml polythene bottle. Any material passing through this filter will act largely as though it were truly dissolved with respect to availability to the biota (Wilson, 1976). An unfiltered sample was also collected in a 150 ml polythene bottle. The difference between the metal concentrations in the filtered and unfiltered samples can be taken as an indication of the 'particulate' fraction.

Both samples were acidified in the field to pH 1.0 using spectrosol grade nitric acid (BDH Chemicals, Poole, Dorset), to ensure the metal ions remained in solution (Smith, 1973). All equipment used for sample collection and storage was washed first with 1 M hydrochloric acid and then distilled deionised water prior to use.

Flame atomic absorption spectroscopy (flame AAS) was used to measure the concentrations of copper, iron, calcium and magnesium. A fine mist of sample is sprayed into a flame where atomization occurs. The resulting metal atoms are excited by a hollow cathode lamp, and absorb energy allowing transition from the ground state to a higher energy level. The amount of energy absorbed is related to the concentration in a similar way to Beers' Law in molecular spectroscopy.

Flame AAS may be subject to chemical interferences. In the case of calcium and magnesium, anions such as phosphate can form less volatile compounds in the sample which hinder the formation of atoms. This is overcome by adding an excess of lanthanum chloride (10,000 mg l^{-1}) as a 'releasing agent', with the interfering anion forming a compound with the lanthanum so releasing calcium and magnesium.

Flame AAS was carried out on a Varian AA-975 Atomic Absorption Spectrophotometer (Varian Techtron Limited, Mulgrave, Australia). Full details of the operating conditions are given in Appendix 1A.

Arsenic concentrations were measured by the more sensitive technique of graphite furnace atomic absorption spectroscopy (graphite furnace AAS). The flame is replaced by an electrothermally heated hollow graphite tube, into which a small volume (typically 10 to 15 µl) of sample is injected. The sample is then slowly dried before the graphite tube is rapidly heated to the atomization temperature.

As the sample is injected into a relatively confined space graphite furnace AAS offers particular advantages over flame AAS when sample size is limited, because of the poor nebulization efficiency and dilution in the flame associated with flame AAS. The very rapid increase to the atomization temperature causes a high concentration of atoms within the graphite tube, resulting in a much higher sensitivity when compared with flame AAS.

Graphite furnace AAS is usually regarded as being more prone to interference than flame AAS. Non-specific absorption is a common problem and is removed by the technique of background correction. In the specific case of arsenic, nickel nitrate (1000 mg l^{-1}) is added to the sample to form nickel arsenide so reducing loss of the analyte during ashing. Such matrix modification also permits the use of higher ashing temperatures so reducing background and other matrix effects.

Arsenic concentrations were measured using a Pye SP9 Atomic Absorption Spectrophotometer with SP9 Computer and PU9095 Video Furnace Programmer (Pye Unicam Limited, Cambridge). Full details of the operating conditions are given in Appendix 1D. A comprehensive account of both flame AAS and graphite furnace AAS is given by Ebdon (1982).

Free ionic copper, i.e. Cu^{2+} ions, in solution was determined using an ion-selective electrode as recommended by Stiff (1971) and Kamp-Nielsen (1972). The cupric ion electrode consists of a membrane containing sulphides of copper and silver. When in contact with a cupric solution an electrode potential is set up across the membrane, and this is related to the level of cupric ions by the Nernst equation:

$$E = E^{\theta} + \frac{RT}{nF} \log_e a_{Cu^{2+}}$$

where E = measured electrode potential

E^{θ} = reference potential (a constant)

R = gas constant

T = absolute temperature

nF = quantity of electricity carried (n = number of electrons transferred; F = Faraday constant)

$a_{Cu^{2+}}$ = activity or 'effective concentration' of cupric ions

The cupric ion concentration was measured directly in the field and also in unfiltered non-acidified water samples brought back to the laboratory. In the laboratory an ionic strength adjuster (ISA) was added to all standards and samples to ensure the background ionic strength was high and constant relative to variable concentrations of cupric ion. 2 ml of 5 M sodium nitrate (ISA) was added to every 100 ml of standard or sample.

In this work measurements were made with an Orion Model 94-29 cupric ion electrode (Russell pH Limited, Fife), with a KCl single junction electrode and Pye Unicam PW9409 digital pH/mV meter. All solutions were allowed to come to ambient temperature and were stirred with a magnetic stirrer. Between measurements the electrode was rinsed with distilled deionised water and blot dried with tissues.

All labware was acid washed and thoroughly rinsed, and the following analytical procedure was used:

1. 2 ml of ISA were added to 100 ml of a 0.10 mg l^{-1} copper standard in a beaker, and left until the reading was stable.

2. (1) above was repeated with a 1.00 mg l^{-1} copper standard. Correct electrode operation was indicated by a difference between the two standards of 28 $\pm$ 2 mV, assuming solution temperature was between 20-25°C.

3. A calibration curve of electrode potential (linear scale) against copper concentration (logarithmic scale) was plotted.

4. 2 ml of ISA were added to 100 ml of sample in a beaker, and the reading recorded when stable. This stage was repeated for all the samples taking the necessary precautions and care to avoid contamination.

Correct electrode operation in the field was checked by taking two acidified standards of 0.10 mg l^{-1} and 1.00 mg l^{-1} copper out to the field in polythene bottles.

3.2.2. Additional water quality variables

Total oxidised nitrogen and orthophosphate were measured by collecting unfiltered non-acidified water samples in 150 ml polythene bottles at each of the sampling stations from November 1984 to October 1985. Analysis was carried out using a Technicon AutoAnalyzer II (Technicon Instruments Co. Ltd., New York).

pH was measured using a Camlab pH Meter Model LD (Camlab Limited, Cambridge) which was calibrated in the field with buffers of pH 5.0 and pH 7.0. Conductivity (K) was recorded using an Electrolytic Conductivity Measuring Set Model MC-1, Mark V (Kent Industrial Measurements Limited, Surrey) which compensates for water temperature and expresses conductivity at 25°C, i.e. K_{25} value.

Dissolved oxygen, % oxygen saturation and water temperature were all measured using a YSI Model 58 Dissolved Oxygen Meter (Yellow Springs Instruments Co. Inc., Yellow Springs, Ohio). This meter was air-calibrated in the field, based on the principle that at a certain altitude there will be a known % oxygen saturation. Water and air temperatures were also recorded on maximum-minimum thermometers (Fisons PLC, Loughborough).

A rectangular-notch thin-plate weir was installed at the top of station 1 (see Plate 3.1) where it was possible to record total discharge, i.e. the continuous adit flow and the seasonal winter flow from the mine shaft. Although a second weir was installed at the adit it was found to yield unreliable data due to leakage through granite fissures around the weir.

The type and size of weir was calculated beforehand so that after installation the area and depth of water in the channel above the weir and conditions below the weir remained unchanged. The weirs were inserted on 17.4.84 after a long dry period when the water level

PLATE 3.1. Rectangular-notch thin-plate weir installed at the top of station 1 to measure stream discharge.


8
7
6
5
4
3
2
1
0
PLEASE DO NOT TOUCH

in the stream was low, and so disturbance to the stream ecosystem was minimal. The installation of the weirs required a certain amount of careful excavation, concreting and sealing to ensure that the entire flow of water passed through weirs of known and constant shape.
A stage board was installed to measure the height of water (h cm) in the notch and this was related to discharge (Q ls^{-1}) using the graph shown in Figure 3.1.

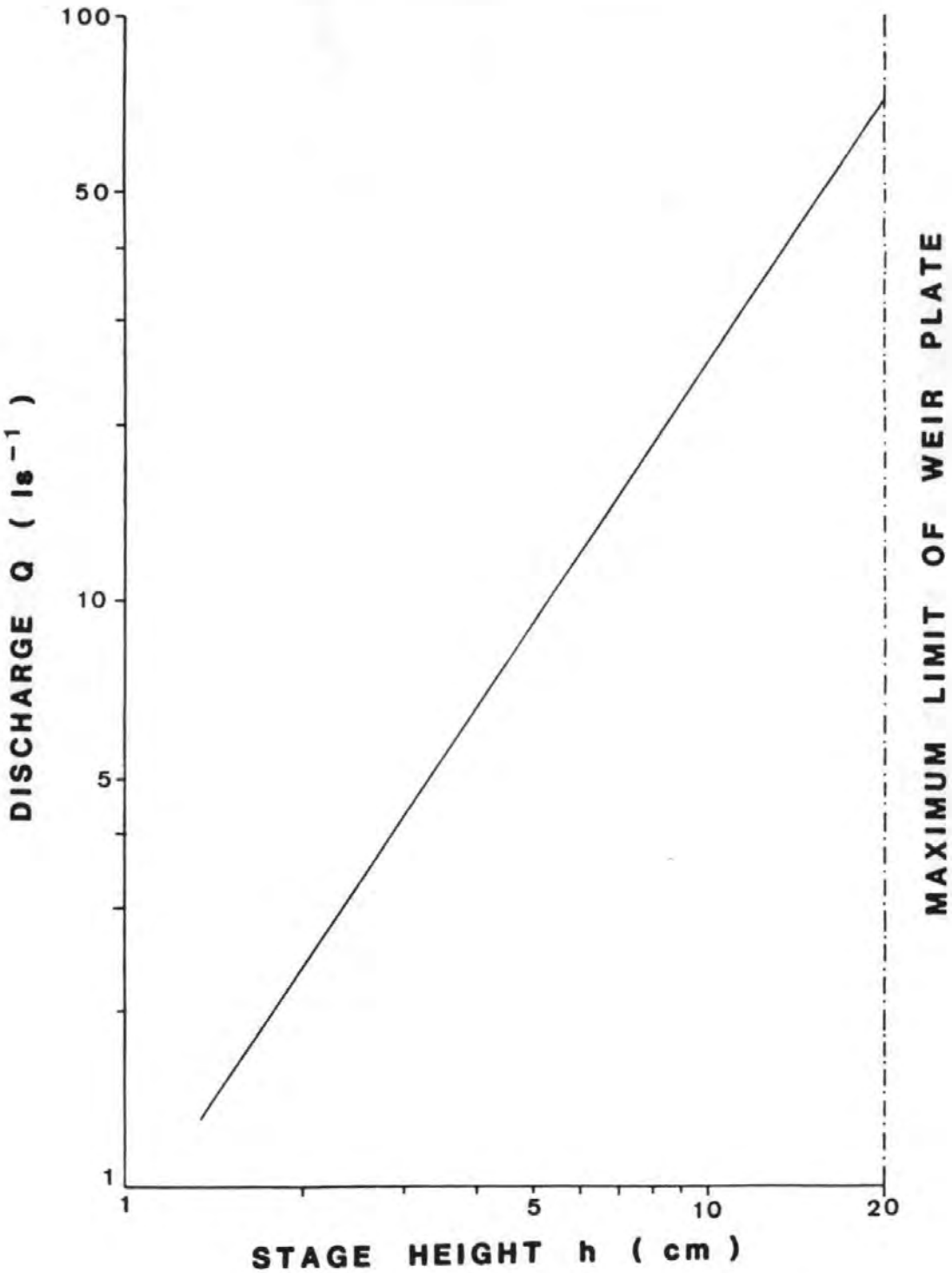


FIGURE 3.1. Relationship between height of water (h) in the rectangular-notch thin-plate weir and discharge (Q).

3.3. Results

The precision of the flame AAS method was determined by taking six filtered and six unfiltered water samples from station 1 in January 1984. The results are presented in Table 3.1, and since there was no significant difference (at $p = 0.05$ level) between the concentration of a metal in the six samples, only one filtered and one unfiltered sample were collected at each station on each occasion.

Neither iron nor arsenic, with detection limits of 0.045 mg l^{-1} and 0.002 mg l^{-1} respectively, were measurable in water from any station during the entire sampling period.

After the first eleven months of the sampling programme the metal concentrations in the filtered and unfiltered samples were compared (see Table 3.2). There proved to be no significant difference (at $p = 0.05$ level) between these two fractions, and subsequently unfiltered water samples were collected.

The results for the metal concentrations, together with other selected physicochemical variables are recorded in Tables A-I (see Appendix 2), and are summarised in Table 3.3. The metal concentrations were determined from unfiltered water samples and unless stated were analysed by flame AAS. Hardness was calculated by applying the formula:

$$CaCO_3 = (Ca \times 2.497) + (Mg \times 4.116)$$

where all the concentrations are in mg l^{-1}.

These data allows us to characterise more fully the water quality of the Darley Brook and to study changes in a number of physicochemical variables along its length. The groundwater at station A had a very high copper concentration (mean 0.89 mg l^{-1}), was 'soft' in nature (mean hardness 8.25 mg l^{-1}) and acidic (mean pH 5.2). In addition the water was poorly oxygenated (mean dissolved oxygen concentration of 5.19 mg l^{-1}), had low levels of orthophosphate and total oxidised nitrogen, and was always between 10°C and 11°C.

TABLE 3.1. Mean concentrations of copper, calcium and magnesium (mg l^{-1}) in replicate (n = 6) unfiltered and filtered water samples collected at station 1 in January 1984.

METAL	CONCENTRATION (mg l^{-1}) MEAN	2 S.D.	*CHARACTERISTIC CONCENTRATION (mg l^{-1})
Cu - UNFILTERED	0.99	0.02	0.040
- FILTERED	0.93	0.01	
Ca - UNFILTERED	1.08	0.02	0.013
- FILTERED	1.04	0.02	
Mg - UNFILTERED	1.16	0.06	0.003
- FILTERED	1.15	0.05	

(*From Ebdon, 1982)

TABLE 3.2. Metal concentrations (mean and range) in unfiltered and filtered water samples collected over 11 months from January to November 1984.

		COPPER mg l^{-1}	CALCIUM mg l^{-1}	MAGNESIUM mg l^{-1}
STATION 1	UNFILTERED	0.87 0.78 - 0.99	1.48 1.04 - 2.37	1.10 0.88 - 1.36
	FILTERED	0.84 0.77 - 0.93	1.45 1.02 - 2.33	1.06 0.86 - 1.20
STATION 2	UNFILTERED	0.79 0.68 - 0.94	1.63 1.02 - 3.09	1.07 0.86 - 1.27
	FILTERED	0.77 0.66 - 0.92	1.61 1.01 - 3.06	1.05 0.85 - 1.22
STATION 3	UNFILTERED	0.67 0.57 - 0.77	1.57 1.05 - 2.54	1.04 0.85 - 1.25
	FILTERED	0.64 0.52 - 0.75	1.55 1.04 - 2.51	1.02 0.85 - 1.20
STATION 4	UNFILTERED	<0.04	3.03 2.10 - 4.02	0.92 0.75 - 1.07
	FILTERED	<0.04	2.99 2.07 - 4.02	0.90 0.74 - 1.02

TABLE 3.3. Concentrations (mean and ranges) of selected physicochemical variables recorded from monthly samples collected from January 1984 to July 1985 (n = 19 for stations 1,2,3 and 4; n = 12 for stations A & C; n = 4 for stations B,D,E, & F). Metal levels were determined from unfiltered samples. *analysis by graphite furnace AAS. **n = 1.

STATION	COPPER mg l^{-1}	CALCIUM mg l^{-1}	MAGNESIUM mg l^{-1}	HARDNESS mg l^{-1} $CaCO_3$	CONDUCTIVITY K_{25} $\mu S\ cm^{-1}$	pH	DISSOLVED OXYGEN mg l^{-1}	% OXYGEN SATURATION	TOTAL OXIDISED NITROGEN mg l^{-1} N	ORTHO-PHOSPHATE mg l^{-1} P
A	0.89 0.76-0.99	1.38 1.12-1.70	1.17 1.05-1.34	8.25 7.12-9.76	79 53-100	5.2 4.9-5.6	5.19 4.35-6.42	45 40-56	1.22 1.00-1.50	0.04 0.02-0.06
B SEASONAL STREAM	0.81 0.73-0.87	1.41 1.27-1.55	1.11 1.00-1.24	8.09 7.29-8.72	75 71-78	5.5 5.2-5.7	10.14 8.87-11.05	91 78-100	1.0**	0.03**
C	0.87 0.75-0.96	1.36 1.11-1.68	1.11 1.00-1.30	7.99 6.89-9.55	79 52-100	5.4 4.9-5.8	6.95 5.90-8.72	62 53-79	1.33 0.95-2.05	0.03 0.02-0.05
1	0.86 0.74-0.99	1.41 1.04-2.37	1.10 0.88-1.36	8.04 6.22-10.69	76 52-101	5.5 4.9-6.1	9.31 7.67-11.02	85 70-106	1.19 0.90-1.70	0.03 0.01-0.06
2	0.79 0.68-0.94	1.53 1.02-3.09	1.06 0.83-1.27	8.19 6.09-11.83	75 52-92	5.6 5.0-6.4	10.47 9.30-12.20	97 86-108	1.39 0.90-2.50	0.03 0.01-0.05
3	0.67 0.57-0.77	1.48 1.05-2.54	1.04 0.80-1.25	7.97 6.12-10.62	68 51-86	5.7 5.0-6.4	9.95 7.19-11.67	91 72-103	1.43 1.00-2.30	0.02 0.01-0.04
D	0.29 0.24-0.32	1.76 1.70-1.80	1.14 1.00-1.30	9.08 8.36-9.80	N O T D E T E R M I N E D				2.12 1.30-3.10	0.03 0.01-0.04
E	0.13 0.11-0.15	2.48 2.23-2.72	1.67 1.55-1.75	13.07 12.37-13.58	N O T D E T E R M I N E D				3.26 2.90-3.80	0.03 0.01-0.06
F	0.029*	2.53 2.31-2.84	1.71 1.55-1.80	13.33 12.42-14.17	N O T D E T E R M I N E D				3.47 2.95-4.15	0.03 0.01-0.05
4	0.025*	2.82 2.07-4.02	0.91 0.75-1.07	10.81 8.33-14.20	80 54-102	6.4 5.7-7.4	10.55 8.60-12.87	96 85-110	1.98 1.45-3.30	0.05 0.02-0.09

52

The water at station B was similar in quality to that at station A, for example the mean copper concentration was 0.81 mg l^{-1}. However, the oxygen content of this seasonal stream was approximately twice that of station A.

There was a downstream decrease in copper concentration along the Darley Brook, which is shown graphically in Figure 3.2A, with the concentration falling from 0.89 mg l^{-1} at station A to 0.029 mg l^{-1} at station F. By contrast hardness was found to increase along the length of the Darley Brook (see Figure 3.2B) from 8.25 mg l^{-1} at station A to 13.33 mg l^{-1} at station F. Total oxidised nitrogen showed a similar trend to hardness, with concentrations rising from 1.22 mg l^{-1} at the adit to 3.47 mg l^{-1} in the lowermost reaches.

The copper concentration at station 4 was below the detection limit of flame AAS (i.e. < 0.04 mg l^{-1}) throughout the sampling period, and subsequent determination by graphite furnace AAS indicated the copper concentration was 0.025 mg l^{-1}. Low hardness values were recorded at station 4 (mean 10.81 mg l^{-1}), and the water had a mean pH of 6.4. The water was well oxygenated (mean dissolved oxygen concentration of 10.55 mg l^{-1}), and had low levels of orthophosphate and total oxidised nitrogen.

Records of air temperature and maximum-minimum water temperatures at stations 1,2,3 and 4 are shown in Figure 3.3. Whilst air temperatures ranged from -5°C to 27°C over the sampling period, there was a much narrower range of stream temperatures (from 6°C to 19°C) so emphasizing the more constant conditions in the water.

Maximum-minimum water temperatures at all four stations exhibited a clear temporal variation, reaching a maximum in summer (July/August) and a minimum in winter (January/February). Water temperatures recorded on the day of sampling are given in Appendix 2H,

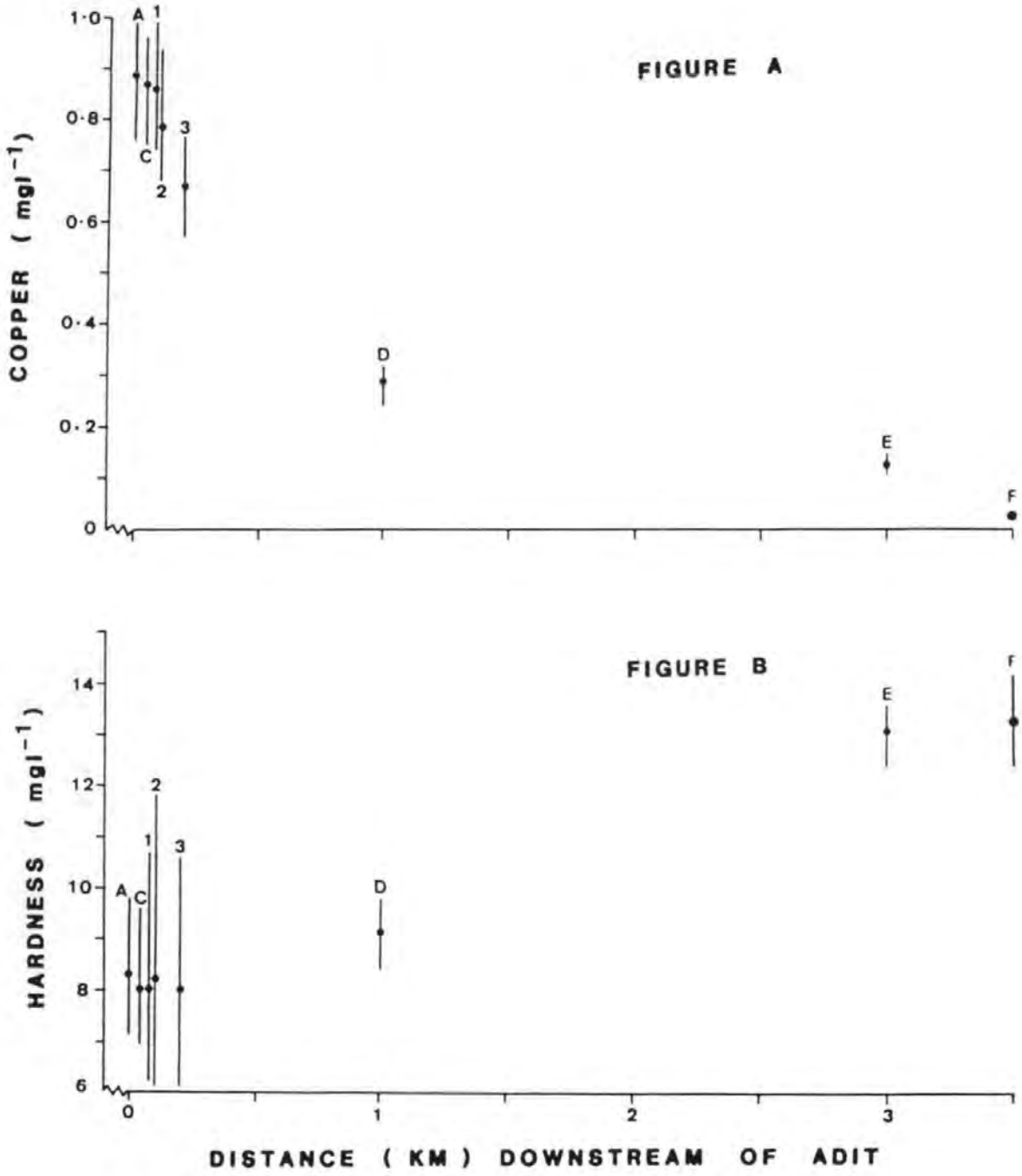


FIGURE 3.2. Range and mean of copper concentrations (Figure A) and hardness (Figure B) in monthly samples at eight stations in the Darley Brook.

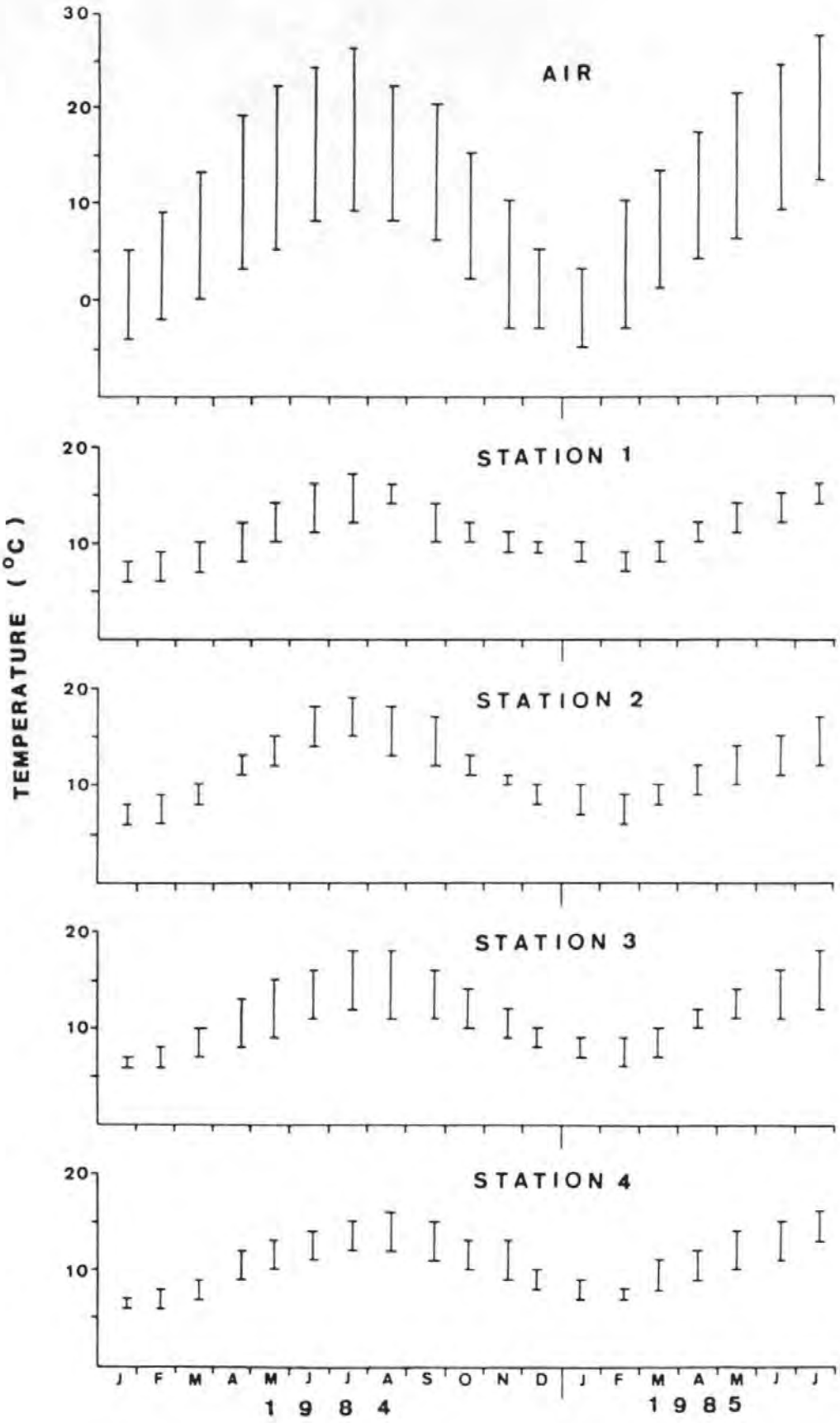


FIGURE 3.3. Maximum and minimum air temperatures, and maximum and minimum water temperatures recorded at stations 1 to 4, from January 1984 to July 1985.

and showed a similar seasonality to the maximum-minimum data.

Figure 3.4 shows stream discharge measured at the weir installed at station C from April 1984 to July 1985. Over this period, with the exception of November to February when the seasonal stream was flowing, discharge is reflecting adit flow. The flow was found to vary from a minimum of 3 l s^{-1} (August 1984) to a maximum of 46 l s^{-1} (December 1984). Since the changes in discharge exhibit a gradual trend, this suggests that the flow at station C is derived predominantly from groundwater. Observations also suggest that the seasonal stream is supplied mainly by overflow of water from the shaft. Thus there is minimal contribution from surface run-off in this upland area.

Relationships between the physicochemical variables measured monthly at each of the four main stations, and at the adit, were examined by means of the Pearson product moment correlation coefficient. The variables that were significantly correlated ($p < 0.05$) at all the upper Darley Brook stations are shown in Table 3.4. Both the copper and magnesium concentrations in unfiltered water were positively correlated with stream discharge, i.e. the concentration of the two metals increases as discharge increases. There was a negative relationship between pH and discharge, i.e. as discharge increases so conditions become more acidic. In addition to the correlations shown in Table 3.4, a negative relationship between dissolved oxygen and water temperature was slightly below the $p = 0.05$ level at stations A, 1 and 2, and was significant at stations 3 and 4, i.e. the oxygen content of the water falls as the water temperature rises.

During the latter half of the sampling programme it was observed that the aluminium levels in the sediment at station 3 were much higher than at stations 1 and 2 (see p 102). It was therefore decided to measure the aluminium concentration in the water to see

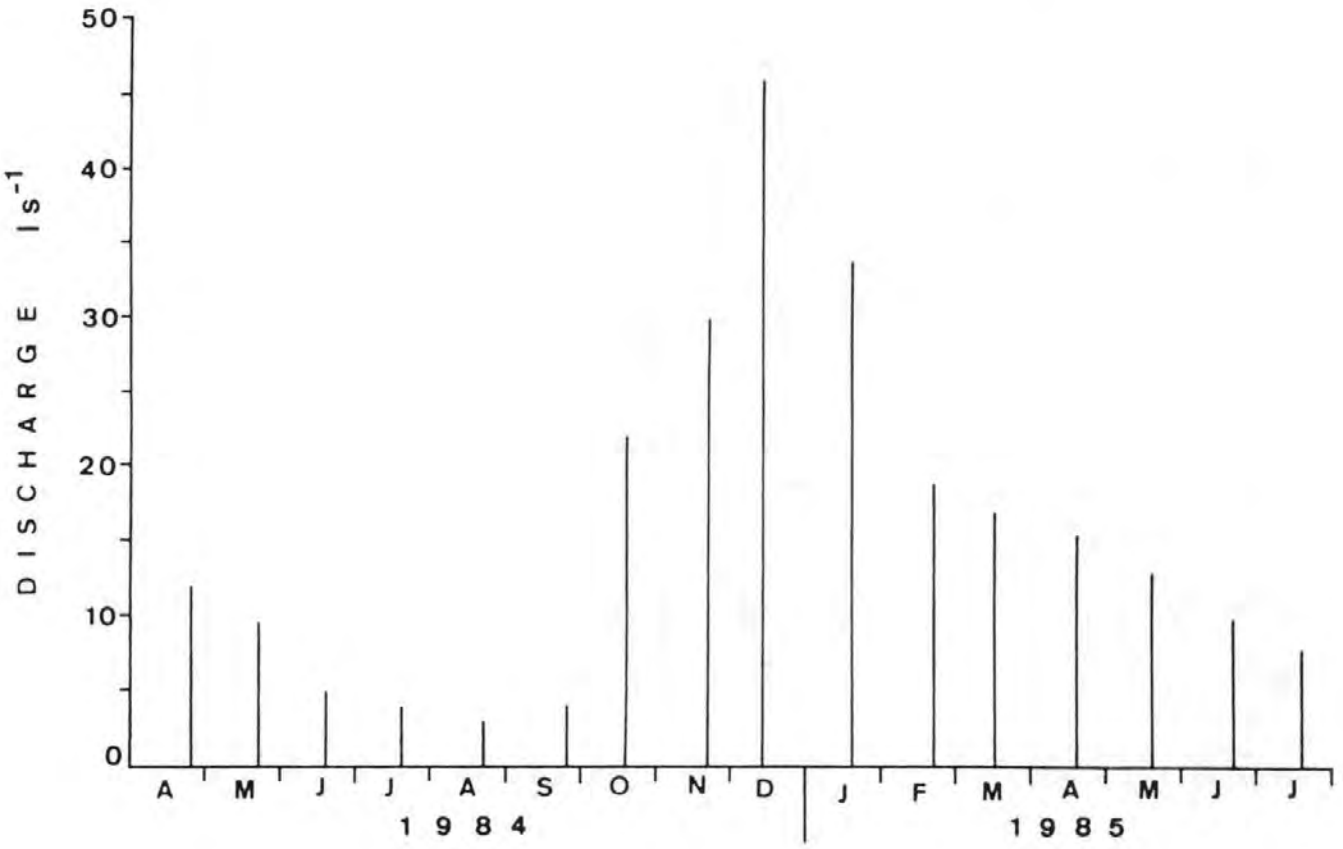


FIGURE 3.4. Stream discharge (ls^{-1}) recorded at the rectangular-notch thin-plate weir at station C from April 1984 to July 1985. (Note that the seasonal stream was flowing between November 1984 and February 1985).

TABLE 3.4. Correlation coefficients between discharge and the concentrations of copper and magnesium in unfiltered water, and pH, at 4 stations in the upper reaches of the Darley Brook (n = 16 for stations 1, 2 and 3; n = 12 for station A). N.S. Not Significant.
$^{*}p < 0.05$. $^{**}p < 0.01$.

	Discharge			
	Station A	Station 1	Station 2	Station 3
Copper	0.777^{**}	0.582^{*}	0.572^{*}	0.636^{**}
Magnesium	N.S.	0.524^{*}	0.505^{*}	0.574^{*}
pH	-0.652^{*}	-0.664^{**}	-0.566^{*}	-0.646^{**}

if there was any significant change at station 3. This analysis was carried out using graphite furnace AAS on unfiltered acidified samples collected along the length of the Darley Brook, and at station 4, in May 1986.

The results, presented in Table 3.5, show that there was little change in the aluminium concentrations between the adit (175 ng ml^{-1}) and station 2 (171 ng ml^{-1}). There was a marked decrease at station 3 (109 ng ml^{-1}), and then a more gradual decrease downstream to a minimum of 62 ng ml^{-1} at station F, just prior to the confluence with the River Lynher. The concentration at station 4 (75 ng ml^{-1}) was similar to that recorded in the lower reaches of the Darley Brook.

As previously mentioned the Cu^{2+} ion is one of the more toxic forms or species of copper to aquatic organisms. The concentrations of free ionic copper measured both in the field and in samples returned to the laboratory, between April and July 1985, are presented in Table 3.6. At the stations on the lower reaches of the Darley Brook and in the control stream, the concentration of Cu^{2+} ions was below the detection limit of the cupric ion electrode i.e. < 0.10 mg l^{-1}.

At the adit the mean concentration of free cupric ions was 0.18 mg l^{-1}, which accounts for 22% of the total copper concentration (mean 0.83 mg l^{-1}) at this station. The Cu^{2+} ion concentration then gradually falls, and at station 2 a mean concentration of 0.11 mg l^{-1} was recorded. Interestingly at station 3 the cupric ion concentration increased to a mean of 0.19 mg l^{-1}, accounting for 30% of the total copper concentration (mean 0.63 mg l^{-1}) at this station.

TABLE 3.5. The concentration of aluminium (ng ml^{-1}) in unfiltered acidified water samples collected from the Darley Brook including the control stream in May 1986. Analysis by graphite furnace AAS.

Station	Aluminium (ng ml^{-1})
A	175
C	172
1	170
2	171
3	109
D	83
E	75
F	62
4	75

TABLE 3.6. Concentration (mg l^{-1}) of copper in water and concentration (mg l^{-1}) of free cupric ions (figures shown in brackets) measured in the field (F) or in unfiltered non-acidified samples in the laboratory (L) between April and July 1985.

	STATION A	STATION C	STATION 1	STATION 2	STATION 3
APRIL (L)	0.90 (0.20)	0.86 (0.17)	0.80 (0.15)	0.77 (0.13)	0.65 (0.25)
MAY (L)	0.87 (0.17)	0.87 (0.13)	0.87 (0.11)	0.83 (0.11)	0.69 (0.19)
JUNE (F)	0.80 (0.23)	0.80 (0.16)	0.79 (0.13)	0.74 (0.12)	0.59 (0.19)
JULY (F)	0.76 (0.13)	0.75 (0.12)	0.74 (0.09)	0.70 (0.07)	0.57 (0.14)
MEAN	0.83 (0.18)	0.82 (0.15)	0.80 (0.12)	0.76 (0.11)	0.63 (0.19)

3.4. Discussion

At the adit (station A) the water quality is relatively constant over time, and reflects the integrated effects of hydrology, mineralogy and oxidising agencies. This constancy in water quality is characteristic of many mine drainage streams (Patterson & Whitton, 1981); again Foster (1982a) found "a stable pattern of copper pollution from mine adits on the River Hayle."

Discharge from the adit shows seasonal variation, and is positively correlated with both copper and magnesium concentrations in the water. This suggests that in the wetter winter time underground chambers fill with water which siphons into the adit watercourse. Thus there will be an increased mass flow of copper during periods of high discharge. The negative correlation between discharge and pH indicates that more acidic water is flushed from greater depths in the winter. A similar explanation was proposed by Koryak et al. (1972) to account for fluctuating pH values in an acid mine drainage stream.

The stream which flows from the mine shaft in winter has similar water characteristics to the adit, although the oxygen content is higher due to aeration before it reaches station B. A comparison between the stations just below its confluence (i.e. stations C and 1) and the adit (see Table 3.3) clearly shows that the stream has a negligible dilution effect on water quality.

At the four main sampling stations the difference between metal concentrations in filtered and unfiltered water samples was very small for all the metals measured, indicating most of the metals are in a 'soluble' form, e.g. only 3-5% of the copper in the upper reaches of the Darley Brook is in a 'particulate' form. By contrast Brown (1977a) found that 70% of all copper in the River Hayle was in the 'particulate' form.

The copper concentration at station 1 (mean 0.86 mg l^{-1}) is higher than that recorded for mine drainage streams in the River Hayle catchment (Brown, 1977a; Foster, 1982a), since there is little organic material in the sediment at station 1 to adsorb copper from the water. Although Butcher (1946) measured copper concentrations upto 1.20 mg l^{-1} in the water of the River Churnet downstream of a copper works, the levels of copper at station 1 are higher than those reported in other investigations on mine drainage streams in the U.K. (Burton & Peterson, 1979; Jones et al., 1985).

In order to assess the full impact of mine drainage waters on aquatic organisms it is necessary to consider temporal variation in the water quality. Whilst a few workers have analysed water samples on a monthly basis (Patterson & Whitton, 1981; Wehr & Whitton, 1983), Brown (1977a) only sampled the waters of the River Hayle in March, August and October. From just these 3 samples Brown suggests that the concentrations of copper, zinc and iron in the water increase during periods of high flow and decrease during lower flows. If the unfiltered copper concentrations measured in these same 3 months at station 1 are examined (see Appendix 2A) then a similar pattern to that observed by Brown is found; however when these samples are considered in context with the other monthly samples it can be seen that this pattern is in fact just part of the small and random variation observed in the copper concentration.

In the Darley Brook, conditions change rapidly over a relatively short distance, only 230 m downstream of the adit the copper concentration has already decreased by an average of 0.19 mg l^{-1}. At station 3 the aluminium concentration also falls rapidly due to the precipitation of alumina. Although the pH conditions further upstream are conducive to the precipitation of aluminium, this does not occur until station 3

because the kinetics of the aluminum-oxide bond and of precipitation are slow. It is thus suggested that copper and aluminium co-precipitate out at station 3, an argument supported by sediment analysis (see p 103).

As stated previously most of the copper in this stream is in a 'soluble' form, this fraction includes the free cupric ion and some organic and inorganic complexes. Stiff (1971) added copper to several UK river water samples and found that the free Cu^{2+} ion accounted for only 0.1 - 0.4% of the total copper concentration. However in this investigation at the adit 17-29% of the copper is in the form of free cupric ions.

Florence (1977) determined the chemical form of copper in four natural freshwaters and found that most of the copper was associated with organic matter, probably organic colloids. The speciation of copper in freshwater is also affected by pH, and work by Sylva (1976) has shown that above pH 5.5 the free Cu^{2+} ion concentration rapidly decreases. It is therefore likely that the high free cupric ion concentrations recorded at the adit are due to the low organic content and acidic nature of the groundwater.

The increase in the concentration of free ionic copper at station 3 is due to the removal of the ligand (possibly the bicarbonate ion) which is complexed with the copper, so liberating Cu^{2+} ions. Furthermore the significant decrease in the total copper concentration at station 3 suggests that it is the hydroxy and carbonate species, and not the free cupric ion, which precipitates out at this station.

To supplement the data on metal levels in the Darley Brook, Table 3.7 summarises the water analyses carried out by South West Water (1984) at station E between April 1983 and March 1984. The data highlights copper as being the heavy metal present in the highest concentration (mean 0.17 mg l^{-1}), and this compares with a mean

TABLE 3.7. Mean and range of concentrations of selected metals, total hardness and pH in water samples collected at station E by South West Water between April 1983 and March 1984 (n = 6).

	pH	HARDNESS $CaCO_3$ mg l^{-1}	Cd mg l^{-1}	Cr mg l^{-1}	Cu mg l^{-1}	Fe mg l^{-1}	Pb mg l^{-1}	Mn mg l^{-1}	Ni mg l^{-1}	Zn mg l^{-1}
MEAN	6.6	27	<0.005	<0.005	0.17	0.10	<0.005	0.04	<0.005	0.05
RANGE	6.1 - 7.2	25 - 29			0.15 - 0.19	0.06 - 0.16		0.02 - 0.08		0.05 - 0.06

concentration of 0.13 mg l^{-1} recorded at station E in the present investigation. No doubt the decrease observed in the copper concentration along the Darley Brook (see Figure 3.2A) is a result of physicochemical agencies including dilution and precipitation.

The concentration of iron at the adit was always below the detection limit of flame AAS (i.e. < 0.045 mg l^{-1}). Smith (1973) studied the effects of pH on the solubility and stability of metal ions in aqueous solution, and found that the concentration of iron in solution decreased markedly when the pH was increased from 3.5 to 5.0. As the water at the adit will have a higher oxygen content than the deeper groundwater, it is likely that any soluble ferrous ions will be oxidised to insoluble ferric ions. Thus the pH conditions at the adit (mean pH 5.2) and the increasing oxygen content of the water will cause iron to readily precipitate out.

Arsenate (oxidation state V) is the stable form of arsenic in aerobic waters, and is readily removed from the water column by adsorption or co-precipitation (Ferguson & Gavis, 1972). The concentration of arsenic at the adit was < 0.002 mg l^{-1}, and it is suggested that arsenic and iron are the first metals to co-precipitate out of the water. Analysis of sediment taken from station 1 supports this hypothesis (see p. 103).

At the adit the water is poorly oxygenated with a mean oxygen saturation of 45% which is comparable to the 50% oxygen saturation of most of the waters at sites of mine drainage entry on the rivers Hayle and Gannel (Foster, 1982a). In the Darley Brook the oxygen content quickly rises due to physical aeration due to turbulence, and to the photosynthetic activity of the aquatic vegetation. However there is an unexpected decrease in the oxygen concentration of the water at station 3 (see Table 3.3), and additional measurements taken in the

outflow channel of the pool have shown that this decrease actually occurs at station 3. The decrease may be accounted for by the formation of aluminium-oxide bonds, and the magnitude of the decrease is not too large because the aluminium concentrations in the water are small and there is reoxygenation of the water from the atmosphere.

Conductivity is largely influenced by geology and land use within a catchment area. The low K_{25} values recorded in the upper reaches of the Darley Brook (all the conductivities were in the range 51 to 101 $\mu S\ cm^{-1}$) are due both to the stream originating as groundwater, and to the nature of the rock through which the groundwater flows. Streams draining the granite masses in SW England generally have low conductivities. Ternan and Murgatroyd (1984) recorded K_{25} values around 45 $\mu S\ cm^{-1}$ in the headwaters of a stream on Dartmoor, and conductivities in the range 68 to 119 $\mu S\ cm^{-1}$ have been measured in the upper part of the River Lynher catchment (South West Water, 1984).

The conductivities recorded at the adit indicate that the total salt concentration of the groundwater is relatively low, i.e. it has a poor buffering capacity. However this water has also been shown to have high concentrations of H^+ ions, which are known to strongly influence the structure of benthic macroinvertebrate communities (Koryak et al., 1972; Sutcliffe & Carrick, 1973; Letterman & Mitsch, 1978). Thus the aquatic organisms living in the upper reaches of the Darley Brook are stressed not only by elevated copper levels, but also by high H^+ ion concentrations.

The toxicity of copper to both fish and macroinvertebrates is influenced by the hardness of the water; in general copper is more toxic in 'soft' waters (Doudoroff & Katz, 1953; Lloyd & Herbert, 1962; Learner & Edwards, 1963; Howarth & Sprague, 1978; Stephenson,

1983). At the adit a mean hardness of 8.25 mg l^{-1} was recorded, and although this increases slightly along the length of the Darley Brook (see Figure 3.2B) due to normal catchment processes, the water is characteristically 'soft' in nature. Thus the high copper concentrations near to the adit are likely to be more toxic to the aquatic biota due to the low hardness of the water.

The aluminium concentration recorded at the adit (175 ng ml^{-1}) is above that known to be toxic to fish at pH 5.2 (100 ng ml^{-1}; Mason, 1984). Brown (1983) has studied the effects of differing pH values (4.5 to 5.4) and calcium levels (0.25 mg l^{-1} to 2.00 mg l^{-1}) on the toxicity of aluminium to brown-trout fry; toxicity was found to be a sensitive function of pH and was moderated by the presence of calcium in solution (due to preferential adsorption of calcium by the gills, so blocking the adsorption of aluminium ions). Thus the calcium concentration at station 1 (mean 1.41 mg l^{-1}) is likely to reduce the toxicity of aluminium, and by station D the aluminium concentration in the water has fallen below the critical level of 100 ng ml^{-1}.

There are low levels of nitrate (range of the mean concentrations is 1.19 to 1.43 mg l^{-1}) and phosphate (0.02 to 0.04 mg l^{-1}) in the water at the upper Darley Brook stations. South West Water (1984) have recorded nitrate concentrations in the range 0.59 to 2.40 mg l^{-1}, and phosphate levels between 0.01 and 0.08 mg l^{-1} in the upper catchment of the River Lynher. Further down the Lynher the concentrations of these two nutrients rises, reflecting the increasing effects of natural catchment processes and man's agricultural activities.

It is informative to compare the water quality of station 4 with that of station 1, and Figure 3.5 shows the mean and ranges of the physicochemical water quality variables measured at the two stations.

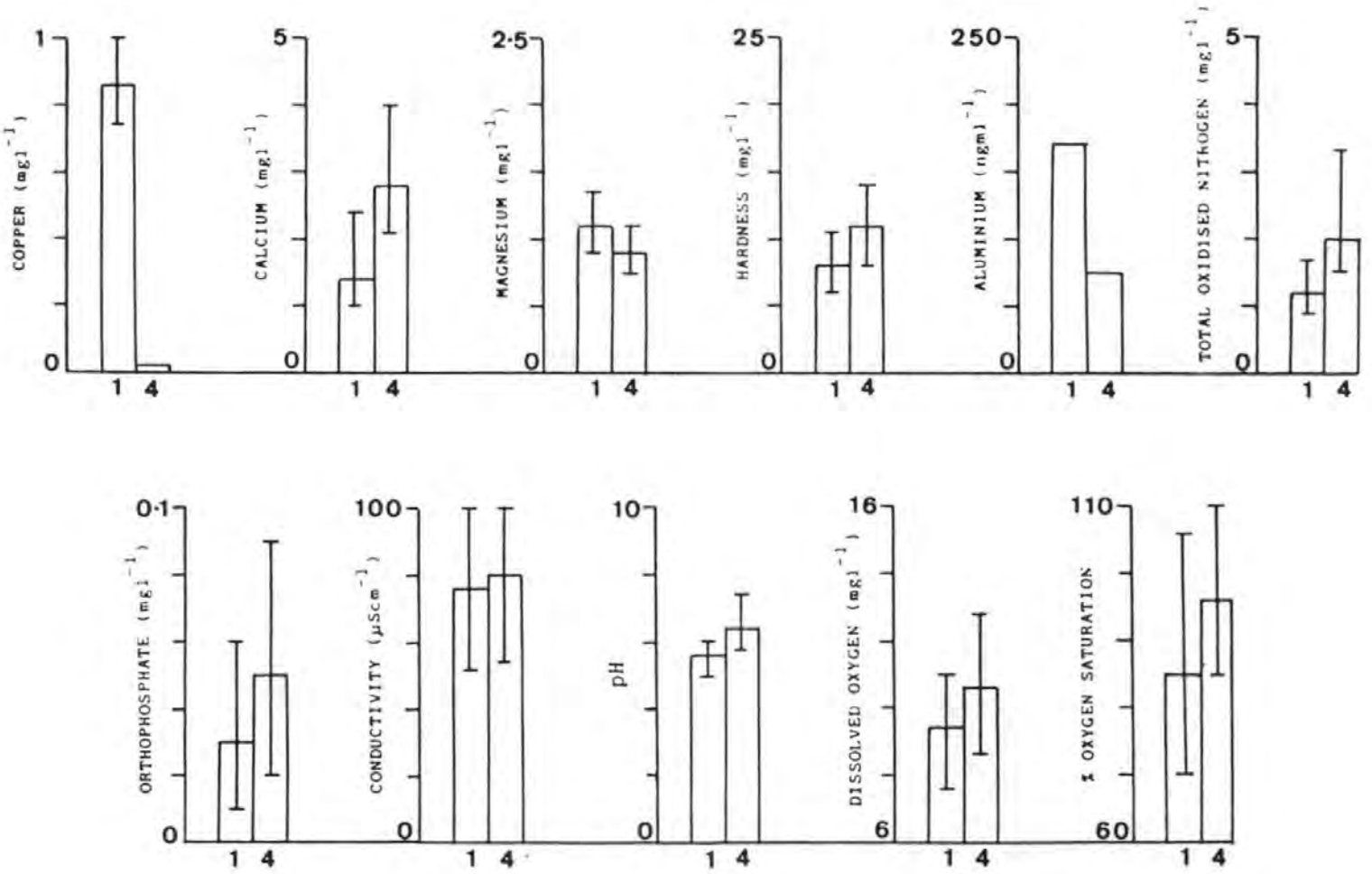


FIGURE 3.5. The mean and range (indicated by vertical bars) of selected physicochemical water quality variables measured between January 1984 and July 1985 at stations 1 and 4. For aluminium n = 1.

The most striking difference is the copper concentration which at station 4 is only 0.025 mg l^{-1} compared to a mean of 0.86 mg l^{-1} at station 1. The aluminium concentration at station 4 is less than half that recorded at station 1. The organisms at station 4 are also not exposed to the higher H^{+} ion concentrations that are associated with the mine drainage water. Overall the water quality at station 4 is similar to that of other non-polluted streams in the River Lynher catchment (South West Water, 1984), and it is therefore reasonable to regard station 4 as a control stream to contrast with the upper reaches of the Darley Brook.

CHAPTER 4

SEDIMENTS

4.1. Introduction

In an aquatic ecosystem metals associated with the sediment can interact with both the overlying water column and the aquatic organisms, and so it is important to quantify the metal loading of this component. An investigation of sediment metal concentrations is useful from an analytical viewpoint since metal levels in particulate matter are frequently many times higher than those in the aqueous phase. Thus metals may be concentrated from the water into the sediments, and the most common particulate sinks for metals have been shown to be iron and manganese oxides and organic substances (Förstner & Wittmann, 1981).

Only a few workers have measured the concentrations of metals in the sediments of metal contaminated streams in South West England, although the work by the Applied Geochemistry Group (e.g. Aston et al., 1974; 1975) has highlighted the elevated levels of a number of metals in stream sediments in this area. The sediments of the River Gannel and Hayle have been analysed for their concentrations of copper, zinc, iron and lead (Brown, 1976; 1977a), whilst Bryan and Gibbs (1983) have measured the levels of several metals including copper in the Fal Estuary.

In the present investigation it has been shown that in the headwaters of the Darley Brook there is a high and sustained copper concentration. Since a number of interactions can occur at the sediment surface-water boundary (e.g. surface exchange, complexation, precipitation and coprecipitation), it is clearly of interest to measure the levels of copper in the sediments of the Darley Brook. Analysis of these sediments for metals allows us to further examine

the earlier hypotheses set-up to explain the low concentrations of both iron and arsenic in the water (see p. 66), and the decrease in aluminium and copper concentrations in the water at station 3 (see p.63).

Elevated metal concentrations in sediment associated with an anthropogenic input have been shown to decrease downstream of the input. Vivian and Massie (1977) recorded lower concentrations of five metals including copper in the sediments downstream of smelting waste, and in another study the concentration of copper in the sediment was found to decrease as distance from a metal processing plant increased (Aulio, 1980). As the Darley Brook has a point source of metal pollution it is possible to measure the concentrations of metals in the sediment at stations progressively downstream from the adit.

Sediments serve as a 'memory' for the conditions that an aquatic ecosystem has been exposed to, and in sediments subject to recent anthropogenic metal inputs there is generally a comparatively high fraction of labile metals. Clearly it is important to consider not only variation in metal levels between stations but also variation over time at one station. In studies which have investigated temporal variation in metal concentrations in sediments, collections have been made seasonally (Burrows & Whitton, 1983), at irregular intervals (Brown, 1977a) or in alternate months (Patterson & Whitton, 1981). It was therefore important that in the present investigation the sampling programme was so designed that any variation over time in metal concentrations in the sediments of the Darley Brook could be examined.

Analysis of sediments is also necessary to understand the relationships between metal levels in the sediment and those in the

aquatic organisms. A useful way of estimating the concentration of metals available to the biota is to subject the sediment to a digestion technique where only the exchangeable metal fraction is leached out. In addition it is informative to measure the total metal concentration in a sediment.

Several workers have shown that the roots of macrophytes contain higher concentrations of metals than the shoots, mainly due to the uptake of these metals from the sediment (Ray & White, 1976; Welsh & Denny, 1977; Schierup & Larsen, 1981; Brix & Lyngby, 1982). Thus metal analysis of sediment taken for example from station 2 would be a prerequisite to studying any interactions between the sediment and the rush Juncus bulbosus.

Sediment metal levels will affect the body concentrations of deposit feeders, as demonstrated in Nereis diversicolor (Bryan, 1974) and in Scrobicularia plana (Bryan & Hummerstone, 1978). Burrows and Whitton (1983) found that the concentrations of one or more metals in a number of insect species showed a significant positive correlation with levels in the water, sediment or both. Thus analysis of sediments is an essential step towards understanding the significance of metal levels in aquatic organisms and towards determining the interactions between aquatic organisms and their surrounding environment.

4.2. Materials and Methods

A surface sediment sample consisting of six sample units was collected every three months at the four main sampling stations (1 to 4 inclusive). Each sample unit was obtained by means of a rigid perspex corer (5 cm diameter with an 0.5 cm wall) specially designed for use in this project (see Plate 4.1). The upper end of the corer was sealed with nylon netting (mesh 170 μm) which allowed free passage of water and prevented scouring during core collection. The lower end had a sharpened edge to help the corer to be worked into the sediment, and was sealed off with a hand. The sediment samples were transported to the laboratory in heat- and water-resistant bags.

The sediments were dried to constant weight at 105°C and then passed through a 200 μm HCl-washed nylon sieve. This sieve size was chosen because particles < 200 μm provide the best discrimination between background and anomalous values (Hawkes & Webb, 1962). Furthermore particles of this size are likely to be ingested by deposit feeders and are therefore biologically significant. This fraction was then prepared for leachable and exchangeable metal analyses by the following procedures:

Leachable metal - between 0.2 and 0.3 g of sediment was weighed into a Kjeldahal flask and digested in 10 ml of Spectrosol grade nitric acid (BDH Chemicals, Poole, Dorset) at 110°C for 30 minutes. After filtering through Whatman no. 42 paper (Whatman Limited, Maidstone), the solution was made upto 50 ml with distilled deionised water (Harding & Whitton, 1978).

Exchangeable metal - approximately 0.5 g of sediment was weighed into a conical flask and shaken with 25 ml of 2.5% Analar grade acetic acid at room temperature for 15 minutes. After standing for 24 hours the

PLATE 4.1. Sampler used for the collection of stream sediment cores.

contents of the flask were filtered through Whatman no. 42 paper, and made upto 50 ml with distilled deionised water (Brown, 1977a).

These two procedures were selected because of their relative simplicity and the information that each yields. The nitric acid digestion is capable of breaking down some mineral lattices, whilst the weaker acetic acid digestion removes primarily metals adsorbed onto sediment particles, and so is a better reflection of the concentration of metals available to the biota and of recent anthropogenic inputs.

For both digests flame AAS was used to measure the concentrations of copper, iron, aluminium, calcium and magnesium. It was found necessary to use a nitrous oxide - acetylene flame (which is both hot and reducing) to breakdown more refractory compounds. However the use of a hotter flame also increases ionization, and so potassium chloride (10,000 mg l^{-1}) was added to release excess electrons and so suppress ionization of the analyte by a simple mass action effect (Ebdon, 1982). Analysis was carried out on a Varian AA-975 Atomic Absorption Spectrophotometer, and full details of the operating conditions are given in Appendix 1B.

Leachable and exchangeable concentrations of arsenic were measured by graphite furnace AAS, with nickel nitrate (1000 mg l^{-1}) added as a matrix modifier. The instrumentation and operating conditions were the same as those used for arsenic determinations in the water samples (see Appendix 1D).

It has previously been mentioned that organic matter acts as a sink for metals and so in addition to metal analyses, the amount of organic material in the sediment was estimated using the 'loss on ignition' procedure outlined by Allen et al. (1974). Approximately 1 g of oven-dried sediment was weighed into a dry pre-weighed crucible.

This was placed in a muffle furnace and the temperature allowed to rise to 450°C for 4 hours. After removal and cooling in a desiccator the crucible was reweighed, and the percentage organic matter was estimated by:

$$\frac{\text{weight loss (g)} \times 100}{\text{initial oven-dry weight (g)}}$$

4.3. Results

The concentrations of copper, iron, calcium and magnesium liberated by the nitric acid leaching were checked against a certified reference material, NIES Pond Sediment. The results are presented in Table 4.1 and show that the determined values were less than the certified values, and so the term 'leachable' was used in preference to 'total' metal concentration.

The metal concentrations measured in sediments collected at stations 1, 2 and 3, and in the control stream (station 4) from January 1984 to July 1985 are presented in Figures 4.1 to 4.6. Each column shows the mean leachable and mean exchangeable metal concentration determined from the six core sample units taken at a particular station on one sampling occasion. The individual leachable and exchangeable metal concentrations in the six sediment sample units, together with the mean concentration and standard error, are given in Tables A to F (see Appendix 3).

The variation in metal concentrations within each sample of six core sample units is shown on most columns by a vertical bar representing the standard error; this gives an indication of the range into which other mean values are likely to occur. For each metal at each station the standard error is small compared to the mean, suggesting that there is only a small amount of spatial variation within a station.

A one-way analysis of variance was carried out on both the leachable and exchangeable metal concentrations of sediment samples at the four stations to investigate any temporal variation in the metal levels. The resulting F-values together with their levels of significance are given in Table 4.2.

There were significant differences at or above the 5% level ($p < 0.05$) between both the leachable and exchangeable concentrations

TABLE 4.1. Comparison of the certified metal concentrations ($\mu g\ g^{-1}$) in NIES Pond Sediment with concentrations ($\mu g\ g^{-1}$) determined by the flame AAS programmes used in sediment metal analyses.

METAL	CERTIFIED VALUE $\mu g\ g^{-1}$		DETERMINED VALUE $\mu g\ g^{-1}$	
	Mean	Uncertainty	Mean	S.E.
COPPER	210	12	85	3
IRON	65300	3500	19400	225
CALCIUM	8100	600	4500	150
MAGNESIUM	8130	-	4060	73

FIGURE 4.1. Mean concentrations ($\mu g\ g^{-1}$) of leachable and exchangeable copper in replicate sediment samples (n = 6) collected at stations 1 to 4 from January 1984 to July 1985. The shaded portion of each column indicates the concentration of the exchangeable metal. Standard error is indicated by a vertical line at the top of each column. E/L shows the mean exchangeable concentration/mean leachable concentration expressed as a percentage. Note that different scales have been used for the concentrations at the four stations.

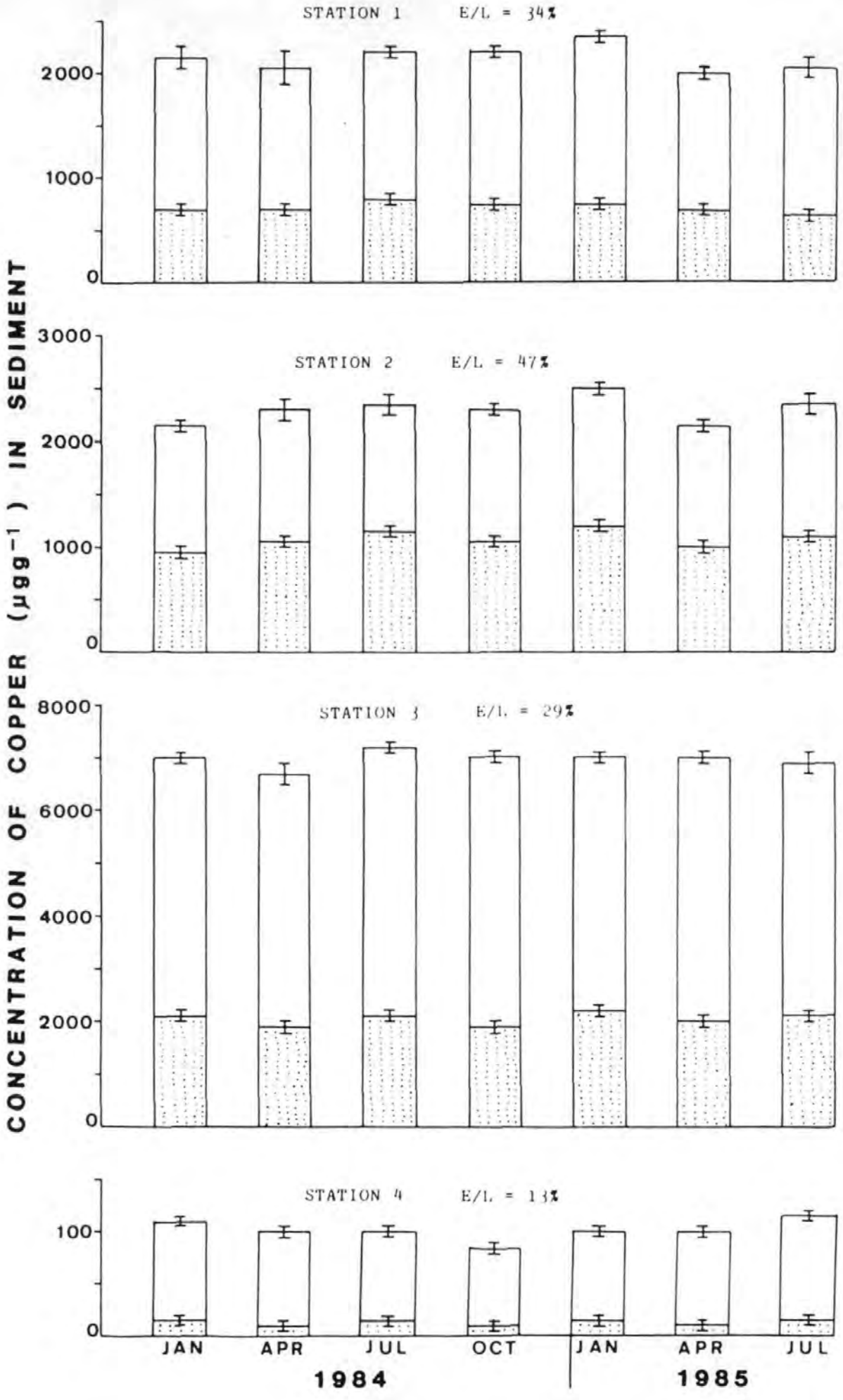

STATION 1 E/L = 34%
STATION 2 E/L = 47%
STATION 3 E/L = 29%
STATION 4 E/L = 13%
CONCENTRATION OF COPPER (µgg⁻¹) IN SEDIMENT
0
1000
2000
3000
4000
6000
8000
100
JAN
APR
JUL
OCT
JAN
APR
JUL
1984
1985

FIGURE 4.2A. Mean concentrations ($\mu g\ g^{-1}$) of leachable and exchangeable iron in replicate sediment samples (n = 6) collected at stations 1 and 2 from January 1984 to July 1985. The shaded portion of each column indicates the concentration of the exchangeable metal. Standard error is indicated by a vertical line at the top of each column. E/L shows the mean exchangeable concentration/mean leachable concentration expressed as a percentage.

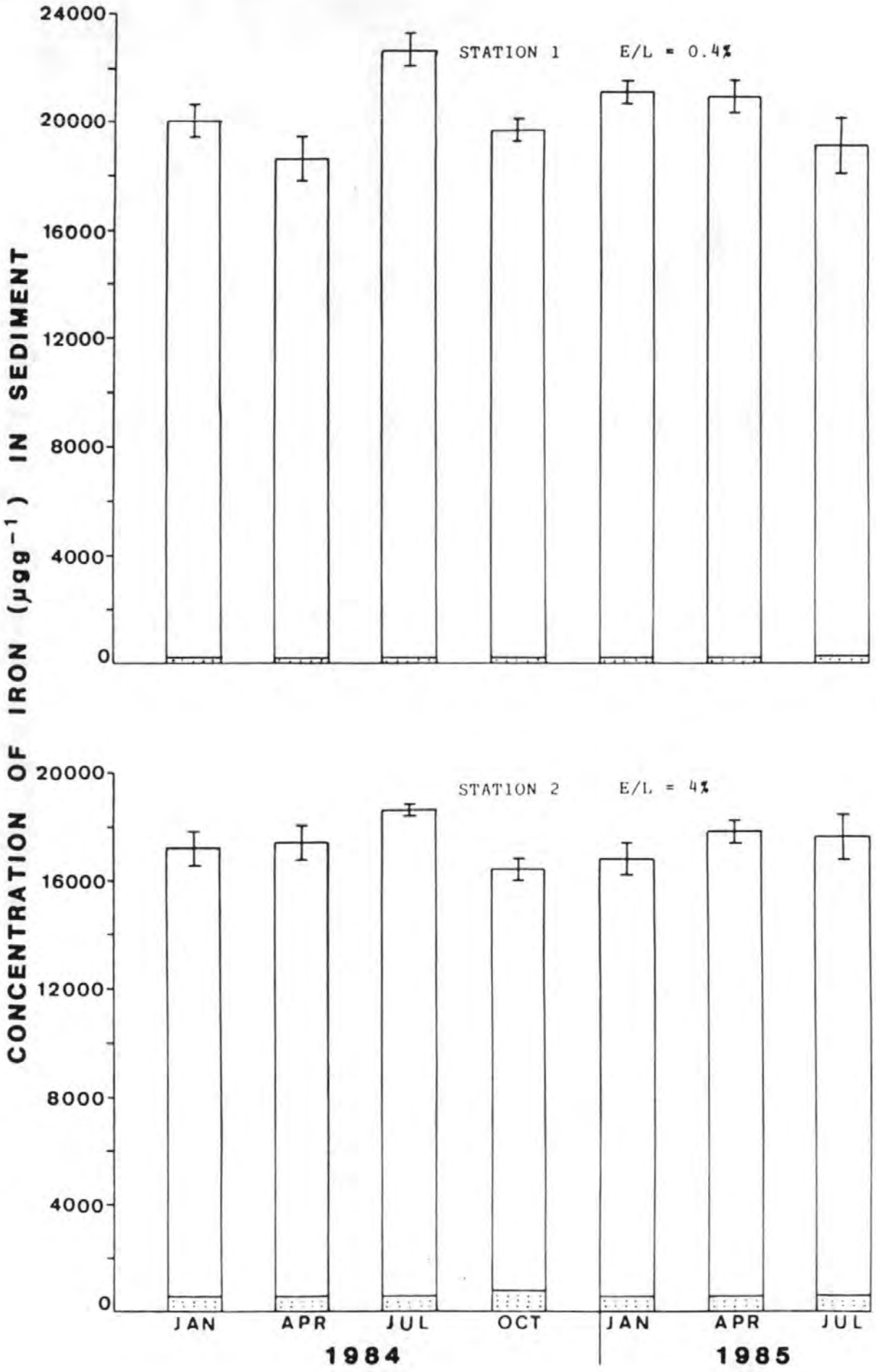

STATION 1
E/L = 0.4%
STATION 2
E/L = 4%
CONCENTRATION OF IRON (µgg⁻¹) IN SEDIMENT
24000
20000
16000
12000
8000
4000
0
JAN
APR
JUL
OCT
JAN
APR
JUL
1984
1985

FIGURE 4.2B. Mean concentrations ($\mu g\ g^{-1}$) of leachable and exchangeable iron in replicate sediment samples (n = 6) collected at stations 3 and 4 from January 1984 to July 1985. The shaded portion of each column indicates the concentration of the exchangeable metal. Standard error is indicated by a vertical line at the top of each column. E/L shows the mean exchangeable concentration/mean leachable concentration expressed as a percentage.

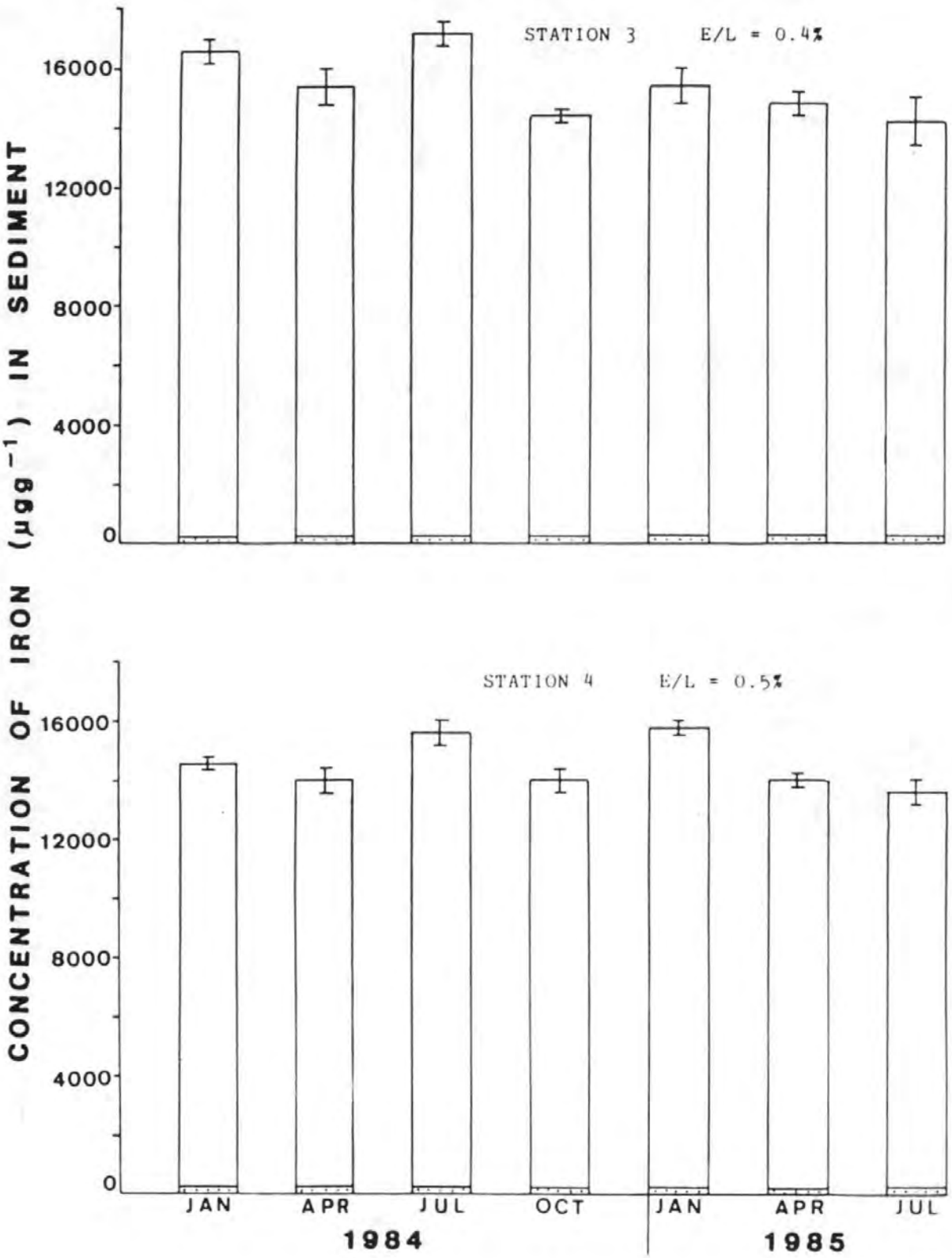

STATION 3 E/L = 0.4%
STATION 4 E/L = 0.5%
CONCENTRATION OF IRON (µgg⁻¹) IN SEDIMENT
16000
12000
8000
4000
0
JAN
APR
JUL
OCT
JAN
APR
JUL
1984
1985

FIGURE 4.3. Mean concentrations ($\mu g\ g^{-1}$) of leachable and exchangeable arsenic in replicate sediment samples (n = 6) collected at stations 1 to 4 from January 1984 to July 1985. The shaded portion of each column indicates the concentration of the exchangeable metal. Standard error is indicated by a vertical line at the top of each column. E/L shows the mean exchangeable concentration/mean leachable concentration expressed as a percentage.

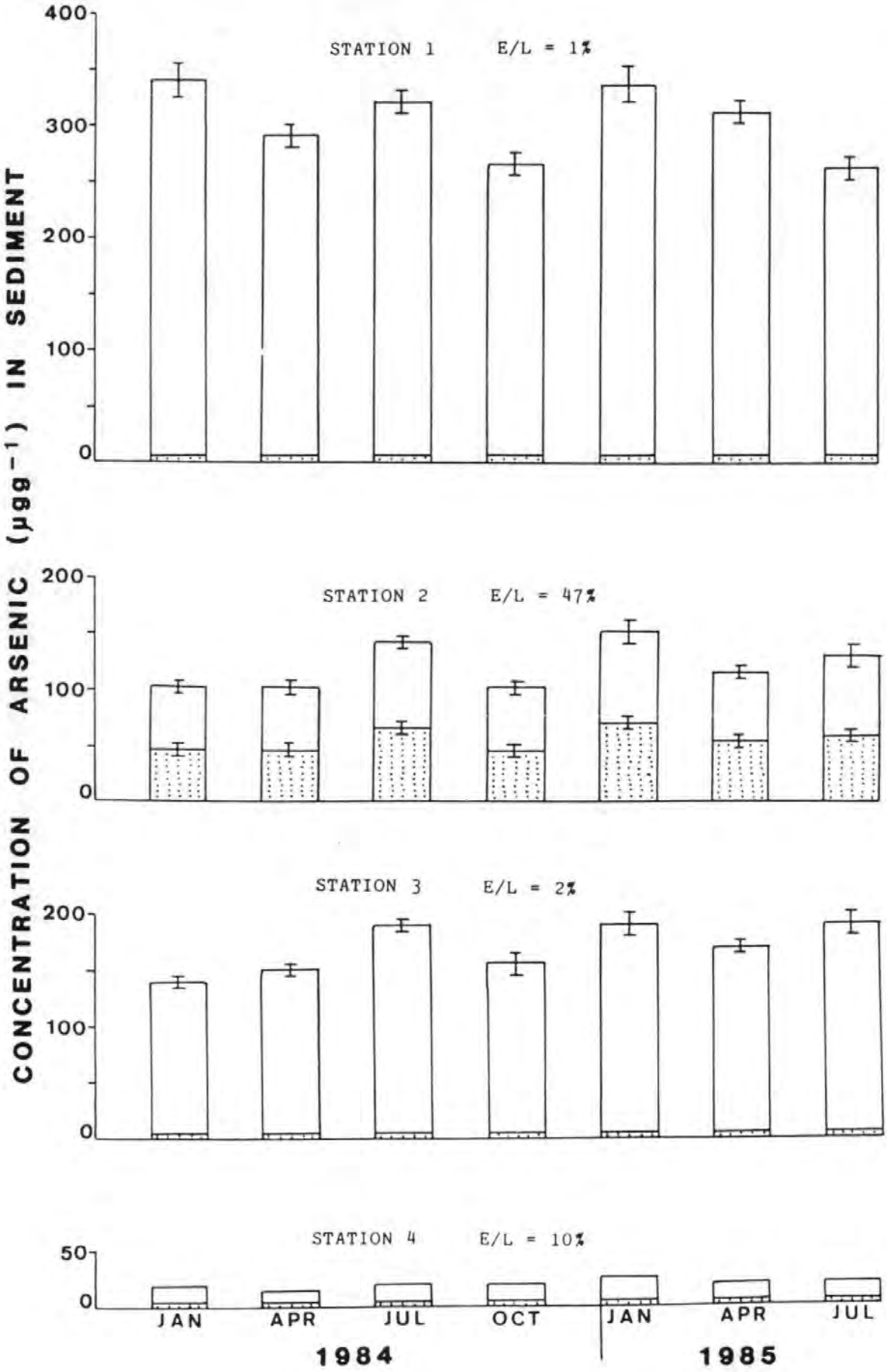

CONCENTRATION OF ARSENIC (µgg⁻¹) IN SEDIMENT
STATION 1 E/L = 1%
STATION 2 E/L = 47%
STATION 3 E/L = 2%
STATION 4 E/L = 10%
400
300
200
100
0
200
100
0
200
100
0
50
0
JAN
APR
JUL
OCT
JAN
APR
JUL
1984
1985

FIGURE 4.4. Mean concentrations ($\mu g\ g^{-1}$) of leachable and exchangeable calcium in replicate sediment samples (n = 6) collected at stations 1 to 4 from January 1984 to July 1985. The shaded portion of each column indicates the concentration of the exchangeable metal. Standard error is indicated by a vertical line at the top of each column. E/L shows the mean exchangeable concentration/mean leachable concentration expressed as a percentage.

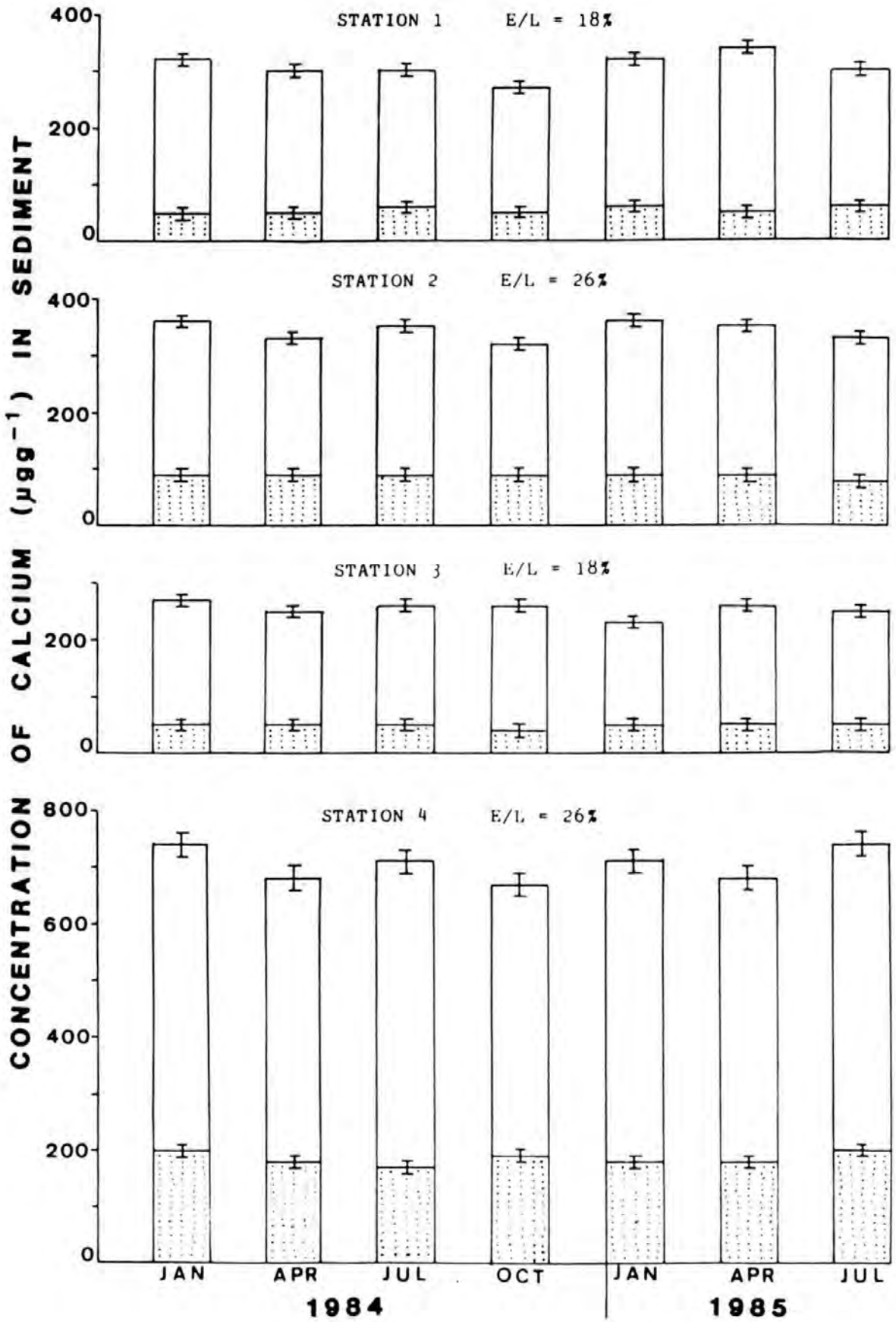

STATION 1 E/L = 18%
STATION 2 E/L = 26%
STATION 3 E/L = 18%
STATION 4 E/L = 26%
CONCENTRATION OF CALCIUM (µgg⁻¹) IN SEDIMENT
400
200
0
800
600
JAN
APR
JUL
OCT
JAN
APR
JUL
1984
1985

FIGURE 4.5. Mean concentrations ($\mu g\ g^{-1}$) of leachable and exchangeable magnesium in replicate sediment samples (n = 6) collected at stations 1 to 4 from January 1984 to July 1985. The shaded portion of each column indicates the concentration of the exchangeable metal. Standard error is indicated by a vertical line at the top of each column. E/L shows the mean exchangeable concentration/mean leachable concentration expressed as a percentage.

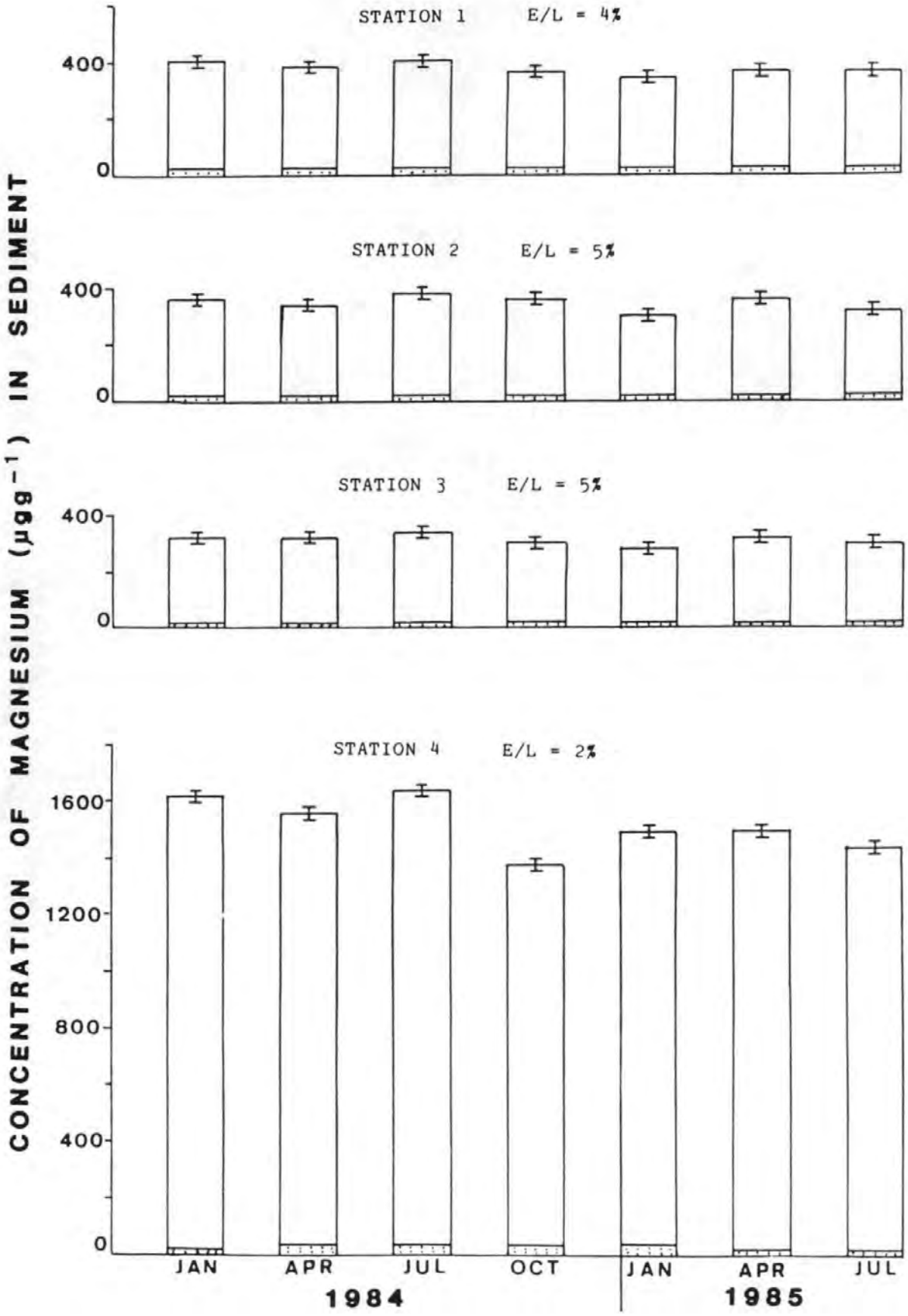
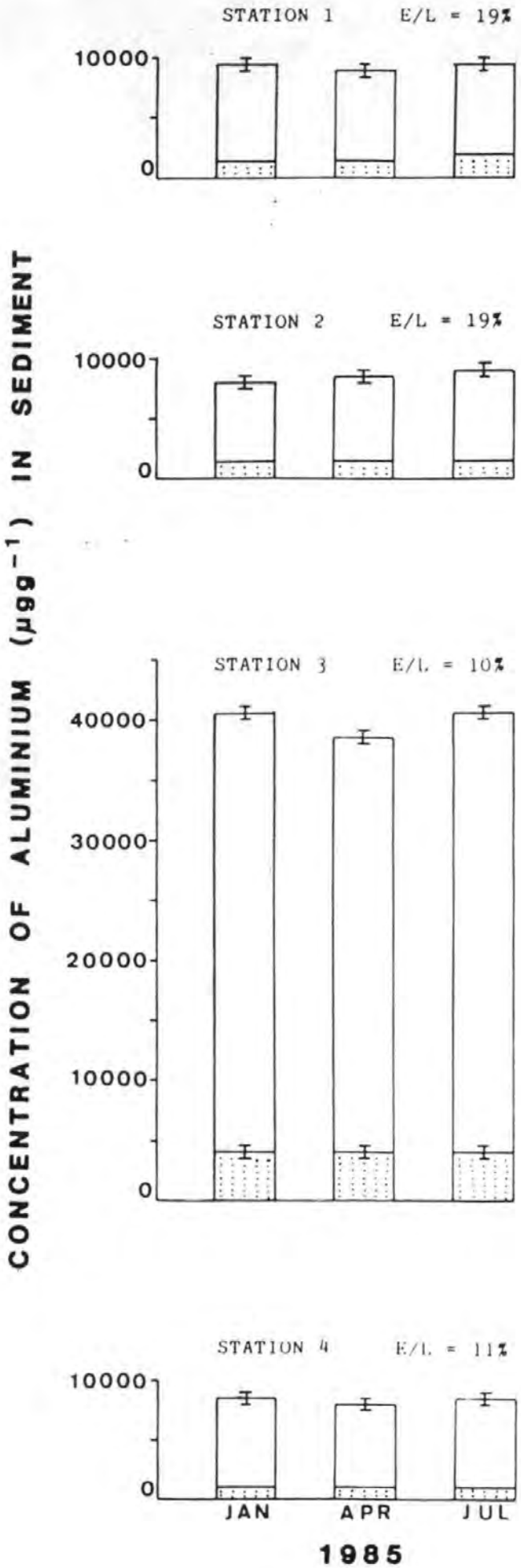

STATION 1 E/L = 4%
400
0
STATION 2 E/L = 5%
400
0
STATION 3 E/L = 5%
400
0
STATION 4 E/L = 2%
1600
1200
800
400
0
CONCENTRATION OF MAGNESIUM (µgg⁻¹) IN SEDIMENT
JAN
APR
JUL
OCT
JAN
APR
JUL
1984
1985

FIGURE 4.6. Mean concentrations ($\mu g\ g^{-1}$) of leachable and exchangeable aluminium in replicate sediment samples (n = 6) collected at stations 1 to 4 from January to July 1985. The shaded portion of each column indicates the conentration of the exchangeable metal. Standard error is indicated by a vertical line at the top of each column. E/L shows the mean exchangeable concentration/mean leachable concentration expressed as a percentage.


STATION 1 E/L = 19%
10000
0
STATION 2 E/L = 19%
10000
0
STATION 3 E/L = 10%
40000
30000
20000
10000
0
STATION 4 E/L = 11%
10000
0
JAN
APR
JUL
1985
CONCENTRATION OF ALUMINIUM (µgg⁻¹) IN SEDIMENT

of all the metals at station 4. Although differences between the leachable concentrations of copper were not significant (at $p = 0.05$ level) at the two contaminated riffle stations 1 and 3, there were significant differences ($p < 0.01$) between the concentrations of exchangeable copper at all the stations. Differences between both the leachable and exchangeable concentrations of arsenic were highly significant ($p < 0.001$) at all the stations. Whilst there was a significant difference ($p < 0.001$) between the leachable concentrations of calcium at station 1, there were no significant differences between the leachable concentrations at stations 2 and 3, nor between the exchangeable concentrations of calcium at the three contaminated upstream stations. Although differences between the exchangeable concentrations of aluminium were highly significant ($p < 0.001$) at station 1, there were no significant differences in the exchangeable concentrations at stations 2 and 3.

It is evident from Figures 4.1 to 4.6 that there were differences in both the mean leachable and mean exchangeable concentrations of a metal between the four stations, and that these differences were consistent on all sampling occasions. To examine this variation between the stations a series of t-tests were carried out on the concentrations in both digests, using the seven mean concentrations recorded over the 19 month sampling period as a data set from which a 'mean of the means' concentration was calculated. The 'mean of the means' leachable and exchangeable metal concentrations at stations 1 to 4 are shown in Table 4.3, and it is these concentrations that will now be described and later discussed.

The sediment at station 3 contained significantly ($p < 0.001$) higher concentrations of leachable copper (6985 $\mu g\ g^{-1}$) and exchangeable copper (2043 $\mu g\ g^{-1}$) compared to stations 1 and 2. Similarly the

TABLE 4.2. F values together with their level of significance (N.S. Not Significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) obtained from one-way analysis of variance tests carried out to investigate temporal variation in leachable and exchangeable metal concentrations in sediments at stations 1 to 4 from January 1984 to July 1985.

METAL	STATION 1		STATION 2		STATION 3		STATION 4	
	LEACHABLE	EXCHANGEABLE	LEACHABLE	EXCHANGEABLE	LEACHABLE	EXCHANGEABLE	LEACHABLE	EXCHANGEABLE
COPPER	N.S. 2.00	*** 5.75	* 3.22	*** 5.51	N.S. 1.17	*** 6.08	*** 5.98	** 4.67
IRON	*** 4.93	* 2.54	N.S. 2.09	*** 13.67	** 4.69	*** 6.38	*** 7.35	*** 19.26
ARSENIC	*** 9.01	*** 29.69	*** 10.51	*** 9.63	*** 7.89	*** 26.26	*** 4.97	*** 6.58
CALCIUM	*** 6.32	N.S. 1.36	N.S. 2.32	N.S. 0.85	N.S. 1.10	N.S. 0.72	* 2.91	* 2.41
MAGNESIUM	** 4.08	*** 7.43	*** 6.79	* 3.02	* 2.76	*** 5.64	*** 13.65	*** 16.58
ALUMINIUM	N.S. 3.03	*** 12.77	* 4.22	N.S. 3.60	** 8.18	N.S. 2.33	* 5.17	* 3.97

TABLE 4.3. Overall mean leachable and exchangeable metal concentrations ($\mu g\ g^{-1}$) calculated from the 7 mean concentrations (n = 3 for aluminium) determined for sediment samples collected between January 1984 and July 1985 at stations 1 to 4. Standard error is shown in brackets.

METAL	STATION 1		STATION 2		STATION 3		STATION 4	
	LEACHABLE	EXCHANGEABLE	LEACHABLE	EXCHANGEABLE	LEACHABLE	EXCHANGEABLE	LEACHABLE	EXCHANGEABLE
COPPER	2139 (45)	725 (21)	2302 (49)	1071 (33)	6985 (53)	2043 (45)	101 (3)	13 (0.8)
IRON	20174 (515)	73 (3)	17394 (277)	647 (38)	15404 (414)	63 (4)	14512 (350)	75 (6)
ARSENIC	303 (12)	4.4 (0.5)	119 (8)	56 (4)	168 (8)	3.1 (0.4)	20 (1)	1.9 (0.2)
CALCIUM	308 (8)	54 (2)	343 (6)	89 (2)	254 (4)	47 (1)	704 (12)	185 (4)
MAGNESIUM	371 (9)	15 (1)	343 (11)	16 (1)	312 (9)	15 (1)	1516 (35)	33 (3)
ALUMINIUM	9441 (156)	1778 (88)	8495 (160)	1637 (51)	39764 (609)	3945 (77)	8387 (195)	973 (53)

highest concentrations of aluminium (39764 μg g^{-1} and 3945 μg g^{-1} in the leachable and exchangeable digests respectively) were also recorded in the sediment at station 3.

The concentration of leachable iron in the sediment was highest at station 1 (20174 μg g^{-1}) and then decreased significantly ($p < 0.01$) downstream to a concentration of 15404 μg g^{-1} at station 3. However the exchangeable iron concentration of 647 μg g^{-1} measured at station 2 was over 8 times higher than the level recorded at either station 1 or 3. Differences in the arsenic concentrations in the sediments at stations 1, 2 and 3 were similar to those described for iron, in that the highest leachable concentration was recorded at station 1 (303 μg g^{-1}) and the highest exchangeable concentration was measured at station 2 (56 μg g^{-1}); however the lowest concentration of leachable arsenic was recorded at station 2.

At station 2 there was a significant ($p < 0.01$) increase in the concentrations of leachable and exchangeable calcium (343 and 89 μg g^{-1} respectively) compared to station 1. This was followed by a significant ($p < 0.001$) decrease at station 3, resulting in levels lower than those recorded at station 1.

Although the highest leachable concentration of magnesium was recorded at station 1 (371 μg g^{-1}) there was no significant difference (at $p = 0.05$ level) between the levels at stations 1 and 2, nor between the levels at stations 2 and 3. However significantly ($p < 0.001$) higher leachable concentrations of magnesium were measured at station 1 compared to station 3. There was no significant difference between the exchangeable magnesium concentrations at stations 1, 2 and 3.

It is informative to compare the metal concentrations in the sediment at station 1 (the nearest station to the adit) with those at station 4. From Table 4.3 it is clear that the sediment at station 1

contained significantly ($p < 0.01$) higher leachable and exchangeable concentrations of copper, arsenic and aluminium. Conversely the leachable and exchangeable concentrations of calcium and magnesium were significantly ($p < 0.001$) higher in the sediment at station 4. Whilst the leachable concentration of iron was significantly ($p < 0.001$) higher at station 1, there was no significant difference (at $p = 0.05$ level) between the exchangeable iron concentrations at stations 1 and 4.

The ratio of mean exchangeable concentration/mean leachable concentration has been calculated for each metal at each station, and is expressed as a percentage in Figures 4.1 to 4.6. The highest ratios for copper, iron, arsenic and calcium were recorded at station 2, e.g. the % mean exchangeable concentration/mean leachable concentration for arsenic was 1% at station 1, 47% at station 2, 2% at station 3 and 10% at station 4. At the three contaminated upstream stations the ratios for magnesium were between 4 and 5% compared to 2% at station 4. The highest ratios for aluminium were recorded at both stations 1 and 2 (19%), with a ratio of 10% at station 3 and 11% at station 4. At station 4 the ratio for copper (13%) was lower than at stations 1 and 3 (34% and 29% respectively), the ratio for iron (0.5%) was the same as stations 1 and 3 (0.4% at both stations), and the ratio for calcium (26%) was higher than at stations 1 and 3 (18% at both stations). These percentages should of course be treated with care, as if the leachable concentration is low the percentage may rise or vice-versa without any significant change in exchangeable metal levels.

Table 4.4 shows the mean percentage 'loss on ignition' values recorded every three months at stations 1 to 4, and also gives an overall mean calculated from the individual mean values. The highest amount of organic material was recorded in the sediment at station 2 (the pool) where the overall mean was 14.3%. In the riffle stations,

TABLE 4.4. Mean percentage 'loss on ignition' calculated from replicate sediment core samples (n = 6) taken at stations 1 to 4 every 3 months from January 1984 to July 1985, together with an overall mean calculated from the 7 individual mean values.

DATE		STATION 1	STATION 2	STATION 3	STATION 4
1984	JAN	7.5	15.1	11.3	7.6
	APR	6.6	14.0	10.8	8.7
	JUL	6.8	14.3	11.5	7.4
	OCT	6.3	14.5	13.7	8.9
1985	JAN	7.2	14.6	12.2	8.2
	APR	6.7	14.2	11.5	7.8
	JUL	6.3	13.7	10.8	7.0
OVERALL MEAN % LOSS ON IGNITION		6.8	14.3	11.7	7.9

although a significantly lower organic content was recorded at stations 1 and 4 (6.8% and 7.9% respectively), a value of 11.7% was found at station 3.

4.4. Discussion

Whilst the leachable and exchangeable concentrations of most metals at a number of stations frequently exhibited significant temporal variation, this showed no obvious trend over time and is partly a reflection of the small amount of variation within a sample (i.e. between the six sediment sample units taken at a station). Furthermore this temporal variation is small and variable when compared to the marked and consistent differences in metal concentrations between the stations, and it is these differences which will now be discussed using the overall mean metal concentrations.

The overall mean leachable copper concentration at station 1 (2139 $\mu g\ g^{-1}$) is over 20 times higher than the overall mean leachable concentration at station 4 (101 $\mu g\ g^{-1}$), a reflection of the very high copper concentration in the mine drainage water at station 1. In other studies on metal contaminated rivers in South West England, Brown has recorded total (nitric/perchloric acid digestion) copper concentrations in the sediments of 1964 $\pm$ 100 $\mu g\ g^{-1}$ in the River Gannel (1976) and 700 to 4900 $\mu g\ g^{-1}$ at sites of mine drainage entry in the River Hayle (1977a). Bryan and Gibbs (1983) recorded copper concentrations (nitric acid digestion) of 1733 to 2540 $\mu g\ g^{-1}$ in the sediments of Restronguet Creek, a part of the Fal Estuary which receives the metal contaminated water of the River Carnon which drains both copper and tin mining areas.

At station 3 in the Darley Brook there is a marked increase in both the leachable and exchangeable copper concentrations, and the overall mean leachable concentration of 6985 $\mu g\ g^{-1}$ here is higher than any other copper concentration that has been reported in sediments in streams and rivers in South West England. The leachable and exchangeable concentrations of aluminium also show an increase at station 3. Indeed the leachable aluminium concentrations are at least four times higher

than the levels recorded at stations 1 and 2. In the previous chapter (see p.63) it was suggested that copper and aluminium may coprecipitate out of the water at station 3. Sediment analysis supports this hypothesis and it is suggested that the light grey-green precipitate which covers the substratum at station 3 is alumina discoloured by copper.

In a study on the River Hayle (Brown, 1977a) the total concentration of copper in the sediments was higher in August than in March and October, due to the increased deposition of 'particulate' copper from the overlying waters. However only 3 to 5% of the copper in the headwaters of the Darley Brook is in a 'particulate' form (compared to 70% in the River Hayle), and a seasonal variation in sediment concentrations of copper was not evident. Indeed the difference between the leachable concentrations of copper recorded during the year is not significant (at $p = 0.05$ level) at either of the upstream riffle stations. Furthermore although mean values for both leachable and exchangeable concentrations of copper are higher in the summer (July) than in the spring (April) or autumn (October) at the three contaminated upstream stations, the highest values were recorded for example in leachable copper at stations 1 and 2 in January 1985, and the concentrations recorded would appear to be part of an ongoing variation through the year with no clear pattern of seasonal variation.

The highest leachable concentrations of iron and arsenic were recorded at station 1, and it is suggested that the common arsenate species is either coprecipitating or being adsorbed onto iron hydroxides. In the River Tamar, arsenic (present as the arsenate species AsO_4^{3-}) was readily scavenged out of the water by iron oxides (Millward & Marsh, 1986). Thus in the Darley Brook iron and arsenic are the first metals to precipitate out of the water, while copper and aluminium coprecipitate out at station 3.

The leachable iron concentration was found to decrease progressively downstream, from an overall mean concentration of 20174 $\mu g\ g^{-1}$ at station 1 to 15404 $\mu g\ g^{-1}$ at station 3. These concentrations are lower than the levels reported in other metal contaminated waters in South West England. Brown (1977a) recorded total concentrations in the sediment in the range 50000 to 420000 $\mu g\ g^{-1}$ at sites of mine drainage entry in the River Hayle, where the concentration of 'particulate' iron in the water was upto 2.0 $mg\ l^{-1}$. In Restronguet Creek, Bryan and Gibbs (1983) have measured levels of between 39200 and 63000 $\mu g\ g^{-1}$ in the sediment, with a concentration of iron of 9 $mg\ l^{-1}$ in the water of the River Carnon which flows into the Creek. It is interesting to note that the lower concentrations of iron recorded in the sediments of the Darley Brook are associated with a lower iron concentration ($< 0.045\ mg\ l^{-1}$) in the water column.

The overall mean leachable concentration of arsenic at station 1 ($303\ \mu g\ g^{-1}$) is comparable to the arsenic concentrations recorded by Bryan and Gibbs (1983) in the sediments of the River Gannel ($233\ \mu g\ g^{-1}$), although in Restronguet Creek the sediments contained between 1076 and 2520 $\mu g\ g^{-1}$ of arsenic in accordance with the comparatively high concentration of arsenic (upto $0.4\ mg\ l^{-1}$) in the water of the River Carnon.

At station 2 the exchangeable arsenic concentrations were at least 12 times higher than at station 1 or 3. There was also a marked increase (upto 10 times) in the exchangeable concentration of iron at station 2 when compared to stations 1 and 3. It is likely that these high exchangeable concentrations are the result of arsenic and iron being adsorbed onto organic material in the sediment at station 2, which has the highest 'loss on ignition' values of any station (see Table 4.4). Although the sediment at station 3 contains comparatively

high amounts of organic material, the exchangeable concentrations of iron and arsenic were low since these metals are so readily adsorbed onto the organic material at station 2 (the pool).

Analysis of water collected at the adit has shown that it has low concentrations of calcium (mean 1.38 mg l^{-1}) and magnesium (mean 1.17 mg l^{-1}), due to the granitic nature of the rock through which the groundwater flows. The levels of both calcium and magnesium in the sediments at stations 1 to 3 reflect the soft nature of the groundwater, with low leachable concentrations of calcium (overall maximum of 343 $\mu g\ g^{-1}$ at station 2) and magnesium (overall maximum of 371 $\mu g\ g^{-1}$ at station 1), and low exchangeable concentrations of calcium (overall maximum of 89 $\mu g\ g^{-1}$ at station 2) and magnesium (overall maximum of 16 $\mu g\ g^{-1}$ at station 2). By comparison in a lead-zinc contaminated stream in Cumbria (Patterson & Whitton, 1981) the concentrations of calcium in the water ranged between 79.9 and 105 mg l^{-1} and in the sediment (nitric acid digestion) were around 2000 $\mu g\ g^{-1}$. Burrows and Whitton (1983) have recorded calcium concentrations of between 7.4 and 31.8 mg l^{-1} in the water of a stream in the River Derwent catchment which received mine drainage, with levels between 6050 and 17700 $\mu g\ g^{-1}$ in the sediment (nitric acid digestion).

In the upper reaches of the Darley Brook the highest leachable and exchangeable concentrations of calcium were recorded at station 2, probably because the comparatively high amount of organic material at this station acts as an important sink for calcium. At the three riffle stations the leachable concentration of magnesium is greater than the leachable calcium concentration, since magnesium is more closely associated with mineral erosion products than calcium and is thus retained in the sediments to a greater degree than calcium (Mackereth, 1966). At station 2, where erosion is likely to be less

than at the riffle stations, similar leachable concentrations of magnesium and calcium were recorded.

The sediments at station 1 contain significantly higher leachable concentrations of copper and aluminium compared to station 4, reflecting the higher concentration of these metals in the mine drainage water. However it is interesting to note that the overall mean leachable copper concentration of 101 $\mu g\ g^{-1}$ recorded at station 4 (where the concentration of copper in the water is 0.025 $mg\ l^{-1}$) is within the range of copper concentrations (8 to 370 $\mu g\ g^{-1}$; nitric/perchloric acid digestion) recorded by Aston et al. (1974) in the uncontaminated River Fowey in Cornwall (where the copper concentration in the water was between 0.002 and 0.021 $mg\ l^{-1}$).

Although neither iron nor arsenic were detectable in the water at stations 1 or 4, the leachable concentrations of these metals are significantly higher at station 1 due to its proximity to the adit where it has been suggested that iron and arsenic precipitate out. The overall mean leachable iron concentration of 14512 $\mu g\ g^{-1}$ at station 4 is lower than the mean concentration of 33000 $\mu g\ g^{-1}$ recorded in the uncontaminated River Fowey (Aston et al., 1974) due in part to the lower concentration of iron in the water at station 4 ($< 0.045\ mg\ l^{-1}$ compared to a mean of 0.21 $mg\ l^{-1}$ in the River Fowey). Aston et al. (1975) recorded arsenic concentrations of upto 30 $\mu g\ g^{-1}$ in the sediments of uncontaminated streams in Cornwall (where the concentration of arsenic in the water was $< 0.01\ mg\ l^{-1}$), and comparable leachable arsenic concentrations were measured at station 4 (overall mean was 20 $\mu g\ g^{-1}$ and the arsenic concentration in the water was $< 0.002\ mg\ l^{-1}$).

The leachable concentrations of calcium and magnesium are significantly higher at station 4 than at station 1. In the case of calcium this difference is probably due to the higher concentration of

calcium in the water at station 4 (mean 2.82 mg l^{-1}) compared to station 1 (mean 1.41 mg l^{-1}). Since the leachable concentration of magnesium at station 4 is four times higher than at station 1 this suggests that the magnesium is more firmly bound up in the sediment at station 4, which would account for the similar concentrations of magnesium measured in the water at these two stations (mean concentrations of 1.10 and 0.91 mg l^{-1} at station 1 and 4 respectively).

The ratio of mean exchangeable/mean leachable metal concentration would be expected to decrease with increasing complex stability, since a metal which forms highly stable complexes is less likely to be removed by a weak acetic acid digestion. Copper has been shown to form an unusually large number of very stable complexes with a wide range of ligands (Irving & Williams, 1948), and the exchangeable/leachable ratio for copper might therefore be expected to be low compared to other metals. However in the present study the highest exchangeable/leachable ratios were recorded for copper at stations 1, 2 and 3. Since copper is present at high concentrations in the water at these stations, it is likely that by a mass-effect many of the adsorption sites on the sediment particles will be occupied by copper, resulting in the high exchangeable/leachable ratios.

At the three upstream stations the highest exchangeable/leachable ratios were recorded for copper and the lowest ratios were recorded for iron, e.g. at station 1 ratios of 34% and 0.4% were determined for copper and iron respectively. By comparison the ratio of available/total metal concentration in sediments at sites of mine drainage entry on the River Hayle (Brown, 1977a) followed the sequence zinc > copper > iron, with values of upto 25% recorded for copper and of upto 1.1% recorded for iron. The exchangeable/leachable ratio was higher for copper, iron, arsenic and calcium at station 2 than at stations 1 or 3, possibly due

to the increased amount of organic material in the sediment at station 2 onto which these metals are readily adsorbed.

CHAPTER 5

PLANTS

5.1. Introduction

At station 1 in the Darley Brook the aquatic vegetation is very prominent covering a large part of the substratum (see Plate 2.1). This vegetation consists of randomly distributed clumps of the leafy liverwort Jungermannia atrovirens (Nees), along with strands of the filamentous green alga Microspora sp. The rush Juncus bulbosus (Linnaeus) grows in the muddy deposit covering the substratum of the pool (station 2), with the tips of this plant often emerging above the water (see Plate 2.2).

Chronic (i.e. long term) metal pollution often leads to the elimination of non-tolerant plant species, resulting in a reduced floral community (Whitton & Diaz, 1980; Say & Whitton, 1981; Foster, 1982a). Whilst most studies on aquatic plants in metal contaminated waters have compared concentrations in species from the same phylum, few workers have compared concentrations in plants from different phyla growing in the same watercourse. One such comparative study was that of Harding et al. (1981) who measured the concentrations of zinc and lead in an alga and two bryophyte species collected at the same sites above and below the input of a mine drainage stream. The Darley Brook offers an opportunity to study the effect of a constant and elevated copper concentration on plants from three different phyla.

Although there is now a certain amount of literature on metal concentrations in plants in metal contaminated freshwater ecosystems, most of the work in the United Kingdom has been carried out in Northern England (Welsh & Denny, 1977; Patterson & Whitton, 1981; Say et al., 1981) and Wales (Mclean & Jones, 1975; Burton & Peterson, 1979;

Jones et al., 1985). These studies have generally focused on lead and zinc as the most important metals, with copper concentrations less well documented. In South West England, Bryan and Gibbs (1983) have measured metal concentrations in selected species of the marine flora of the Fal estuary. However, apart from the work by Foster (1982a) on the algal community of the River Hayle, there are no other detailed records of copper concentrations in plants in the metal contaminated streams of South West England.

It is well established that plants in metal contaminated waters contain elevated metal concentrations with respect to the levels in the water. Thus it is likely for example that the chironomid larvae at stations 1 and 2 in the Darley Brook are consuming a metal enriched diet, whether they feed on living plants or plant debris. In turn these chironomid larvae form a significant prey item in the diet of the invertebrate predators in this part of the stream. Therefore to fully investigate the movement of metals through the food chain, to study any bioaccumulation and to understand the fate of metals, it is important to measure their levels in the plants of the Darley Brook.

The sources of metals to aquatic plants are provided by the water column and the substratum. However the relative importance of these sources is likely to vary for different species and types of plant depending on the growth form. In this investigation the alga Microspora consists largely of filaments suspended in the water column; J. atrovirens has an extensive mat of chlorophyllous tissue and a smaller rhizoidal region with which it is loosely anchored to a solid substratum (i.e. the tops of large stones). With both of these species it is the water passing over the plants which is likely to provide the main source of metals. In rooted angiosperms (such as J. bulbosus at station 2) the sediment is an important source of metals, and so it is necessary to measure the levels in both root and shoot fractions.

Because As aquatic plants accumulate metals from the water and/or sediment they may have a use as 'monitors', since metal concentrations in a plant can provide an integrated record of the nature and extent of metal contamination within a system. Whitton et al. (1981) have compiled a package of ten plant species or genera which could be used to monitor metal levels in freshwaters. However only a few workers have studied how metal concentrations in plants vary over time (Patterson & Whitton, 1981; Say et al., 1981; Lyngby & Brix, 1982; Wehr & Whitton, 1983), which is clearly an important consideration in selecting 'monitor' species. Temporal variation in the metal levels in plants may result in variation of metal concentrations in the consumers of this plant material.

5.2. Materials and Methods

At station 1 J. atrovirens grows in clumps approximately 10 to 30 cm along the longest axis. As far as possible only clumps that were fully submerged were collected each month from January 1984 to July 1985. Although Microspora is often found growing in association with J. atrovirens at station 1, only algal filaments growing freely in the water were collected every 3 months over the same 19 month period. At station 2 J. bulbosus was collected from within a small area at the same times of the year as the alga.

All plant material was washed in stream water to remove large sediment particles and other contaminating material, prior to being placed in separate plastic bags for transport back to the laboratory. In the laboratory the plants were further washed in distilled water. The alga and liverwort were kept entire, whilst J. bulbosus was divided into 'root'(rhizome and roots) and 'shoot' (stems and flowers) fractions. Samples of the alga were also preserved in Lugol's iodine for subsequent checks on identification.

The plant material was placed in separate porcelain dishes, dried at 105°C to constant weight, and then ground with a pestle and mortar. At this stage, particularly in the Microspora sample, it was easy to remove with fine forceps any sediment particles which had not been previously washed out. Four replicates each weighing between 0.4 and 0.5 g of each type of plant sample were weighed in separate Kjeldahal flasks, and digested in 10 ml Spectrosol grade nitric acid at 110°C for 30 minutes (Harding & Whitton, 1978). These conditions are the same as those used in the sediment analysis (see p. 74), allowing a direct comparison to be made between metal concentrations in the plants and in the sediments. A similar nitric acid digestion is also commonly employed by other workers investigating metal

concentrations in plants. The resulting digests were filtered separately through Whatman no. 42 paper, and made upto 50 ml with distilled deionised water.

The concentrations of copper, iron, calcium and magnesium in all the digests were measured by flame AAS on the same instrumentation used for the metal analysis of water samples, and under the operating conditions outlined in Appendix 1C. A nitrous oxide-acetylene flame was used in all cases to break down the more refractory compounds. Potassium chloride (10,000 mg l^{-1}) was added to all samples as an ionization suppressor.

Arsenic concentrations in all the plants were measured by graphite furnace AAS, since the sensitivity of flame AAS proved too low. The instrumentation and operating conditions (see Appendix 1D) were identical to those used for the measurement of arsenic in water samples. Nickel nitrate (1000 mg l^{-1}) was added to all the samples as a matrix modifier.

When it became evident that aluminium was another metal of interest in the Darley Brook, particularly with respect to levels in the sediment, it was decided that the measurement of aluminium concentrations in the plants would be desirable. Thus from January to July 1985 the concentrations of aluminium in all the plants were measured by flame AAS, again using a nitrous oxide-acetylene flame and potassium chloride (see Appendix 1C).

Observations were made on the variation over time of the amount of plant material at station 1. Thus an estimation by eye was carried out each month of the percentage plant cover along the entire 60 m length of station 1. Furthermore a record was made as to whether the alga or liverwort covered the greatest area.

5.3. Results

Throughout the sampling period the aquatic vegetation of J. atrovirens and Microspora at station 1 always covered at least 60% of the substratum. In March of both years there was an increase in the amount of Microspora, with the percentage plant cover rising to 70% in March 1984 and to 80% in March 1985. In both years most of this alga died out in April or May, leaving J. atrovirens as the dominant plant (see Plate 5.1). In the autumn of 1984 there was another increase in the amount of Microspora (see Plate 5.2) with the percentage plant cover reaching a maximum value of 80% in October. Again most of this algal growth subsequently died out in November, with J. atrovirens dominant through the winter.

The precision of the flame AAS method, and the concentrations of copper, iron, calcium and magnesium leached by the nitric acid digestion, was checked against a NIES certified reference Chlorella sample. The results are presented in Table 5.1 and show that for all the metals the variation about the determined mean concentration was very small, with the magnesium concentrations showing the greatest variation of just 1% of the determined mean concentration. In all cases the certified metal concentrations were greater than the determined metal concentrations since the Chlorella sample was totally digested in nitric acid, in contrast to the 30 minute digestion employed in this study.

The metal concentrations in the 4 replicates of J. atrovirens, Microspora and in the root and shoot fractions of J. bulbosus are recorded in Tables A to K (see Appendix 4). From this data the mean metal concentration and standard error was calculated for each plant sample on each sampling occasion, and these mean concentrations (together with their standard errors) are shown plotted against time in Figures 5.1 to 5.6.

PLATE 5.1. Riffle vegetation at station 1 showing the alga Microspora sp., and in the centre of the photograph the leafy liverwort Jungermannia atrovirens.

PLATE 5.2. Riffle vegetation at station 1 showing the filamentous nature of the green alga Microspora sp.

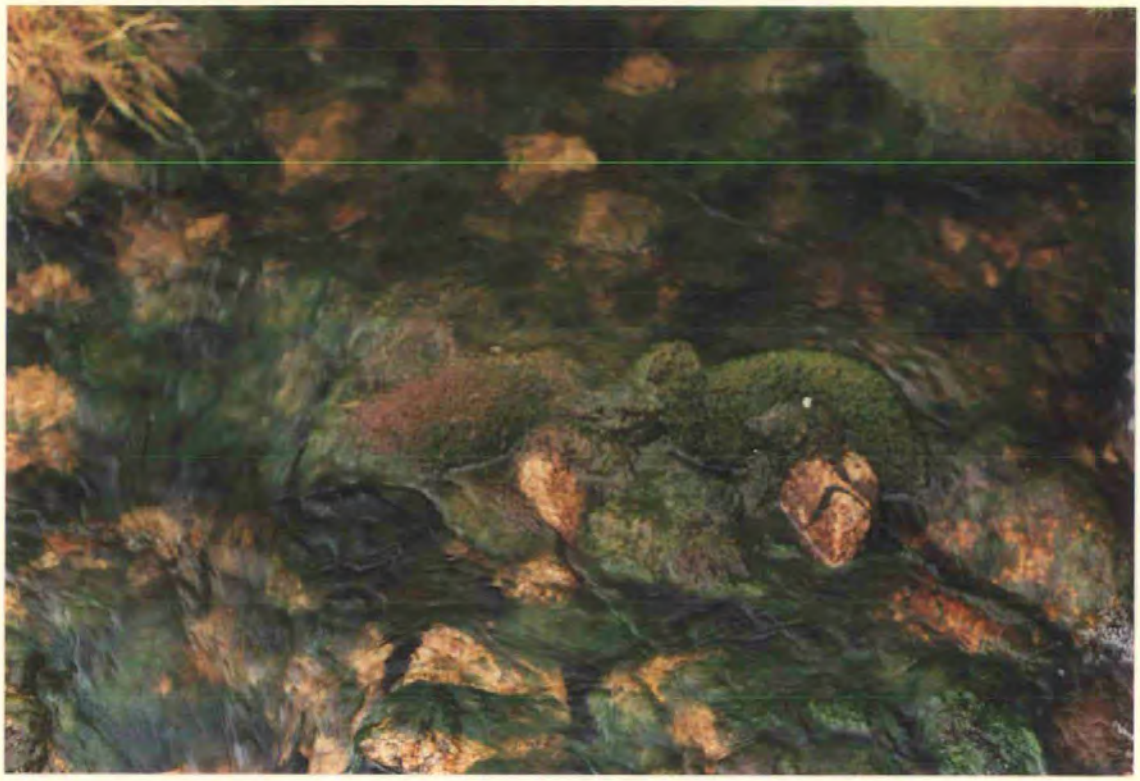


TABLE 5.1. Comparison of the certified metal concentrations ($\mu g\ g^{-1}$) in NIES Chlorella sample with concentrations ($\mu g\ g^{-1}$) determined by the flame AAS programmes used in plant metal analyses.

METAL	CERTIFIED VALUE $\mu g\ g^{-1}$ MEAN	DETERMINED VALUE $\mu g\ g^{-1}$ MEAN	S.D.
COPPER	3.5	2.9	0
IRON	1850	1520	8
CALCIUM	4150	4000	23
MAGNESIUM	3300	2495	25

FIGURE 5.1. Mean concentrations ($\mu g\ g^{-1}$) of copper in replicate samples (n = 4) of Jungermannia atrovirens, Microspora sp. (from station 1) and Juncus bulbosus root and shoot fractions (from station 2) from January 1984 to July 1985. Standard errors are indicated by vertical bars.

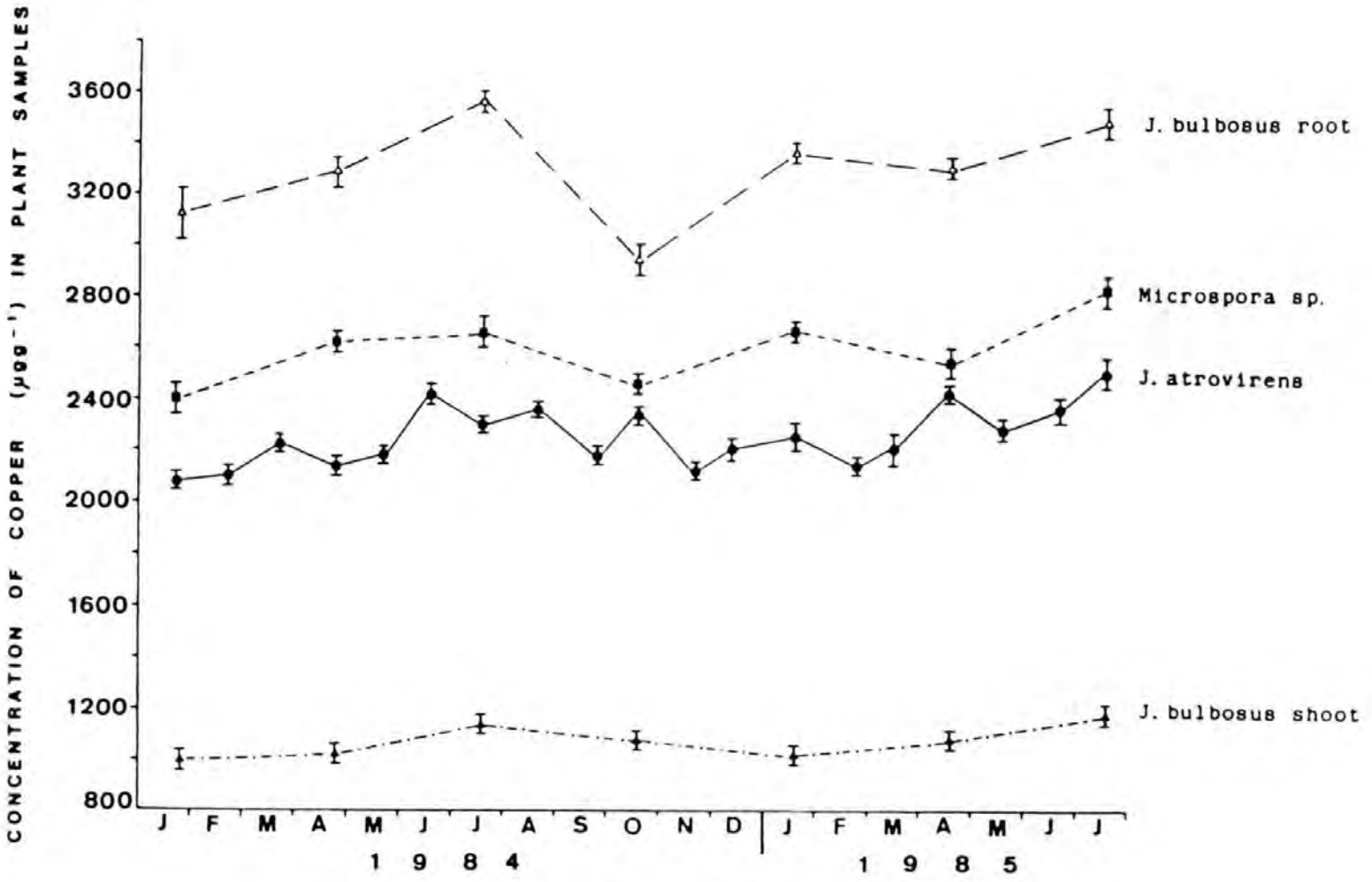

CONCENTRATION OF COPPER (µgg⁻¹) IN PLANT SAMPLES
3600
3200
2800
2400
2000
1600
1200
800
J. bulbosus root
Microspora sp.
J. atrovirens
J. bulbosus shoot
J F M A M J J A S O N D J F M A M J J
1 9 8 4
1 9 8 5

FIGURE 5.2. Mean concentrations ($\mu g\ g^{-1}$) of iron in replicate samples (n = 4) of Jungermannia atrovirens, Microspora sp. (from station 1) and Juncus bulbosus root and shoot fractions (from station 2) from January 1984 to July 1985. Standard errors are indicated by vertical bars.

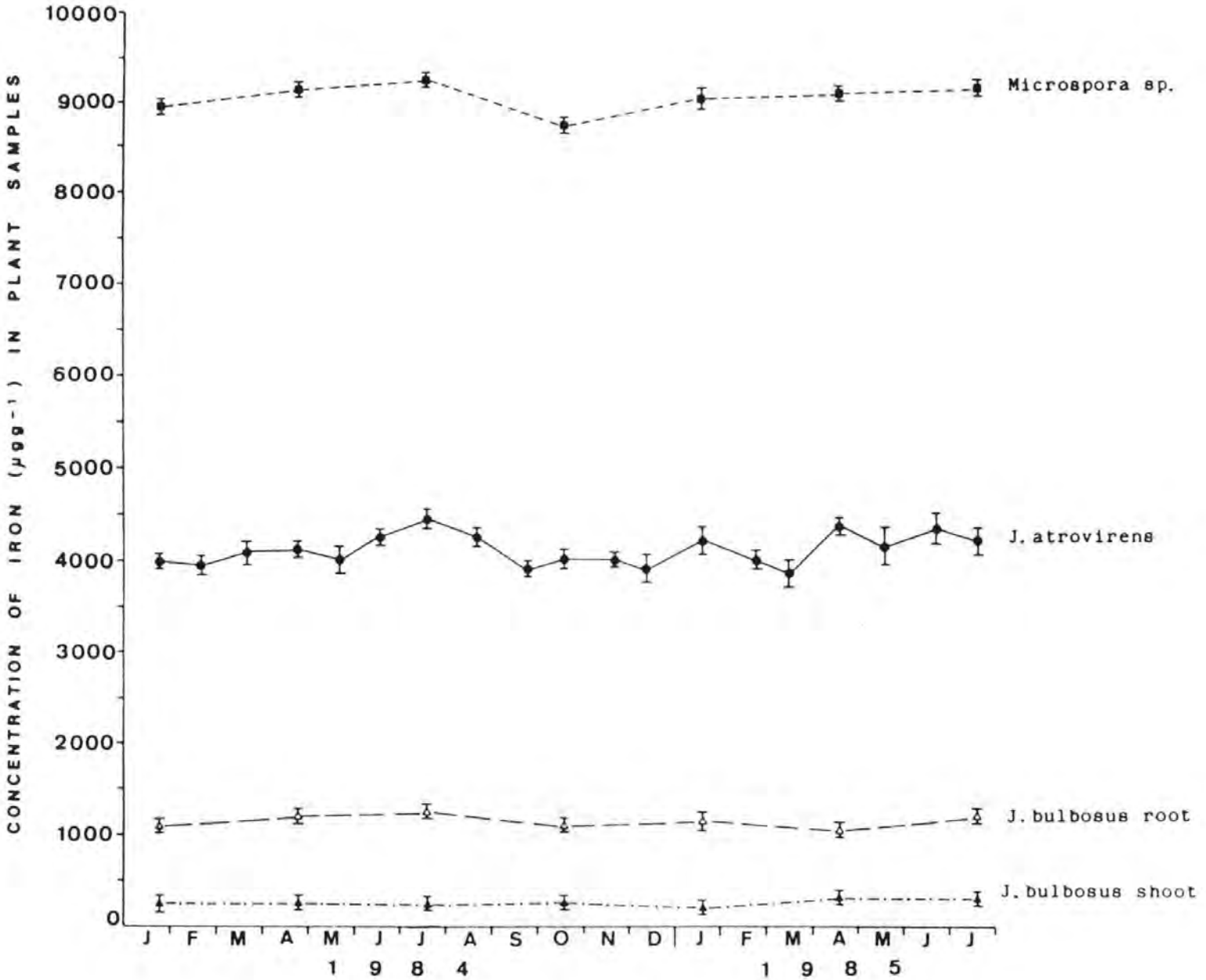

CONCENTRATION OF IRON (µgg⁻¹) IN PLANT SAMPLES
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
J F M A M J J A S O N D J F M A M J J
1 9 8 4
1 9 8 5
Microspora sp.
J. atrovirens
J. bulbosus root
J. bulbosus shoot

FIGURE 5.3. Mean concentrations ($\mu g\ g^{-1}$) of calcium in replicate samples (n = 4) of Jungermannia atrovirens, Microspora sp. (from station 1) and Juncus bulbosus root and shoot fractions (from station 2) from January 1984 to July 1985. Standard errors are indicated by vertical bars.

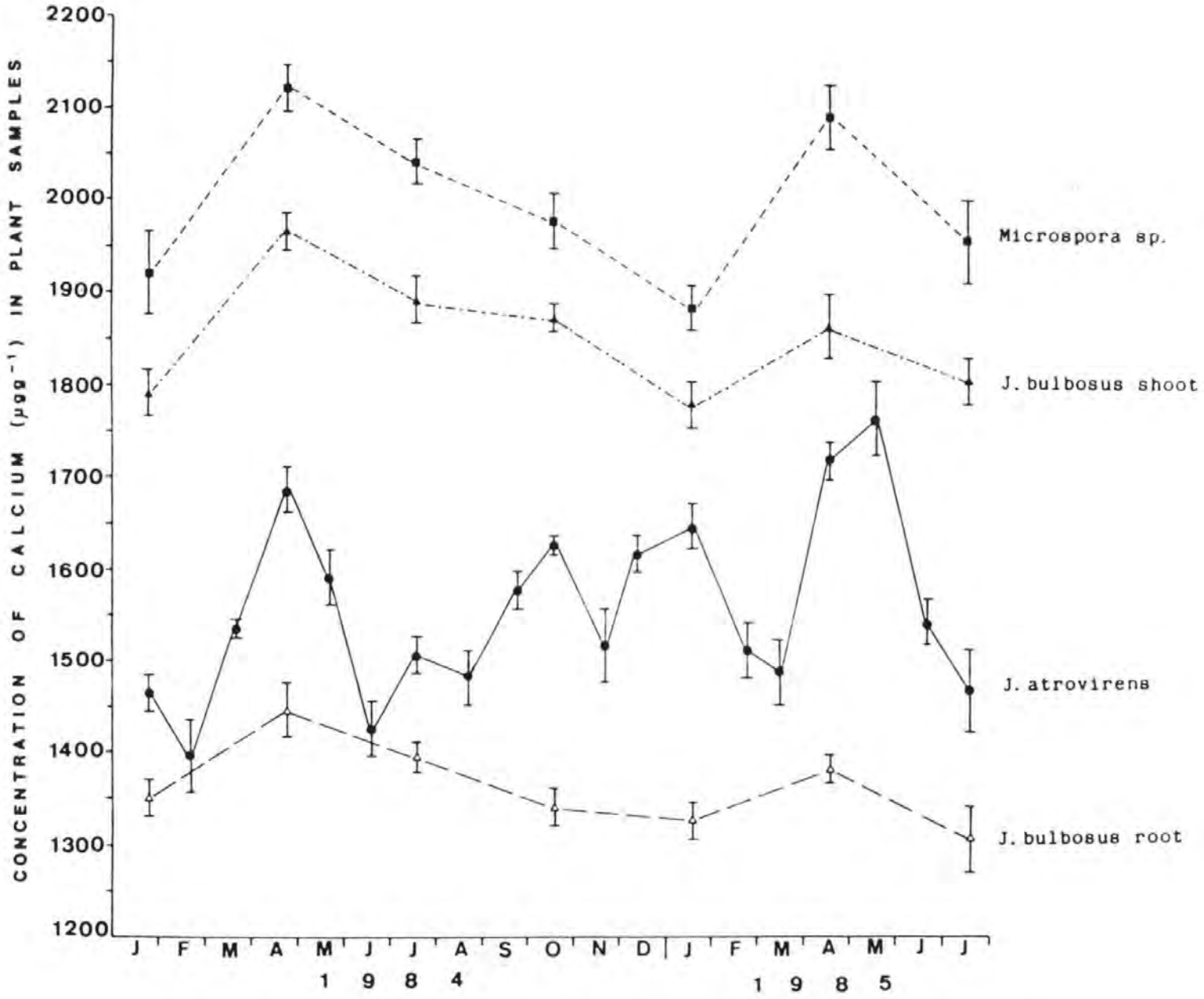

CONCENTRATION OF CALCIUM (µgg⁻¹) IN PLANT SAMPLES
2200
2100
2000
1900
1800
1700
1600
1500
1400
1300
1200
J F M A M J J A S O N D J F M A M J J
1 9 8 4
1 9 8 5
Microspora sp.
J. bulbosus shoot
J. atrovirens
J. bulbosus root

FIGURE 5.4. Mean concentrations ($\mu g\ g^{-1}$) of magnesium in replicate samples (n = 4) of Jungermannia atrovirens, Microspora sp. (from station 1) and Juncus bulbosus root and shoot fractions (from station 2) from January 1984 to July 1985. Standard errors are indicated by vertical bars.

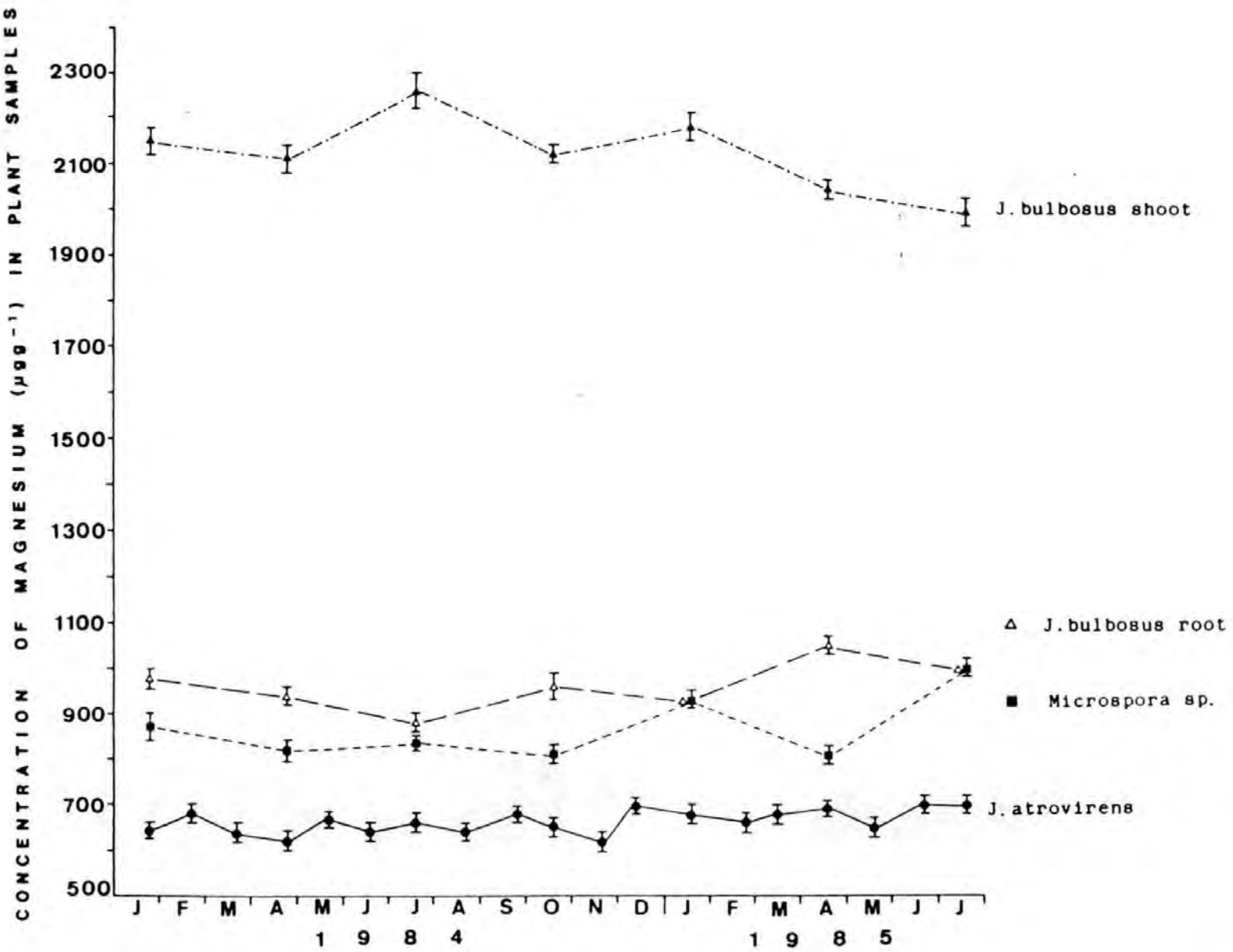

CONCENTRATION OF MAGNESIUM (µgg⁻¹) IN PLANT SAMPLES
2300
2100
1900
1700
1500
1300
1100
900
700
500
J. bulbosus shoot
J. bulbosus root
Microspora sp.
J. atrovirens
J F M A M J J A S O N D J F M A M J J
1 9 8 4
1 9 8 5

FIGURE 5.5. Mean concentrations ($\mu g\ g^{-1}$) of arsenic in replicate samples (n = 4) of Jungermannia atrovirens, Microspora sp. (from station 1) and Juncus bulbosus root and shoot fractions (from station 2) from January 1984 to July 1985. Standard errors are indicated by vertical bars.

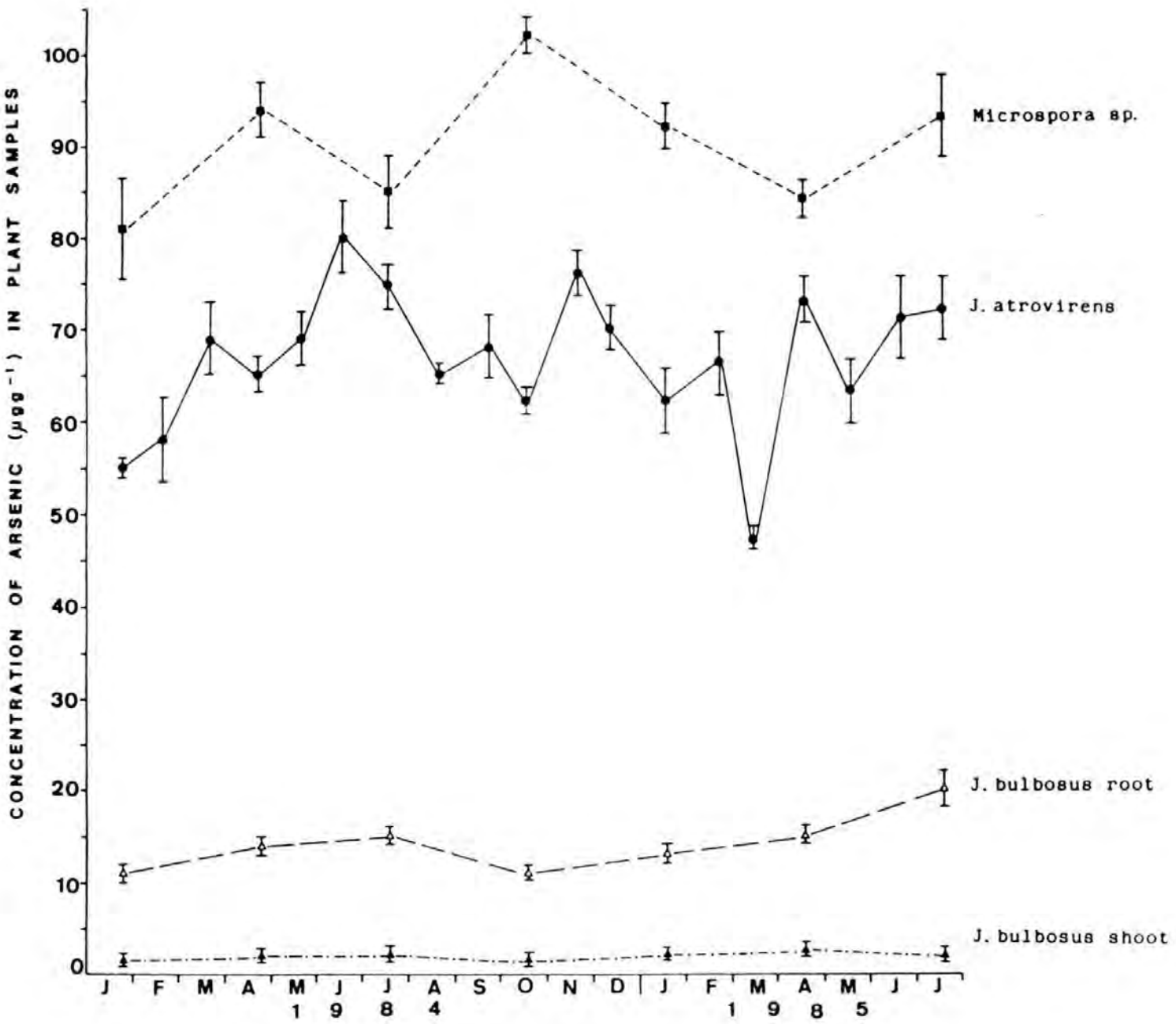
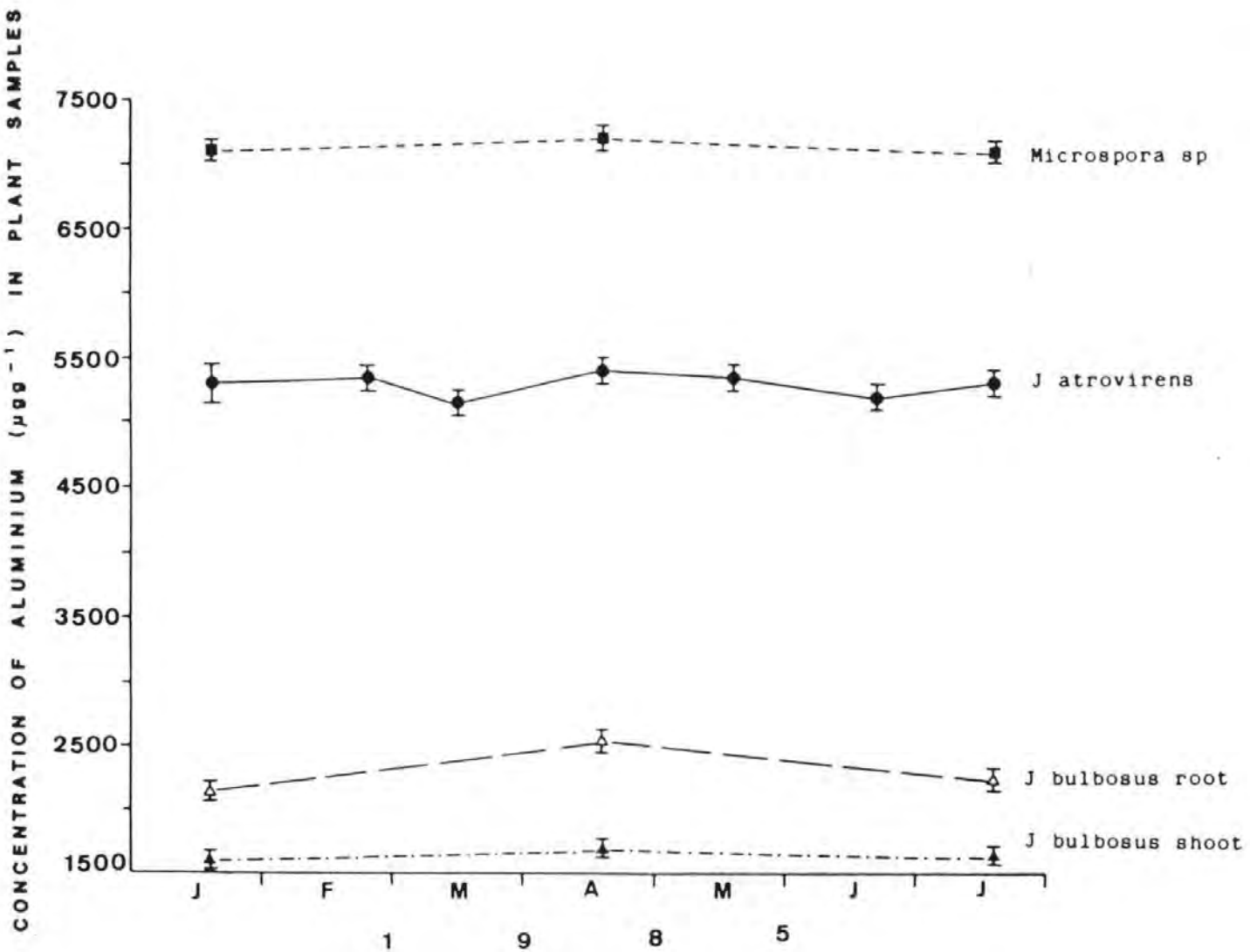

CONCENTRATION OF ARSENIC (μgg⁻¹) IN PLANT SAMPLES
100
90
80
70
60
50
40
30
20
10
0
Microspora sp.
J. atrovirens
J. bulbosus root
J. bulbosus shoot
J F M A M J J A S O N D J F M A M J J
1 9 8 4
1 9 8 5

FIGURE 5.6. Mean concentrations ($\mu g\ g^{-1}$) of aluminium in replicate samples (n = 4) of Jungermannia atrovirens, Microspora sp. (from station 1) and Juncus bulbosus root and shoot fractions (from station 2) from January to July 1985. Standard errors are indicated by vertical bars.


CONCENTRATION OF ALUMINIUM (µgg⁻¹) IN PLANT SAMPLES
7500
6500
5500
4500
3500
2500
1500
Microspora sp
J atrovirens
J bulbosus root
J bulbosus shoot
J
F
M
A
M
J
J
1985

All three species of plant were found to contain elevated concentrations of the metals measured in the water at stations 1 and 2. The degree to which a metal was accumulated differed between the plant samples, and in the case of iron, arsenic and aluminium there was no overlap between the concentrations measured in the four plant samples. For example the range of mean iron concentrations (see Figure 5.2) was 8750 to 9250 $\mu g\ g^{-1}$ in Microspora, 3850 to 4450 $\mu g\ g^{-1}$ in J. atrovirens, 1050 to 1250 $\mu g\ g^{-1}$ in J. bulbosus root and 200 to 300 $\mu g\ g^{-1}$ in J. bulbosus shoot.

With both copper and calcium there was clear separation of individual mean values, but because of seasonal variation there was an overlap in the range of mean concentrations between some of the plant samples (e.g. the mean concentrations of calcium in Microspora and J. bulbosus shoot). In the case of magnesium overlap was only recorded on two occasions, when the mean concentrations for J. bulbosus root and Microspora were the same.

A series of two-way analysis of variance tests were carried out to assess whether there were any significant differences in the metal concentrations in the root and shoot fractions of J. bulbosus. The concentrations of copper ($F = 8613$), iron ($F = 7180$), arsenic ($F = 933$) and aluminium ($F = 479$) were all significantly higher ($p < 0.001$) in the root fraction. However the concentrations of magnesium ($F = 9102$) and calcium ($F = 1553$) were significantly higher ($p < 0.001$) in the shoot fraction.

To investigate any temporal variation in plant metal concentrations, one-way analysis of variance tests were carried out on each metal in all four plant samples. The results, presented in Table 5.2, show that the concentrations of copper, iron, calcium and magnesium all varied significantly ($p < 0.01$) with time in all four plant samples.

TABLE 5.2. F values together with their level of significance (N.S. Not Significant, $^{**}p < 0.01$, $^{***}p < 0.001$) obtained from one-way analysis of variance tests carried out to investigate temporal variation in the metal concentrations of Jungermannia atrovirens, Microspora sp. and Juncus bulbosus root and shoot fractions.

METAL	J. atrovirens	Microspora sp.	J. bulbosus root	J. bulbosus shoot
COPPER	15.54^{***}	9.56^{***}	13.68^{***}	4.82^{**}
IRON	2.63^{**}	11.40^{***}	7.86^{***}	4.56^{**}
CALCIUM	13.03^{***}	7.01^{***}	4.88^{**}	7.82^{***}
MAGNESIUM	3.04^{***}	14.53^{***}	8.34^{***}	11.33^{***}
ARSENIC	6.55^{***}	4.05^{**}	9.02^{***}	$1.74^{N.S.}$
ALUMINIUM	$0.69^{N.S.}$	$1.20^{N.S.}$	26.04^{***}	$1.84^{N.S.}$

Arsenic concentrations also showed significant temporal variation ($p < 0.01$) with the exception of concentrations in J. bulbosus shoot which were not significantly different (at $p = 0.05$ level) over time. Only aluminium concentrations in J. bulbosus root showed significant ($p < 0.001$) temporal variation.

The sequence of copper accumulation in the plants (see Figure 5.1) was J. bulbosus root (range of mean concentrations was 2940 to 3560 $\mu g\ g^{-1}$) > Microspora (2400 to 2820 $\mu g\ g^{-1}$) > J. atrovirens (2080 to 2500 $\mu g\ g^{-1}$) > J. bulbosus shoot (1000 to 1180 $\mu g\ g^{-1}$). In all these plant samples the copper concentrations were higher in the summer than in the preceding winter, for example in J. bulbosus root the mean copper concentration was 3120 $\mu g\ g^{-1}$ in January 1984 and increased to 3560 $\mu g\ g^{-1}$ in July 1984.

Iron, arsenic and aluminium showed the same sequence of metal accumulation in the plants, namely Microspora > J. atrovirens > J. bulbosus root > J. bulbosus shoot. There was a marked difference between the concentrations of iron in the four plant samples (see Figure 5.2), for example the concentrations in Microspora (range of mean concentrations was 3850 to 4450 $\mu g\ g^{-1}$) were upto 40x greater than those measured in J. bulbosus shoot (200 to 300 $\mu g\ g^{-1}$). Although the concentrations of iron in the four plant samples were found to vary significantly with time, these variations were not pronounced. Indeed in J. bulbosus shoot the mean concentration remained at 250 $\mu g\ g^{-1}$ throughout 1984, fell to 200 $\mu g\ g^{-1}$ in January 1985 and subsequently increased to 300 $\mu g\ g^{-1}$. Maximum mean concentrations in both Microspora and J. bulbosus root were recorded in July of both years. In J. atrovirens the highest concentration in 1984 was recorded in July, but in 1985 the maximum concentration was measured in both April and June.

The differences between the concentrations of arsenic (see Figure 5.5) in Microspora, J. bulbosus root and J. bulbosus shoot were

of similar magnitude to the differences between the iron concentrations in these three plant samples. However compared to values for iron, J. atrovirens had a higher concentration of arsenic in proportion to the other plant samples. Arsenic concentrations were highest in the root of J. bulbosus in July of both years, whilst concentrations in the shoot fraction remained between 1.5 and 2.5 $\mu g\ g^{-1}$ throughout the sampling period. Concentrations in both Microspora and J. atrovirens were more variable and showed little discernible pattern over time.

Each plant sample was found to have its own distinct range of aluminium concentrations (see Figure 5.6), with concentrations ranging from between 7100 and 7200 $\mu g\ g^{-1}$ in Microspora to between 1600 and 1700 $\mu g\ g^{-1}$ in J. bulbosus shoot. Although the aluminium concentrations in J. bulbosus root were found to vary significantly with time, it should be pointed out that this significance was only based on data from three sampling occasions.

From Figure 5.3 it can be seen that the sequence of calcium concentrations in the four plant samples was Microspora > J. bulbosus shoot > J. atrovirens > J. bulbosus root. The difference between the highest range of mean calcium concentrations (1880 to 2120 $\mu g\ g^{-1}$ in Microspora) and the lowest range (1305 to 1445 $\mu g\ g^{-1}$ in J. bulbosus root) was smaller than the difference between the highest-lowest range of any other metal measured in the plant samples. In all the plants the calcium concentrations reached a maximum in the spring of both years (April in 1984, and April in 1985 except for J. atrovirens for which a higher concentration was recorded in May). The pattern of temporal variation in Microspora and in the root and shoot fractions of J. bulbosus was identical, i.e. there was an increase from a minimum concentration in January to a maximum concentration in April in both years, and in 1984 this was followed by a gradual decrease in

concentration. In J. atrovirens the increase and decrease associated with the maximum concentration in spring was steeper than that observed in the other plant samples. Furthermore the change in calcium concentrations in J. atrovirens from the summer of 1984 to the spring of 1985 was the opposite to that recorded in the other plant species.

The concentration of magnesium in the plant samples (see Figure 5.4) followed the sequence J. bulbosus shoot > J. bulbosus root > Microspora > J. atrovirens. Levels in J. bulbosus shoot (range of mean concentrations was 1990 to 2260 $\mu g\ g^{-1}$) were noticeably elevated compared to the other plant samples (where concentrations ranged between 620 and 1050 $\mu g\ g^{-1}$). Although there was significant ($p < 0.001$) temporal variation in the magnesium concentrations of all the plant samples, this variation did not follow any obvious pattern over time.

5.4. Discussion

In the headwaters of the Darley Brook there is a reduced floral community due mainly to the high copper level that the ecosystem has been subjected to for approximately 150 years. Clearly the plants that are present must be able to tolerate the elevated concentrations of both copper and hydrogen ions in the water; indeed Microspora is known to be generally resistant to zinc, copper and lead pollution (Whitton, 1970; Foster, 1982a).

At station 1 the high plant cover (never < 60%) of J. atrovirens and Microspora may be explained by the reduction of grazing invertebrates along this riffle section. It is interesting to note that massive growths of another species of Jungermannia, namely J. vulcanicola, have been observed in an acidic stream subject to mercury contamination (Satake et al., 1983). Patterson and Whitton (1981) estimated the percentage cover of the green alga Mougeotia in a metal polluted stream and observed that the cover was greater in summer than in winter, reflecting the seasonal variation in radiant energy. However in the Darley Brook there was a marked increase in plant cover at station 1 in March 1984 and 1985 and in October 1984, due to an increase in algal biomass. The calcium concentration in the water at this station also increases in spring (reaching a maximum in May 1984 and April 1985) and in autumn (maximum in November 1984), and this could be associated with a change in algal growth.

Relationships between the monthly metal concentrations in J. atrovirens and the monthly physicochemical water variables recorded at station 1 were examined using the Pearson product moment correlation coefficient. This coefficient was also used to investigate correlations between the quarterly metal concentrations in Microspora and the respective physicochemical water variables of station 1. Since

J. bulbosus has a root system it may accumulate metals from the sediment as well as from the water, and so relationships between quarterly metal concentrations in both fractions of the plant with the respective physicochemical water variables and quarterly exchangeable sediment metal levels at station 2 were tested by the Pearson coefficient. However it should be stressed that such relationships between metal concentrations in the plants and in the water and/or sediment are being considered here on the basis of temporal changes at one particular station.

At station 1 the copper concentration in the water has been shown (see Table 3.4) to be positively correlated to discharge, i.e. there is an increased mass flow of copper during periods of high discharge. However there was no significant correlation between the quarterly copper concentrations in the water and in Microspora, indeed the copper concentrations in the alga are higher in the summer even though the concentrations in the water are lower. This is probably a reflection of the effect of increased plant growth and activity during the summer, and this is discussed later on in this section. Foster (1982a) demonstrated significant positive correlations between the concentrations of copper, lead and iron in the water and in the algal communities at a number of different sites along the rivers Hayle and Gannel.

There was no significant correlation between the monthly copper concentrations in J. atrovirens and in the water, and the concentrations in J. atrovirens followed a seasonal pattern of a summer maximum and a winter minimum in each year. This again suggests that the concentration of a metal in a plant may in part be determined by factors outside a body of water, such as plant growth. The concentrations of metals in bryophytes have been shown to reflect the concentrations in the

water of metal contaminated streams, for example Say et al. (1981) found a positive relationship between zinc levels in the water (concentrations ranged from 0.01 to 10 mg l^{-1}) and those in the shoot tips (100 to 10000 $\mu g\ g^{-1}$) of several mosses in the River Etherow.

In studies where aquatic angiosperms have been fractioned the underground roots and rhizomes generally contain higher metal levels than the shoots and above ground parts. Ray and White (1976) found that the root concentrations of copper, cadmium and lead in two vascular plants were higher than the shoot concentrations. Similarly copper, cadmium and lead were accumulated mainly in the roots of the reed Phragmites australis, with a decrease in the concentrations in the above ground parts (Schierup & Larsen, 1981). In the Darley Brook the root fraction of J. bulbosus contained significantly higher concentrations of copper, iron, arsenic and aluminium.

There is evidence in the literature that metals may be transported from the root to the shoot. Peter et al. (1979) found clear evidence for such translocation of copper in two species of Potamogeton, and the distribution of copper within the eelgrass Zostera marina was explained in part by translocation within the plant (Brix & Lyngby, 1982). It is also likely that in submerged species metals can be adsorbed or absorbed by the shoots (Welsh & Denny, 1977; 1980). Translocation and direct accumulation from the water may explain why both the calcium and magnesium concentrations were significantly higher in the shoots of J. bulbosus than in the root fraction.

There were no significant correlations between the metal concentrations measured quarterly in the root and shoot fractions of J. bulbosus and the respective concentrations recorded either in the water or sediment at station 2. The concentrations of copper in both

fractions of J. bulbosus, and the concentrations of iron and arsenic in the root fraction reached a maximum in the summer of both years, probably due to increased plant growth at this time of year.

Copper concentrations in Microspora (upto 2820 $\mu g\ g^{-1}$) taken from station 1, where the maximum concentration of copper in the water was 0.99 mg l^{-1}, are lower than the concentrations recorded by Foster (1982a) in algae (upto 8000 $\mu g\ g^{-1}$) taken from the River Hayle (upto 0.5 mg l^{-1} copper in the water). However copper concentrations in J. atrovirens (range 2080 to 2500 $\mu g\ g^{-1}$) also sampled at station 1 are considerably higher than concentrations reported in other liverworts from metal contaminated waters. Burton and Peterson (1979) measured copper concentrations upto 196 $\mu g\ g^{-1}$ in Solenostoma crenulata growing in stream water with a maximum concentration of 0.2 mg l^{-1} copper, and concentrations upto 160 $\mu g\ g^{-1}$ were recorded in Scapania undulata in streams where the copper concentrations in the water were upto 0.10 mg l^{-1} (Jones et al., 1985). A copper concentration of 27 $\mu g\ g^{-1}$ was measured in J. vulcanicola growing in an acidic stream with a concentration of < 0.01 mg l^{-1} copper in the water (Satake et al., 1983).

In the present study the plant sample with the lowest copper concentrations was the shoot fraction of J. bulbosus (range of concentrations was 1000 to 1180 $\mu g\ g^{-1}$) growing at station 2. These concentrations are however higher than those recorded in the shoots of other aquatic angiosperms, perhaps due to the high copper concentration (mean 0.79 mg l^{-1}) in the water at station 2. Ray and White (1976) measured copper concentrations upto 171 $\mu g\ g^{-1}$ in the stems and leaves of Potamogeton richardsoni growing in stream water with a copper concentration of 0.01 mg l^{-1}, and a concentration of 490 $\mu g\ g^{-1}$ was recorded in the shoots of Myriophyllum alterniflorum

growing in a lake with a concentration of 0.004 mg l^{-1} in the water (Welsh & Denny, 1980).

The copper concentrations in all three plants were higher in the summer than in the preceding winter (see Figure 5.1). This temporal variation is probably a result of growth within the plant; i.e. in the summer when growth is rapid there is an increase in metal uptake and in the number of active binding sites, whilst in winter growth is minimal and metal uptake is markedly reduced. Larsen and Schierup (1981) found that the copper concentrations in the stems of P. australis reached a maximum during the growth season, and then decreased possibly because of translocation of copper to the rhizomes. By contrast copper concentrations in both the above- and below-ground parts of Z. marina reached a maximum when growth had ceased, and were at a minimum at the end of the growing season due to the increase in plant biomass (Lyngby & Brix, 1982).

Thus the aquatic vegetation at stations 1 and 2 in the Darley Brook contains very high copper concentrations which are greater in the summer than in the winter. It would further be expected that both the elevated metal concentrations in these plants, and any temporal variation in the concentrations, would have important effects on the metal concentrations in the consumers of this plant material.

In the Darley Brook where the concentration of iron in the water at stations 1 and 2 was < 0.045 mg l^{-1}, the concentrations of iron in all three plants are generally lower than those reported in comparable species of plants in other metal contaminated streams and rivers. The concentrations of iron in Microspora (range of mean concentration was 8750 to 9250 µg g^{-1}) collected at station 1 are considerably lower than the concentrations of upto 450000 µg g^{-1} recorded by Foster (1982a) in algae taken from the rivers Hayle and Gannel, where the iron concentration in the water was upto 10 mg l^{-1}.

The iron concentrations in J. atrovirens (range 3850 to 4450 μg g^{-1}) sampled at station 1 are lower than other iron concentrations recorded in aquatic liverworts. Burton and Peterson (1979) measured iron concentrations between 2325 and 14180 μg g^{-1} in S. crenulata growing in water with an iron concentration of < 0.15 mg l^{-1}, and concentrations upto 100000 μg g^{-1} were measured in S. undulata taken from the River Ystwyth where the concentration of iron in the water was upto 0.2 mg l^{-1} (Mclean & Jones, 1975). However an iron concentration of only 628 μg g^{-1} was recorded by Satake et al. (1983) in J. vulcanicola growing in an acidic stream with a concentration of 0.02 mg l^{-1} iron in the water. Whilst the mean iron concentrations in the root fraction of J. bulbosus ranged from 1050 to 1250 μg g^{-1} and in the shoot fraction ranged from 200 to 300 μg g^{-1}, there are to the author's knowledge no comparable studies on iron levels in other aquatic angiosperms.

In Microspora and J. bulbosus root the highest mean iron concentrations were recorded in July of both years, while in J. atrovirens maximum mean concentrations were measured in July 1984 and in April and June 1985. It is likely that in the summer these plants accumulate more iron as a result of increased plant growth and activity. Wehr & Whitton (1983) studied the accumulation of several metals including iron over a 14 month period in three aquatic mosses, and found that metal accumulation mirrored temporal changes in water chemistry but did not follow any detectable seasonal pattern.

Whilst there are a number of studies recording metal concentrations in aquatic plants, surprisingly few workers have measured arsenic concentrations in the flora of metal enriched waters. In the headwaters of the Darley Brook the arsenic concentration in the water is < 0.002 mg l^{-1}, and the mean concentrations in Microspora range from 81 to 102 μg g^{-1}. Arsenic concentrations of 14 μg g^{-1} (Lancaster

et al., 1971) and 20 to 40 μg g^{-1} (Reay, 1971) have been measured in the green alga Enteromorpha nana taken from water with an arsenic concentration of upto 0.1 mg l^{-1}.

Atri (1983) has reviewed the literature on arsenic concentrations in aquatic macrophytes and found there to be a wide range in the reported concentrations from 2 to > 1000 μg g^{-1}. Metal analysis of Myriophyllum exalbescens collected near a base metal smelter, where the concentration of arsenic in the water was unspecified, revealed arsenic concentrations of between 5 and 40 μg g^{-1} in the stems and leaves (Franzin & McFarlane, 1980). These last values are higher than the concentrations in J. bulbosus, where maximum concentrations of 20 μg g^{-1} and 2.5 μg g^{-1} were recorded in the root and shoot fractions respectively. It is interesting to note that the ratio of arsenic concentrations in Microspora : J. bulbosus root : J. bulbosus shoot is almost identical to the ratio of iron concentrations in these plant samples. Mean concentrations of arsenic in J. atrovirens were in the range 47 to 80 μg g^{-1}, and to the author's knowledge there are no reports in the literature on arsenic concentrations in aquatic bryophytes.

The aluminium concentration in J. atrovirens remained relatively constant over the seven month sampling period (see Figure 5.6); indeed the maximum deviation from the overall mean concentration of 5294 μg g^{-1} was only 3% in March 1985 (mean concentration was 5150 μg g^{-1}). In aluminium, as in iron and arsenic, Microspora accumulated the highest concentrations (overall mean was 7124 μg g^{-1}), followed by J. atrovirens, and then J. bulbosus root (overall mean was 2305 μg g^{-1}) and shoot (overall mean was 1634 μg g^{-1}) fractions. The author has been unable to find any published accounts on aluminium concentrations in plants in metal contaminated freshwater ecosystems.

Calcium concentrations in Microspora (range of mean concentrations was 1880 to 2120 $\mu g\ g^{-1}$) taken from station 1 where the mean calcium concentration in the water was 1.41 mg l^{-1}, are similar to the lower values recorded in the green alga Mougeotia (most of the calcium concentrations were between 2000 and 4000 $\mu g\ g^{-1}$) growing in mine drainage water with a considerably higher calcium concentration of upto 105 mg l^{-1} in the water (Patterson & Whitton, 1981). The mean calcium concentrations measured in J. atrovirens (ranged from 1395 to 1760 $\mu g\ g^{-1}$) collected at station 1, are comparable with the calcium concentration of 1800 $\mu g\ g^{-1}$ recorded in J. vulcanicola (Satake et al., 1983) taken from a stream which had a higher calcium concentration in the water (upto 16.6 mg l^{-1}) than that recorded at station 1.

The magnesium concentration in J. atrovirens (range of mean concentrations was 620 to 700 $\mu g\ g^{-1}$) collected at station 1 where the mean concentration of magnesium in the water was 1.10 mg l^{-1} were slightly lower, but comparable to, the concentration of 857 $\mu g\ g^{-1}$ recorded by Satake et al. (1983) in J. vulcanicola growing in a stream with magnesium concentrations of upto 1.40 mg l^{-1} in the water.

There are not detailed records on calcium and magnesium concentrations in aquatic angiosperms growing in metal enriched freshwaters. Interestingly in this study the concentrations of both of these metals were higher in the shoot fraction of J. bulbosus than in the root fraction (in contrast to the other metals where concentrations in the root were higher). Earlier on in this discussion it was suggested that the higher shoot concentrations could be due to both translocation and direct accumulation from the water.

The calcium concentrations in all the plants in the Darley Brook reached a maximum in April or May of both sampling years (see Figure 5.3). Analysis of water samples collected at stations 1 and 2

during the present investigation showed that the calcium concentration in the water increased in spring (April or May) in 1984 and in 1985. It is therefore suggested that the maximum calcium concentrations in the plants are a reflection of the increasing calcium concentration in the water. However it should be noted that although the calcium concentration in the water at stations 1 and 2 also increased in autumn (November) 1984, this was not mirrored in the concentrations recorded in any of the plant samples. Patterson and Whitton (1981) observed that the calcium concentrations in Mougeotia growing in a mine drainage stream remained relatively constant over a 12 month period, with the exception of a marked increase in August for which they offer no explanation.

Whitton & Shehata (1982) demonstrated in the laboratory that heavy metal tolerance in the blue-green alga Anacystis nidulans was metal specific, and they suggested that in field sites contaminated by several metals A. nidulans would need to acquire separate mechanisms of tolerance for each metal. By contrast work on heavy metal resistance in Chlorophyta taken from the rivers Hayle and Gannel revealed many multiple resistances and co-tolerances, e.g. copper tolerants were also lead resistant (Foster, 1982b).

A number of heavy metal tolerance mechanisms have been identified in aquatic plants. Foster (1977) demonstrated reduced permeability to copper in tolerant strains of the green alga Chlorella vulgaris. Similarly Mclean and Jones (1975) using Zn^{65} showed reduced permeability at the cell wall and a lower intake rate in the liverwort S. undulata. Copper was found to be bound to the cytoplasmic membrane and cell wall in Chlorella pyrenoidosa (Steeman Nielsen et al., 1969), whilst Mouvet (1984) identified electron dense bodies (possibly containing chromium and copper) in the cell wall and vacuoles of the bryophyte Fontinalis antipyretica.

In the present study the extent to which metals were accumulated by the plants may in part be due to differences in tolerance mechanisms within a single species and between different species. The growth form of a plant may also affect metal uptake, for example Microspora contained higher concentrations of all the metals than J. atrovirens, possibly because the algal filaments present a larger surface area to the water for metal adsorption and absorption than the thallus of the liverwort.

CHAPTER 6

QUANTITATIVE ECOLOGY OF THE MACROINVERTEBRATES

6.1. Plectrocnemia conspersa

6.1.1. Introduction

Larvae of the caddis fly Plectrocnemia conspersa (Curtis) are characteristically found in small acid and cool streams such as those on the moors of Devon and Cornwall. However this species also occurs at lower altitudes and under a wider range of conditions. The larvae of P. conspersa spin nets for the capture of prey. Although the type of net spun depends on the prevailing physical conditions (Townsend & Hildrew, 1979a),it normally consists of a horizontal 'retreat' tube which opens at one or both ends to an extensive coarse meshed array of threads which serve to attach the net to all close objects. Since the nets have a large surface area and are easily damaged by fast flow rates, P. conspersa is generally reported to occur in low water velocities usually up to a maximum of 20 cm s^{-1} (Edington, 1968).

A prominent feature of the species-poor communities in certain metal contaminated streams of South West England, especially those affected by copper, is the relative abundance of P. conspersa. There are however no records on the life cycle and population characteristics of this species in these waters. Indeed, apart from Brown (1976) who investigated the tolerance of the isopod Asellus meridianus to lead and copper in two Cornish rivers, there have been few detailed investigations on any of the tolerant organisms associated with the metal enriched waters of South West England.

The life cycle of P. conspersa has previously been studied by Tachet (1967) using laboratory reared animals, from which one or two generations a year were possible, depending on the environmental conditions.

More recently Hildrew and Townsend (1982) followed its life cycle in a small iron rich stream, and provided evidence for one generation in the year in spite of the fact that all the larval instars were present for most of the year. The species is known to have an extended flight period (Crichton et al., 1978) with varying peaks of activity in different areas of Great Britain, and for this reason further studies of the life cycle are desirable.

Although Hildrew and Townsend (1976; 1982) carried out a quantitative study on a population of P. conspersa which was subject to elevated iron concentrations, they did not compare this population with one in a metal uncontaminated stream. The present investigation was undertaken to enable a quantitative comparison of the density and life cycle of two populations of P. conspersa subject to different water qualities; at station 1 in the Darley Brook where there is a significant and elevated copper concentration, and at station 4 (a tributary of the Darley Brook) which is relatively clean and uncontaminated by copper.

6.1.2. Materials and Methods

Invertebrates were randomly sampled at the comparable riffle stations 1, 3 and 4 by superimposing a grid on a plan of each station (60 m x 1.5 m) and dividing it into 360 0.5 m^2 squares. Ten sample units were collected on each occasion using random number tables. Samples were also taken in the pool (station 2) to determine whether the riffle samples alone gave a fair representation of the overall population density of P. conspersa at the upstream site (station 1).

A rigid plastic cylinder sampler (area 0.075 m^2, height 29 cm, diameter 31 cm) was specially constructed for use in this study (see Plate 6.1). The lower edge was serrated to facilitate working the sampler into the substrate. The upstream side of the sampler was perforated (30 holes each 4 cm in diameter) and covered by 1 mm mesh nylon to prevent material being washed into the sampler. On the opposite (downstream) side a rectangular hole (15 cm x 22 cm) was reinforced with angled metal strips designed to hold a removable 400 µm mesh nylon net.

The sampling procedure was similar to that outlined by Surber (1970). The sampler was worked into the substrate keeping the net in a downstream position. All stones in the sampler were carefully rubbed, removed from the water and examined before being discarded. Stones only partly inside the sampler were turned over in situ and rubbed on both surfaces. The remaining gravel was then stirred to a depth of 8 cm with a trowel to extract interstitial invertebrates (Williams & Hynes, 1974). The net was then removed from the sampler, washed through in the stream water and all the contents transferred into a plastic bag. A small quantity of water was added to the bag, which was sealed with an elastic band for transport back to the laboratory.

In the laboratory the contents of each sample unit were passed through a series of sieves (4 mm, 2 mm, 1 mm mesh) with a plastic

PLATE 6.1. Macroinvertebrate cylinder sampler in position on stream bottom.

PLATE 6.2. Compartmentalised perspex trays used for the storage of P. conspersa larvae.

container at the bottom of the stack. The material in this container was passed through a 125 μm sieve. The contents of each sieve were washed into separate white plastic trays and hand sorted using forceps, and also where necessary a large diameter lens.

Any coleopteran larvae and adults in these sample units were removed and preserved in 70% alcohol for later identification. To avoid cannibalism each larva of P. conspersa was placed in a separate cell in a compartmentalised tray filled with water taken at the site of collection (see Plate 6.2). A number of the larvae were used for metal analysis, and the subsequent treatment of these larvae is outlined in Chapter 7 (see p.218). Head width measurements (see Figure 6.1) were used to separate the larval instars.

At stations 1 and 4 any pupal cases found attached to stones in each of the ten sample units were transferred to a plastic bag with a small quantity of water. The pupal cases were subsequently opened and the enclosed stage identified from its external appearance (Wiggins, 1977); i.e. prepupa (a larva sealed within a pupal case), pharate pupa (a developing pupa within a larval cuticle; this stage commences with the middle and hind larval legs straightened and directed towards the posterior end), and pupa which was sexed into male (narrower abdomen, pair of ventral inferior appendages) or female (broader abdomen, ventral vulvar scale).

Field notes were made on the occurrence of egg masses and emerging adults of P. conspersa at stations 1 and 4. In addition pupal cases from these two stations were brought back to the laboratory and placed in one of two aquaria. Each aquarium was partly filled with aerated water (collected from station 1 or 4) and contained a small amount of aquatic vegetation and a stone. The top of the aquarium was covered with a piece of perforated plastic (the holes being covered by 1 mm mesh nylon). Using this laboratory set-up it was possible to record observations of egg masses and emerging adults.

6.1.3. Results

All larvae of the genus Plectrocnemia identified in the Darley Brook during the course of this investigation were P. conspersa. No individuals of P. geniculata or P. brevis were ever found.

The frequency distribution of 567 measurements of head capsule width of P. conspersa larvae collected at station 1 is presented in Figure 6.1. The measurements were found to fall into 5 distinct and non-overlapping groups, which correspond to 5 larval instars. The range of head capsule widths was found to increase in progressive larval instars, e.g. in instar II the range was 0.11 mm compared to 0.82 mm in instar V. A similar frequency distribution of head capsule widths was observed for larvae collected at station 4.

The relationship between head capsule width and dry body weight was examined in 58 larvae collected in January 1985 from station 1. These two variables were significantly correlated ($r = 0.964$, $p < 0.001$), and the relationship between the two is shown graphically in Figure 6.2. It was possible to distinguish 4 separate groups of measurements, corresponding to larval instars II, III, IV and V, although the discrimination between instars IV and V was less clear than for the other larval instars.

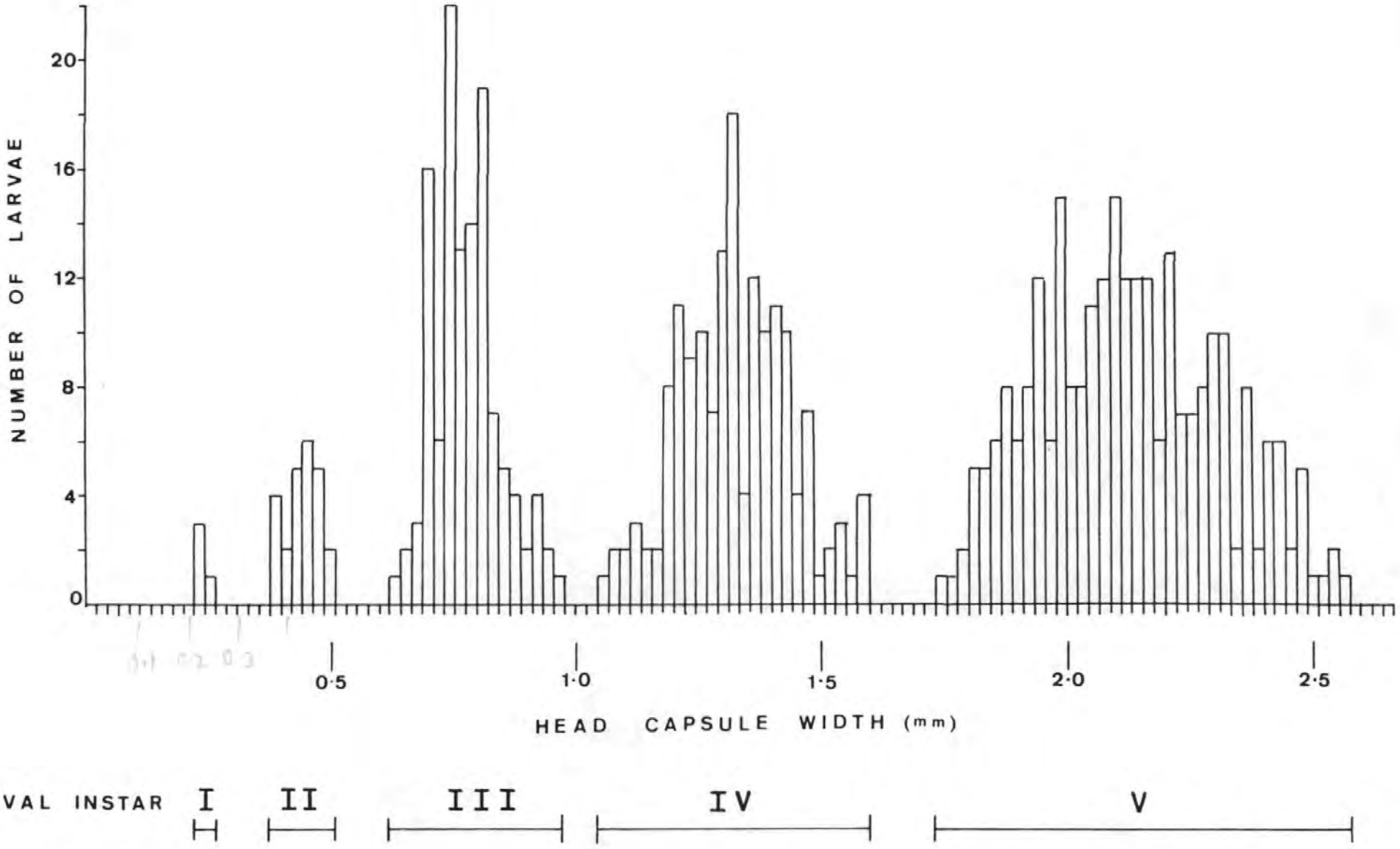
The relationship between head capsule width (H mm) and dry body weight (W mg) was well defined by the following regression equation:

$$\log_{10} W = a + b \log_{10} H$$

where the values of 'a' and 'b' were 0.489 and 2.86 respectively.

Estimates of the mean number of larvae in each instar per 1000 cm^2 (based on the counts from the ten sample units given in Appendix 5A to 5C) at the comparable riffle stations 1, 3 and 4 are shown in Figures 6.3 to

FIGURE 6.1. Frequency distribution of head capsule widths of P. conspersa; the five larval instars are indicated by I, II, III, IV and V.


NUMBER OF LARVAE
0
4
8
12
16
20
0·5
1·0
1·5
2·0
2·5
HEAD CAPSULE WIDTH (mm)
LARVAL INSTAR
I
II
III
IV
V

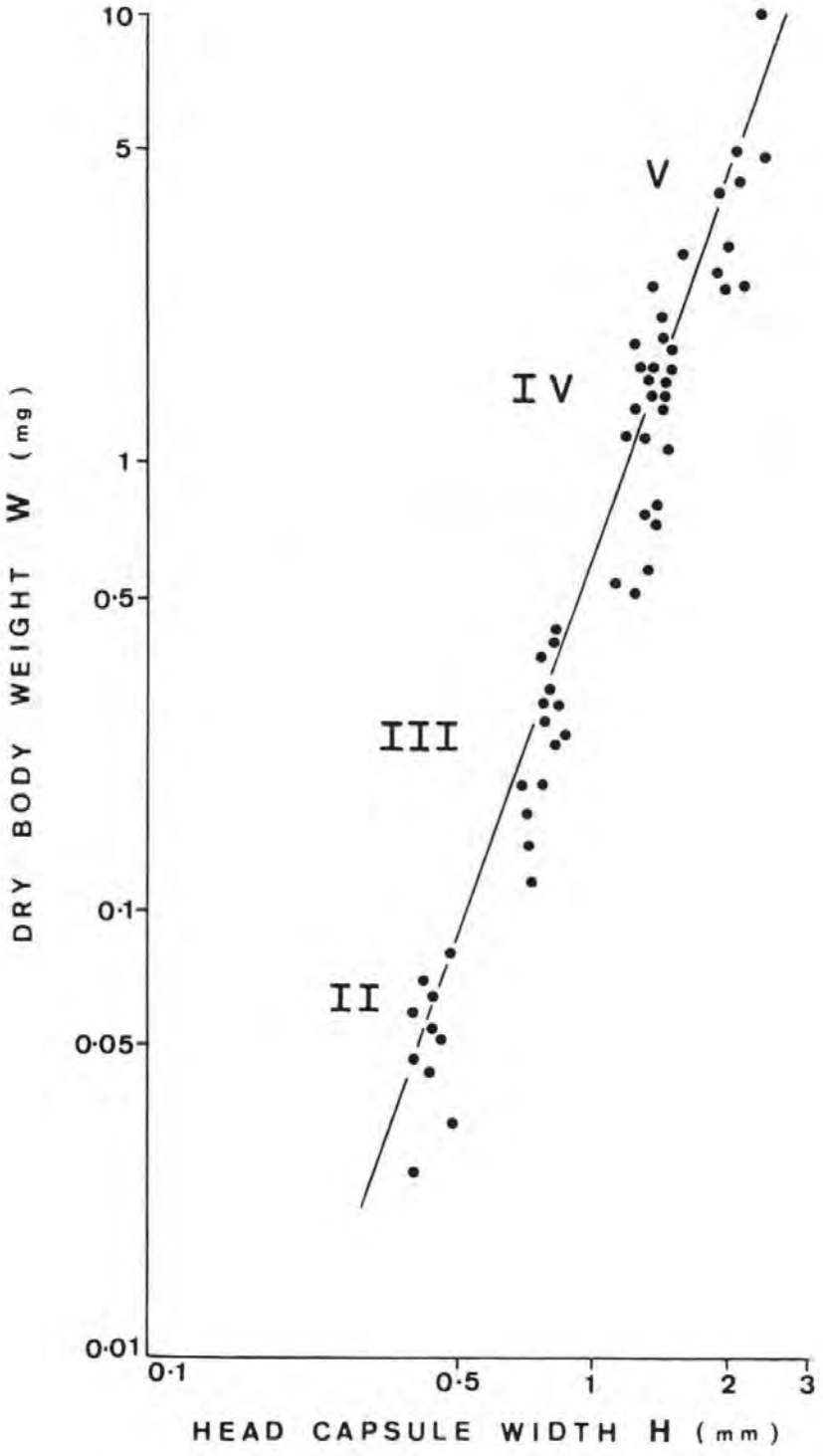


FIGURE 6.2. Relationship between dry body weight (W mg) and head capsule width (H mm) for larval instars II to V of P. conspersa collected at station 1 in January 1985.

6.5 respectively. Similar estimates of the mean number of prepupae, pharate pupae and pupae per 1000 cm^2 (based on selected data taken from Tables 6.1 and 6.2), together with frequency estimates of emerging adults, are shown for stations 1 and 4.

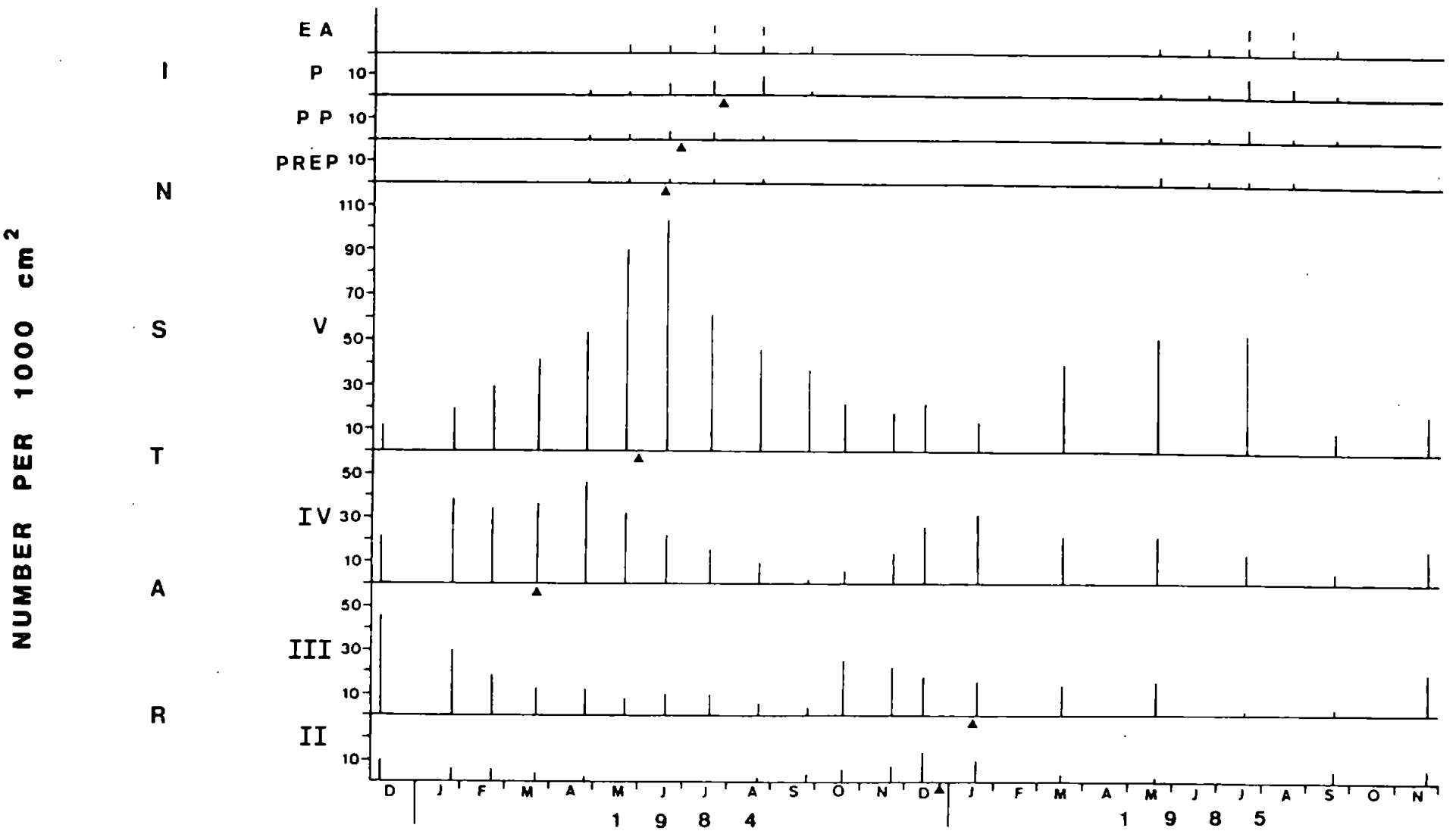
Instar I larvae were found in small numbers in the adit channel upstream of station 1. However in spite of experimenting with the sampling and extraction procedures, instar I larvae were not found at any of the riffle sampling stations and they are therefore omitted from Figures 6.3 to 6.5.

Throughout both years the densities of larvae in instars II, III, IV and V were higher at station 1 than at station 3 or 4, and these differences in densities were greatest in instar V larvae, e.g. in June 1984 the mean number of instar V larvae was 104 m^{-2}, 19 m^{-2} and 21 m^{-2} at stations 1, 3 and 4 respectively. The density of larvae in each instar was similar at stations 3 and 4 during most of the sampling period, apart from July 1985 when a noticeably higher number of instar V larvae was recorded at station 4 (37 m^{-2} compared to 12 m^{-2} at station 3).

In December 1983 when the first samples were taken, all four larval instars (II, III, IV and V) were present at the three riffle stations, the majority being in the third instar. At station 1 the number of instar II and III larvae then decreased, indeed no instar II larvae were found at this station (or at station 3) in May, June and July 1984. Instar II larvae were also absent in these three months at station 4 (as well as in April 1984) and instar III larvae were not found in June and July.

The number of instar IV larvae at station 4 gradually increased from December 1983, and in March 1984 this was the dominant larval instar at this station at a mean density of 12 m^{-2}. The maximum number of instar IV larvae at stations 1 and 3 was recorded in April 1984 (mean

FIGURE 6.3. Mean number of larvae (instars II to V), prepupae (PREP), pharate pupae (PP) and pupae (P) of P. conspersa per 1000 cm^2 at station 1 from December 1983 to November 1985, and frequency estimates of emerging adults (EA) ı rare/occasional, ¦ common. ▲ Median time.


NUMBER PER 1000 cm²
INSTAR
EA
P
PP
PREP
V
IV
III
II
10
110
90
70
50
30
10
D J F M A M J J A S O N D J F M A M J J A S O N
1 9 8 4
1 9 8 5

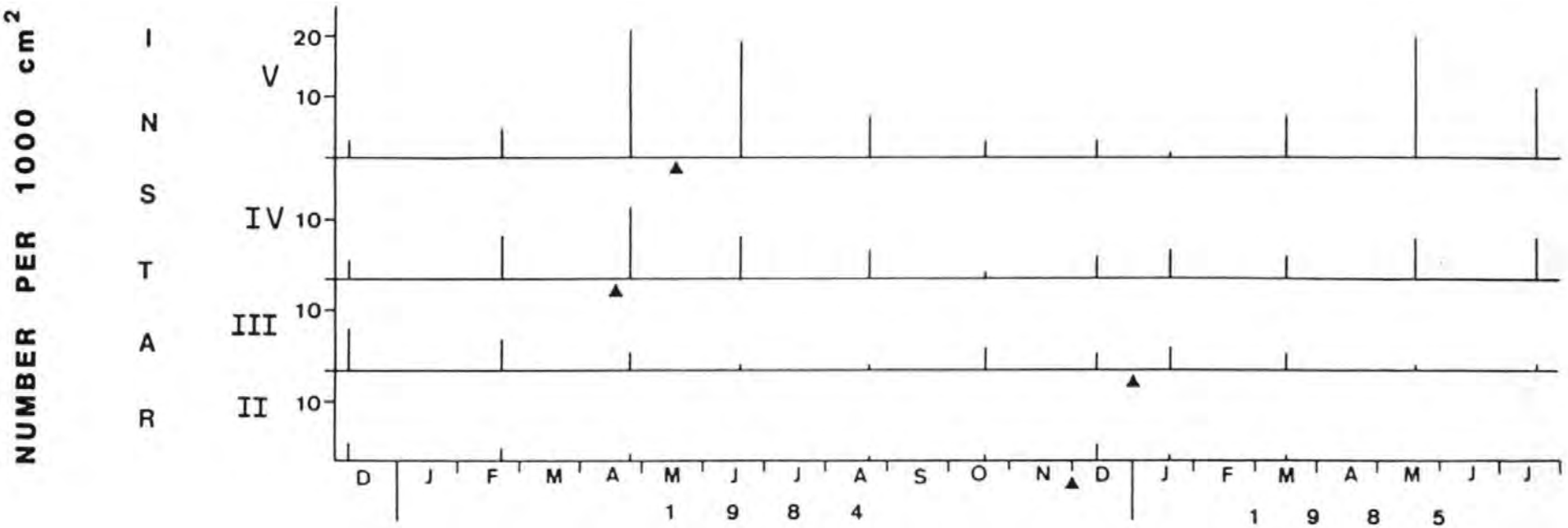


FIGURE 6.4. Mean number of larvae (instars II to V) of P. conspersa per 1000 cm^2 at station 3 from December 1983 to July 1985. ▲ Median time.

FIGURE 6.5. Mean number of larvae (instars II to V), prepupae (PREP), pharate pupae (PP) and pupae (P) of P. conspersa per 1000 cm^2 at station 4 from December 1983 to November 1985, and frequency estimates of emerging adults (EA) ı rare/occasional, ı common. ▲ Median time.

NUMBER PER 1000 cm²
I
N
S
T
A
R
E A
P
P P
PREP
V
IV
III
II
10
40
30
20
D J F M A M J J A S O N D J F M A M J J A S O N
1 9 8 4
1 9 8 5

densities of 46 m^{-2} and 12 m^{-2} respectively), although instar V larvae was the dominant larval instar in this month at these two stations. In 1984 at stations 1 and 4 the number of instar V larvae reached a maximum for the year in June (104 m^{-2} and 21 m^{-2} respectively), whilst the highest numbers of this instar at station 3 were recorded in April 1984 (mean density of 21 m^{-2}).

At stations 1 and 4 prepupae and pharate pupae were first found in April 1984. Pupae were present at station 1 from April to September 1984, reaching a maximum density of 10 m^{-2} in August. At station 4 pupae were found between May and August 1984, with the highest numbers recorded in July (mean density of 4 m^{-2}). The data for pupal case stages (see Tables 6.1 and 6.2) shows that in both years male pupae were present 4 to 7 weeks before female pupae at stations 1 and 4. Furthermore in the laboratory it was observed that male adults emerged several days before females. At station 1 there was a peak in adult emergence in July and August 1984, whilst at station 4 a peak was recorded in July 1984.

In the laboratory some females laid egg masses within a few days of emerging, i.e. newly emerged females were sexually mature and ready to oviposit within a few days. The firm plate-like clear gelatinous egg masses (up to 15 mm diameter) contained numbers of cream oval-shaped eggs, and under laboratory conditions at room temperature the duration of the egg stage was approximately 20 days. Although egg masses were obtained at stations 1 and 4 from June to August in both years, the numbers were relatively few compared to the very large number of chironomid egg masses. The egg masses of P. conspersa were usually found at or just below the water surface on partly submerged stones in slow moving water.

TABLE 6.1. Number of pupal cases of P. conspersa in 10 sample units collected at station 1 from April to September 1984 and from May to September 1985. The cases were either empty (E) or contained an individual in one stage of pupation (PreP prepupa, PP pharate pupa, P pupa). * indicates data used in the estimation of mean number of prepupae, pharate pupae and pupae per 1000 cm^2.

	DATE	PUPAL CASE COUNTS	E	PreP	PP	P	
						Male	Female
1984							
	25/4*	0,0,0,0,0,0,2,0,1,0	0	1	1	1	0
	6/5	0,0,0,0,2,1,0,1,1,0	0	2	1	2	0
	21/5*	2,1,2,2,0,0,0,0,0,0	1	2	2	2	0
	4/6	0,0,0,0,0,2,3,2,0,0	2	1	2	2	0
	18/6*	1,2,0,1,4,3,0,0,0,2	4	2	3	3	1
	2/7	0,2,4,0,6,1,1,0,0,0	4	3	3	3	1
	18/7*	0,2,1,2,3,5,0,0,0,2	4	2	3	3	3
	30/7	7,13,8,9,9,1,0,0,2,0	33	2	4	5	5
	20/8*	5,4,0,1,2,12,0,22,7,4	46	1	2	4	4
	31/8	14,8,3,6,13,1,0,0,1,0	30	1	3	5	7
	10/9	10,2,0,7,5,0,1,1,0,0	17	0	3	2	4
	24/9*	2,0,0,3,0,0,1,0,1,0	5	0	0	0	2
1985							
	17/5*	0,0,2,1,0,0,2,0,0,0	0	3	1	1	0
	4/6	4,0,0,1,3,0,0,0,0,0	0	3	2	3	0
	21/6*	0,0,3,2,1,1,0,1,0,2	4	2	2	2	0
	5/7	2,5,2,0,1,4,2,1,9,0	13	2	3	5	3
	18/7*	0,12,1,1,3,7,4,2,0,0	16	2	4	4	4
	31/7	0,0,0,8,1,1,4,6,1,0	8	1	2	5	5
	19/8*	1,4,1,2,0,0,0,2,0,3	4	2	2	2	3
	3/9	0,0,0,3,2,1,0,0,3,0	3	1	1	1	3
	18/9*	1,0,0,0,0,0,0,0,1,2	2	0	1	1	0

TABLE 6.2. Number of pupal cases of P. conspersa in 10 sample units collected at station 4 from April to August 1984 and from May to September 1985. The cases were either empty (E) or contained an individual in one stage of pupation (PreP prepupa, PP pharate pupa, P pupa). *indicates data used in the estimation of mean number of prepupae, pharate pupae and pupae per 1000 cm^2.

	DATE	PUPAL CASE COUNTS	E	PreP	PP	P	
						Male	Female
1984							
	25/4*	0,0,0,0,0,2,0,0,0,0	0	1	1	0	0
	6/5	1,1,0,0,0,0,0,0,0,0	0	1	0	1	0
	21/5*	0,0,0,1,0,0,0,0,2,0	0	1	1	1	0
	4/6	0,0,0,2,0,0,1,1,0,0	1	1	1	1	0
	18/6*	1,0,0,0,0,1,0,4,0,0	0	2	2	1	1
	2/7	0,0,0,1,0,0,1,4,1,0	0	2	1	2	2
	18/7*	1,0,0,5,0,0,0,0,1,0	0	2	2	1	2
	30/7	0,0,0,0,0,0,2,0,0,0	0	1	0	1	0
	20/8*	0,0,2,0,0,0,0,0,0,0	0	0	1	0	1
	31/8	0,0,0,1,0,0,0,0,0,0	1	0	0	0	0
1985							
	17/5*	0,0,0,0,1,0,0,0,0,0	0	0	1	0	0
	4/6	1,0,0,0,1,0,0,0,0,0	0	1	0	1	0
	21/6*	1,1,0,0,0,0,3,0,0,0	1	2	1	1	0
	5/7	0,0,0,0,2,0,0,0,0,3	2	0	1	1	1
	18/7*	0,0,0,2,1,0,3,0,0,0	0	2	1	1	2
	31/7	0,0,0,3,0,0,0,0,0,3	1	1	2	1	1
	19/8*	0,0,0,0,0,2,4,0,2,0	2	1	2	2	1
	3/9	0,0,0,0,1,2,0,0,0,0	2	0	0	0	1
	18/9*	2,0,0,0,0,0,0,0,0,0	0	0	1	0	1

In August 1984 all four larval instars (i.e. II, III, IV and V) were once again found at all three riffle stations, and they continued to occur in all the samples collected in autumn and winter. In 1984 at station 4 August was the last month in which pupae and emerging adults were recorded, whilst at station 1 they were also observed in September.

In 1985 there was a similar pattern of larval growth to that previously described for stations 1, 3 and 4. A maximum pupal density of 10 m^{-2} was recorded in July 1985 at station 1, where a peak in adult emergence was observed in July and August. At station 4 the maximum number of pupae (mean density of 4 m^{-2}) was recorded in July and August, whilst there was a peak in adult emergence in August.

To compare larval growth at the three riffle stations, the mean numbers of each instar in different months were treated as a frequency distribution. Calculation of 2-unit running means helped to give smooth curves, and the median time for each larval instar was determined planimetrically. These median times are shown in Figures 6.3 to 6.5. It was evident that there was a steady and comparable progression in larval growth at stations 1, 3 and 4, with the following median times: instar II (November/December), instar III (December/January), instar IV (March/April) and instar V (May/June).

Taylor (1961) showed that the relationship between the arithmetic mean ($\overline{X}$) and the variance (S^2) of samples of invertebrates often obeys the power law:

$$S^2 = a\,\overline{X}^{\,b}$$

which can be fitted to the data using the linear regression equation:

$$\log_{10} S^2 = \log_{10} a + b \log_{10} \overline{X}$$

where 'a' and 'b' are constants. The constant 'a' is largely a scaling factor related to sample size, whilst 'b' is an index of aggregation with values of less than 1 indicative of a regular distribution, exactly 1 a random distribution and greater than 1 a contagious distribution (Elliott, 1977).

This model was fitted separately to each larval instar and to the total catches of larvae at each of the three riffle stations. It was also fitted to the pupal case counts at stations 1 and 4. The resulting values of 'a' and 'b' are given in Table 6.3. The 'b' value varied through the larval instars, with the highest values for instars II and III recorded at station 4 (1.34 and 1.21 respectively), and for instars IV and V recorded at station 1 (1.28 and 1.47 respectively). The least variation between the larval 'b' values was found at station 4. All the values of 'b' at station 3 were lower than the corresponding 'b' values at station 4.

When the total catches of larvae were compared, the larvae at station 1 had a higher 'b' value (1.65) than at the other two stations (b = 1.28 and 1.31 at stations 3 and 4 respectively). To test if the difference between the 'b' values for the total catches of larvae at stations 1 and 4 was significant, a t-test was carried out as follows (Southwood, 1978):

1. To calculate the degrees of freedom

$$U = \frac{\dfrac{\text{Variance } b_1}{\text{Variance } X_1}}{\dfrac{\text{Variance } b_1}{\text{Variance } X_1} + \dfrac{\text{Variance } b_4}{\text{Variance } X_4}} = 0.929$$

$$F = \frac{1}{\dfrac{U^2}{n_1-2} + \dfrac{(1-U)^2}{n_4-2}} = 20 \text{ degrees of freedom}$$

TABLE 6.3. Values of the constants 'a' and 'b' (with one standard deviation) in the equation $\log_{10}S^2 = \log_{10}a + b \log_{10}\overline{X}$ for each larval instar, total catches of larvae and pupal cases of P. conspersa at stations 1, 3 and 4 (n = number of samples).

	INSTAR	n	a	b	(S.D.)
STATION 1					
	II	15	1.266	1.018	(0.168)
	III	19	1.196	0.960	(0.152)
	IV	19	1.400	1.278	(0.089)
	V	19	1.026	1.465	(0.253)
	TOTAL LARVAE	19	0.577	1.654	(0.162)
	PUPAL CASES	21	2.007	1.635	(0.082)
STATION 3					
	II	8	0.671	0.824	(0.020)
	III	11	1.382	1.133	(0.152)
	IV	11	0.920	0.768	(0.272)
	V	11	1.514	1.002	(0.152)
	TOTAL LARVAE	11	1.217	1.276	(0.200)
STATION 4					
	II	13	2.037	1.335	(0.181)
	III	16	1.586	1.208	(0.267)
	IV	19	1.367	1.205	(0.091)
	V	19	1.383	1.171	(0.085)
	TOTAL LARVAE	19	1.333	1.314	(0.054)
	PUPAL CASES	19	2.888	1.441	(0.101)

2. t-test

$$t = \frac{b_1 - b_4}{\sqrt{\frac{\text{Variance } b_1}{\text{Variance } X_1} + \frac{\text{Variance } b_4}{\text{Variance } X_4}}} = 0.334$$

i.e. t = 0.334 with 20 degrees of freedom which is not significant at the p = 0.05 level, and so the larvae at station 1 are not significantly more aggregated than those at station 4.

The relationship between sample variance and arithmetic mean for the total larval counts, and for instar V larvae at stations 1, 3 and 4 are shown graphically in Figures 6.6, 6.7 and 6.8 respectively. The variance to mean relationship for the pupal case counts at stations 1 and 4 are also plotted. All lines were fitted by Bartlett's three-group method (Southwood, 1978). At all three stations the spread of the points about the regression line was greatest for the instar V larvae.

From Table 6.3 it can be seen that the values of 'b' for the pupal cases at stations 1 (1.64) and 4 (1.44) were higher than the 'b' values of the instar V larvae (1.47 and 1.17 at stations 1 and 4 respectively). This is also demonstrated in Figures 6.6 and 6.8, in that the regression slope for the pupal cases is steeper than for the final instar larvae. However a t-test (t = 0.191, 19 degrees of freedom) showed there was no significant difference at the p = 0.05 level between the 'b' values of instar V larvae and pupal cases at station 1. Similarly there was no significant difference (t = 0.666, 27 degrees of freedom) between the 'b' values of instar V larvae and pupal cases at station 4.

Before other statistical tests can be applied to the larval counts it is necessary to transform the data to dissociate the mean from the variance and obtain a normal distribution. A suitable transformation

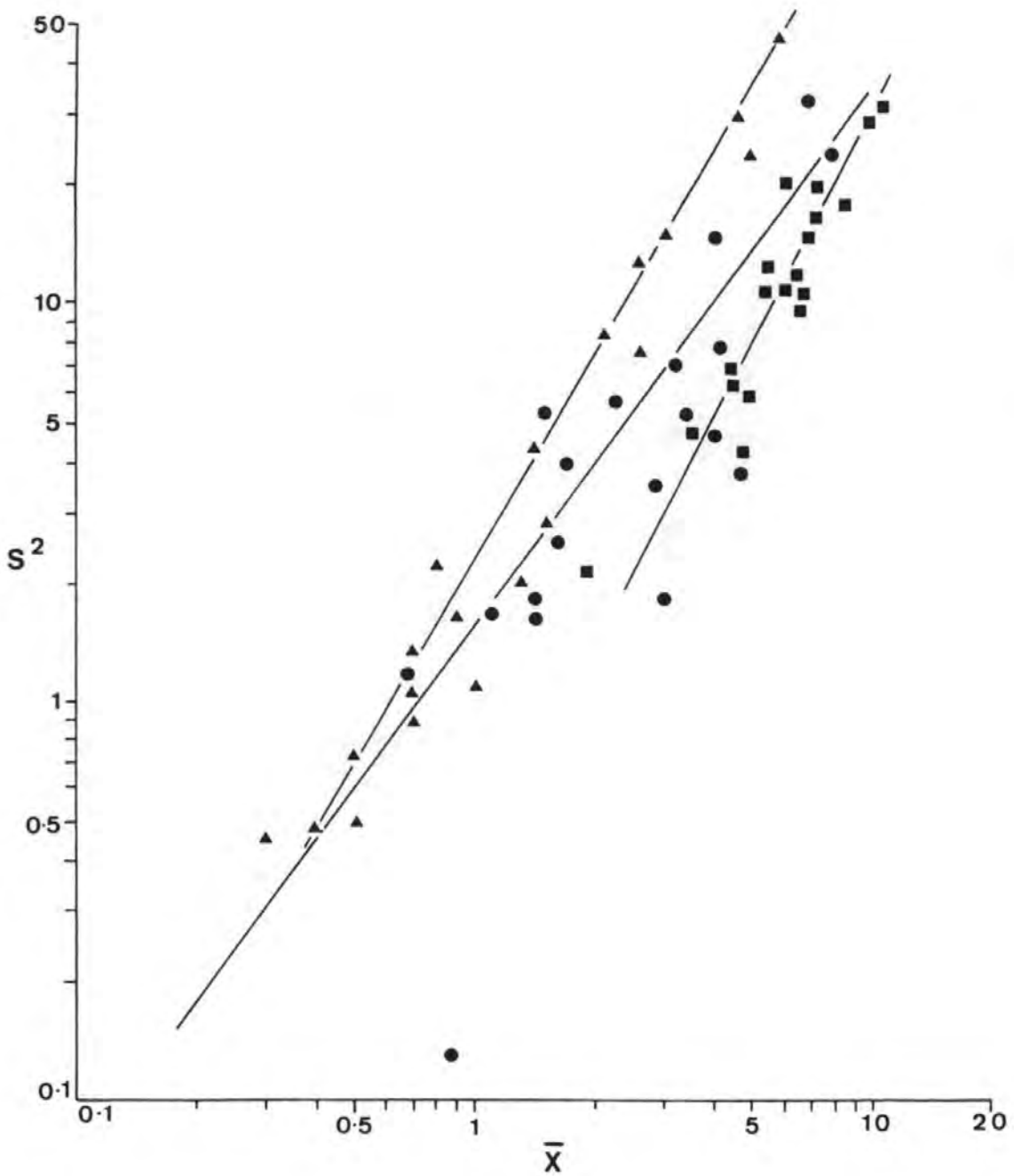


FIGURE 6.6. Relationship between the sample variance (S^2) and arithmetic mean ($\bar{X}$) for pupal cases (▲), larval instar V (●) and all larval instars combined (■) of P. conspersa collected at station 1.

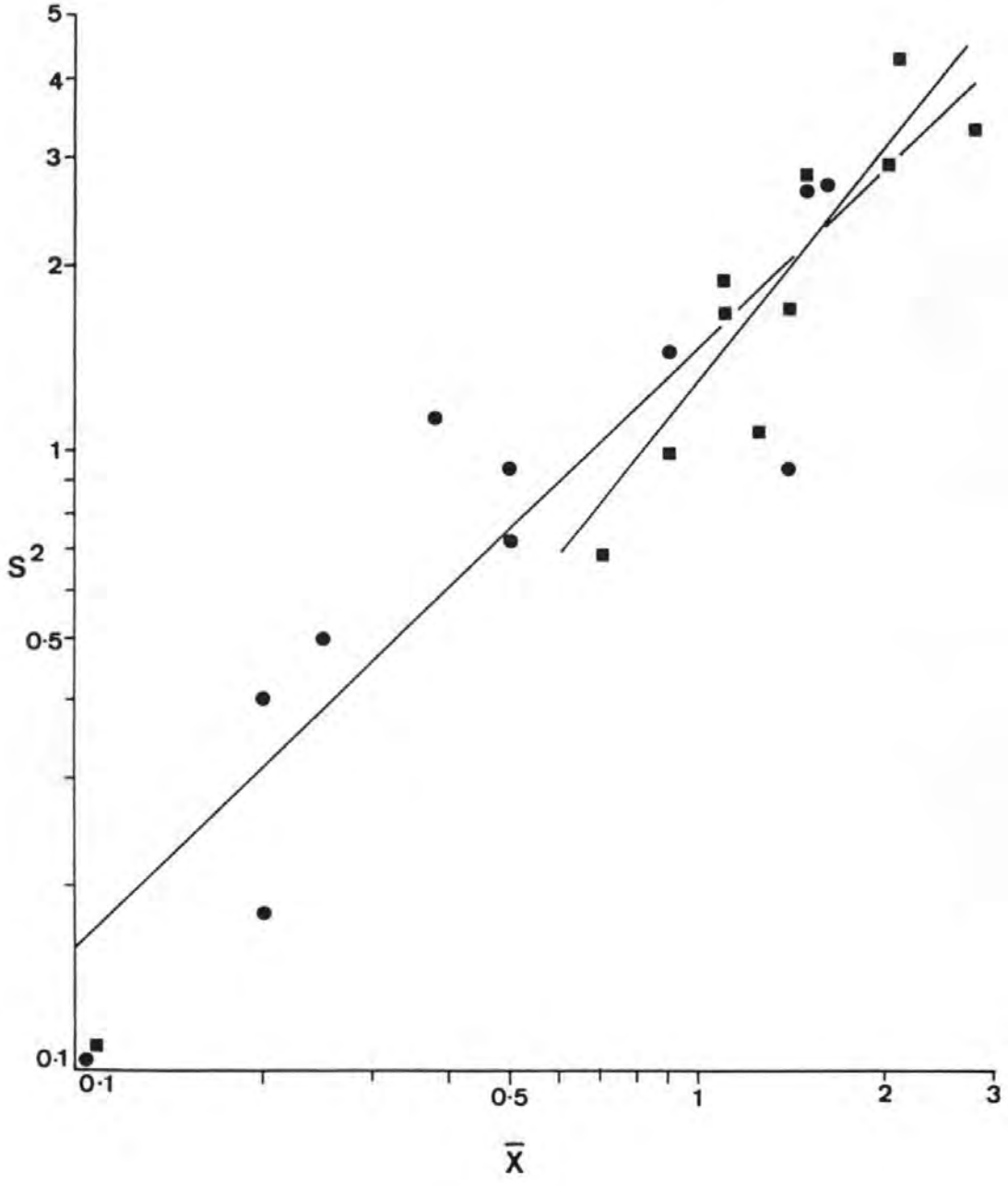


FIGURE 6.7. Relationship between the sample variance (S^2) and arithmetic mean ($\bar{X}$) for larval instar V (●) and all larval instars combined (■) of P. conspersa collected at station 3.

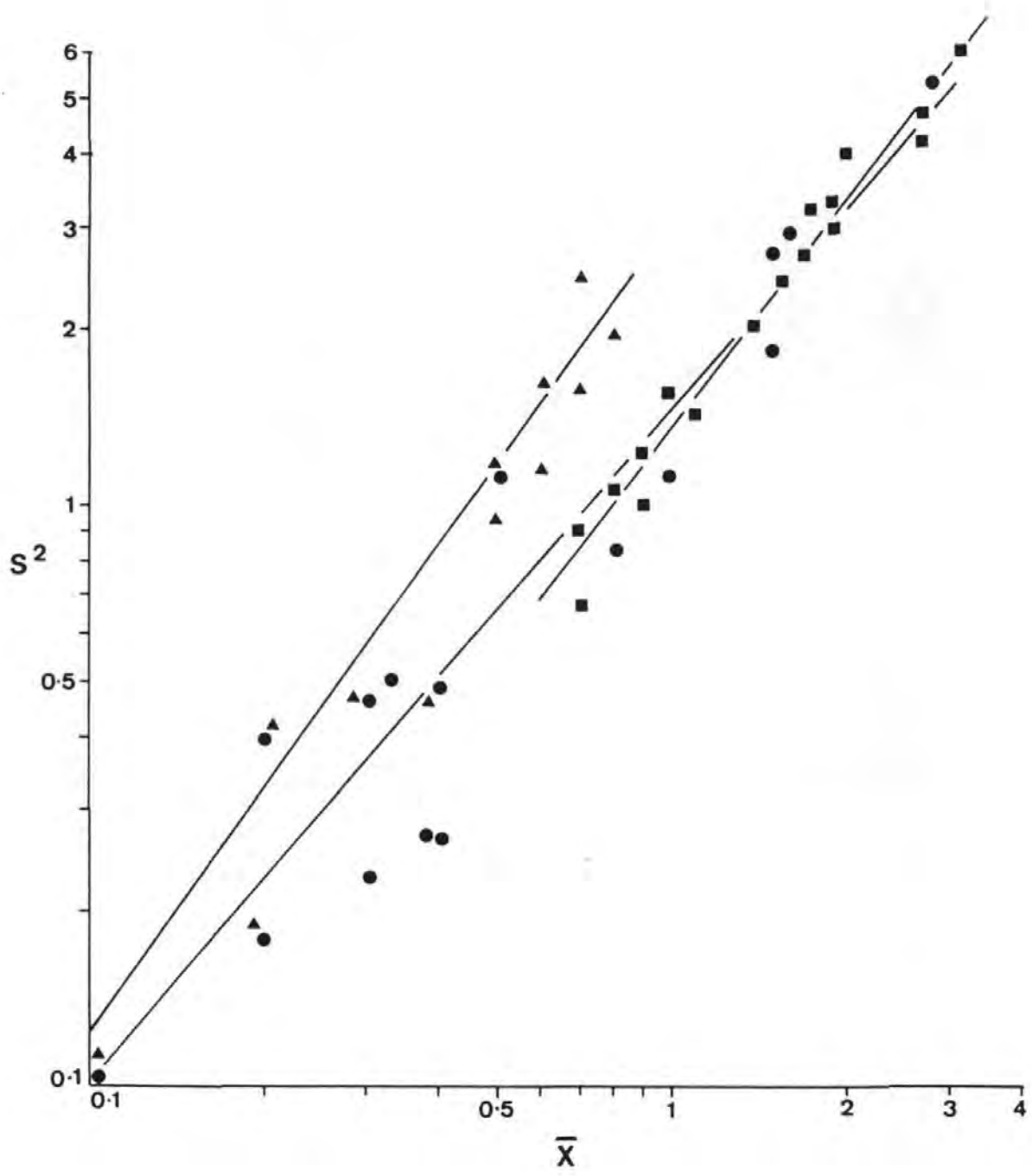


FIGURE 6.8. Relationship between the sample variance (S^2) and arithmetic mean ($\overline{X}$) for pupal cases (▲), larval instar V (●) and all larval instars combined (■) of P. conspersa collected at station 4.

'p' can be obtained from Taylor's power law, which has been shown to fit the data at all three riffle stations, where $p = 1 - \frac{b}{2}$. The data for total larval counts from stations 1,3 and 4 were combined, with the resulting values of 'a' (1.272), 'b' (1.235) and 'p' (0.382). Thus the total larval count X in each sample unit was transformed by $X^{0.38}$.

Two-way analysis of variance tests were carried out on the transformed total larval counts of stations 1, 3 and 4 from April 1984 to November 1985, when the number of sample units was 10 (months prior to April 1984 were omitted since n = 8 or 9). The resulting F values together with a full breakdown of the variance are given in Table 6.4. There was a significant difference ($p < 0.001$) in the number of larvae between stations 1 and 3, and between stations 1 and 4; however the number of larvae at stations 3 and 4 was not significantly different at the 5% level. Furthermore within a station there was a significant difference ($p < 0.01$ or $p < 0.001$) over time. The interaction between time and space was not significant, indicating that the timing of events at the three riffle stations was similar. This supports the earlier observation that the median times for the larval instars were comparable at stations 1, 3 and 4.

Estimates of the mean number of larvae per 1000 cm^2 (based on transformation of the total larval counts, as described above) at stations 1, 3 and 4 are shown in Figure 6.9. The 95% confidence limits were calculated by the methods outlined below. The first method (1A and 1B) is based on the assumption that after transformation the variances are similar throughout the data set, and thus the most reliable estimate of this variance is the 'error' value calculated from the two-way analysis of variance test. A second method (2) was applied to the data which was not used in the two-way analysis of variance test because the number of sample units was less than 10.

TABLE 6.4. F values together with their level of significance (N.S. Not Significant) obtained from two-way analysis of variance tests carried out to investigate temporal and spatial variation in the transformed total larval counts of P. conspersa at stations 1, 3 and 4 from April 1984 to November 1985.

A. Stations 1, 3 and 4

SOURCE	DF	SS	MS	F	SIGNIFICANCE
TIME	8	11.33	1.42	3.94	$p < 0.001$
SPACE	2	66.86	33.43	92.86	$p < 0.001$
INTERACTION	16	3.09	0.19	0.53	N.S.
ERROR	243	88.25	0.36		

B. Stations 1 and 3

SOURCE	DF	SS	MS	F	SIGNIFICANCE
TIME	8	7.82	0.98	3.06	$p < 0.01$
SPACE	1	51.77	51.77	161.78	$p < 0.001$
INTERACTION	8	0.81	0.10	0.31	N.S.
ERROR	162	51.49	0.32		

C. Stations 1 and 4

SOURCE	DF	SS	MS	F	SIGNIFICANCE
TIME	14	18.66	1.33	4.03	$p < 0.001$
SPACE	1	71.67	71.67	217.18	$p < 0.001$
INTERACTION	14	4.60	0.33	1.00	N.S.
ERROR	270	88.22	0.33		

D. Stations 3 and 4

SOURCE	DF	SS	MS	F	SIGNIFICANCE
TIME	8	9.40	1.18	2.68	$p < 0.01$
SPACE	1	0.05	0.05	0.11	N.S.
INTERACTION	8	1.42	0.18	0.41	N.S.
ERROR	162	70.48	0.44		

Method 1A - used for the estimation of 95% confidence limits at stations 1, 3 and 4 in April, June, August, October and December of 1984, and January, March, May and July of 1985.

variance of each sample = 0.36

variance of mean = $\frac{0.36}{10}$ = 0.036

standard error of mean = $\sqrt{0.036}$ = $\pm$ 0.19

95% confidence limits = $t_{0.05}$ x standard error

= 1.96 x 0.19

= $\pm$ 0.37

e.g. April 1984 at station 1

	Transformed	Backtransformed	1000 cm^2 (x 13.33)
mean	2.19	7.87	105
lower limit	1.82	4.84	65
upper limit	2.56	11.87	158

Method 1B - used for the estimation of 95% confidence limits at stations 1 and 4 in May, July, September and November of 1984, and September and November of 1985.

variance of each sample = 0.33

variance of mean = $\frac{0.33}{10}$ = 0.033

standard error of mean = $\sqrt{0.033}$ = $\pm$ 0.18

95% confidence limits = 1.96 x 0.18

= $\pm$ 0.35

The procedure is then identical to Method 1A.

Method 2 - used for the estimation of 95% confidence limits at stations 1 and 4 from December 1983 to March 1984, and at station 3 in December 1983 and February 1984.

e.g. December 1983 at station 1.

mean of transformed counts = 1.99

standard error of mean = $\sqrt{\frac{\text{variance of transformed counts}}{n}} = \pm 0.145$

95% confidence limits = $1.99 \pm 2.37\ (0.145)$

= 1.65 to 2.33

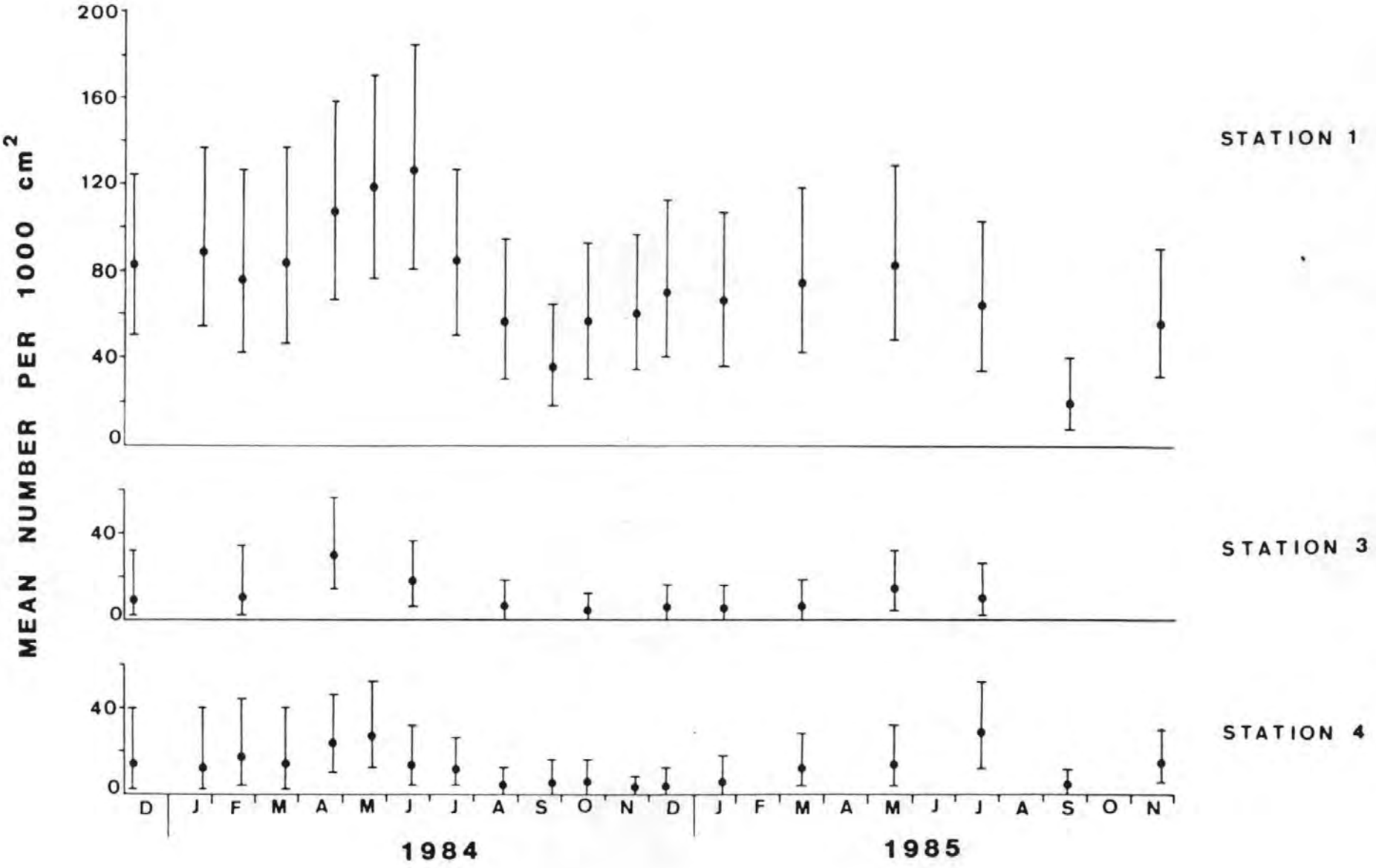
	Transformed	Backtransformed	1000 cm^2 (x 13.33)
mean	1.99	6.12	82
lower limit	1.65	3.74	50
upper limit	2.33	9.26	123

In both methods it should be noted that the derived means are slightly lower than the arithmetic means of the original counts.

Reference to Figure 6.9 shows that at station 1 the mean number of larvae remained relatively constant from December 1983 to March 1984, the numbers then increased to a maximum of 126 m^{-2} in June. There was a subsequent decrease in numbers, which coincided with the peaks in pupation and adult emergence, falling to a minimum of 36 m^{-2} in September. A slow but sustained increase in numbers of larvae was then observed during the next few months, perhaps due in part to the delayed hatching of eggs. In 1985 there was a similar pattern, with a maximum in May of 82 m^{-2} and a minimum of 20 m^{-2} in September.

In contrast to the high numbers recorded at station 1, far fewer larvae were found at station 3. A maximum of 30 m^{-2} was recorded in April 1984, with a decrease to 6 m^{-2} in August. Thereafter numbers of

FIGURE 6.9. Mean number (with 95% confidence limits) of larvae of P. conspersa per 1000 cm^2 at stations 1, 3 and 4 from December 1983 to November 1985.


MEAN NUMBER PER 1000 cm²
200
160
120
80
40
0
STATION 1
40
0
STATION 3
40
0
STATION 4
D J F M A M J J A S O N D J F M A M J J A S O N
1984
1985

larvae remained uniformly low at between 4 and 6 m^{-2} over the winter, and only increased to 14 m^{-2} in May 1985.

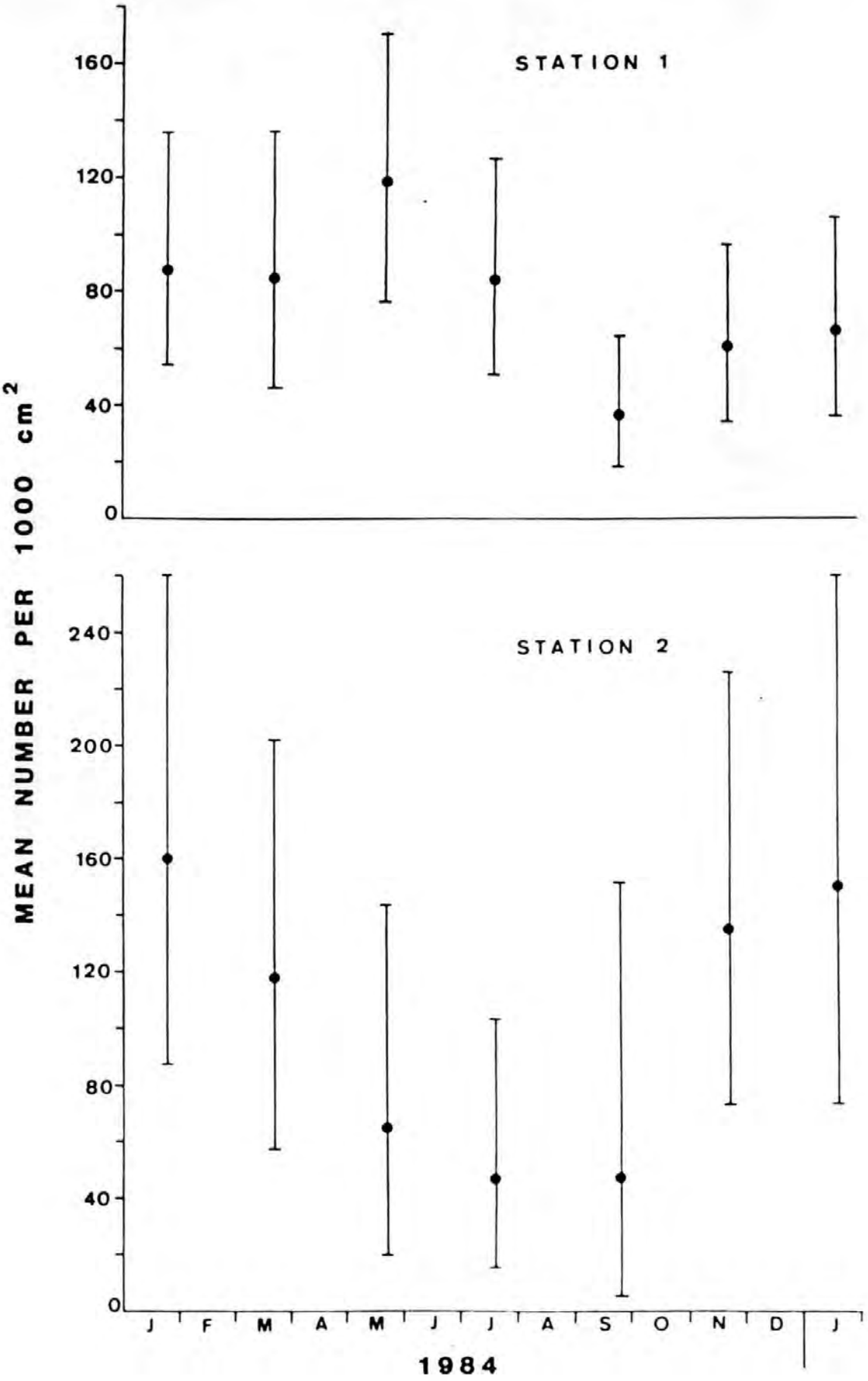
The size of the larval population at station 4 was similar to that at station 3, with mean numbers varying from maxima of 28 m^{-2} (in May 1984 and July 1985) to minima of 2 m^{-2} and 4 m^{-2} (in November 1984 and September 1985 respectively). The general direction of changes in the mean numbers of larvae parallelled those at station 1.

The total larval counts at station 2 (see Appendix 5D) were also transformed by raising each count to the power 0.38. A one-way analysis of variance test was then carried out between the transformed counts of stations 1 and 2, which showed that there was a significant ($F = 3.43$, $p < 0.001$) difference with time between the two stations.

To further investigate this difference the mean number of larvae (together with the 95% confidence limits) per 1000 cm^2 at station 2 was calculated by using Method 2 previously described. The resulting values are plotted with those of station 1 in Figure 6.10. The 95% confidence limits at station 2 were larger than those at station 1 partly due to the smaller number of samples ($n = 4$) taken at station 2.

It can be seen from Figure 6.10 that the population density was significantly greater at station 2 in January (with mean numbers of larvae of 160 m^{-2} and 150 m^{-2} in 1984 and 1985 respectively) than at station 1 (88 m^{-2} and 66 m^{-2} in 1984 and 1985 respectively). By contrast the opposite situation was found in May and July of 1984 with mean densities of 66 m^{-2} and 48 m^{-2} respectively at station 2, compared to means of 118 m^{-2} and 84 m^{-2} respectively at station 1.

FIGURE 6.10. Mean number (with 95% confidence limits) of larvae of P. conspersa per 1000 cm^2 at stations 1 and 2 from January 1984 to January 1985.


STATION 1
STATION 2
MEAN NUMBER PER 1000 cm²
0
40
80
120
160
200
240
J
F
M
A
M
J
J
A
S
O
N
D
J
1984

6.1.4. Discussion

In this study it was possible to distinguish five larval instars in P. conspersa using head capsule width. Table 6.5 shows how these measurements compare with the values recorded by other workers studying this species. All the mean head capsule widths recorded for each instar by Tachet (1967) fall within the size range measured for the corresponding instar in the present investigation. Although the head capsule widths recorded by Edington and Hildrew (1981) are similar to those found in the larvae from station 1, it is interesting to note that the range of head width measurements they recorded in each instar is slightly higher than the range recorded in the same instar in the present investigation.

As dry body weight was significantly correlated with head capsule width (see Figure 6.2) it too could be used to distinguish between larval instars, although subsequent measurements in this investigation showed that the weight of each instar varied from month to month. In the regression equation relating head capsule width (H) to dry body weight (W) i.e. $\log_{10} W = a + b \log_{10} H$, the values of 'a' and 'b' were 0.489 and 2.86 respectively. By comparison Hildrew and Townsend (1976) also related these two variables using the same equation in P. conspersa, and found the values of 'a' and 'b' were 2.58 and 2.80 respectively.

Although instar I larvae were collected from the adit channel, none were found at any of the sampling stations. Elliott (1981) was also unable to collect instar I larvae of another net-spinning caddis Philopotamus montanus, and he suggested that this was due to the rapid development (14 to 18 days) of this instar. Tachet (1967) has demonstrated that the length of the instar I larval stage of P. conspersa (25 ± 6 days) is the shortest of all the larval instars. Thus the absence in this study of instar I larvae may be due to the rapid development of this instar, although in their study on P. conspersa

TABLE 6.5. Comparison of head capsule widths (mm) of larval instars I to V of P. conspersa measured in the present investigation (at station 1) with other head width measurements recorded for larvae of this species.

LARVAL INSTAR	HEAD CAPSULE WIDTH (mm)			
	Present investigation Range	Tachet (1967) Mean (Standard Deviation)		Edington & Hildrew (1981) Range
I	0.24 - 0.27	0.25	(0.01)	0.25 - 0.40
II	0.40 - 0.51	0.41	(0.03)	0.45 - 0.70
III	0.64 - 0.98	0.78	(0.05)	0.80 - 1.10
IV	1.07 - 1.60	1.44	(0.07)	1.30 - 1.75
V	1.76 - 2.58	2.35	(0.15)	1.85 - 2.70

in an iron rich stream, Hildrew and Townsend (1976; 1982) were able to collect instar I larvae.

In 1984 (when samples were collected each month at stations 1 and 4) instar II larvae were present at station 1 throughout the year except from May to July, and at station 4 this instar was not found from April to July. All the other larval instars occurred throughout the year at both stations, with the exception of instar III larvae at station 4 which were not collected in June and July. Hildrew and Townsend (1982) recorded the presence of all five larval instars of P. conspersa in samples collected every two months over a one year period.

At all three riffle stations there is a clear single progression of larvae through the instars, and this is reflected in the one annual median value for each instar. Furthermore the rate of growth is not dissimilar at these three stations, since the median times for each larval instar are comparable (see Figures 6.3, 6.4 and 6.5).

At stations 1 and 4 growth and development culminates in just one main period of pupation in each year, i.e. April to September at station 1 and April to August at station 4 in 1984, and May to September at both stations in 1985. At station 1 there was a peak in adult emergence in July and August of both years, whilst at station 4 a peak was recorded in July 1984 and August 1985. Furthermore since male pupae were present 4 to 7 weeks before females in these two streams, it is reasonable to assume that a number of male adults emerge before females.

Crichton et al. (1978) found that the number of P. conspersa adults caught in light traps in Scotland, Wales and Northern England reached a single maximum in early August, although there were two peaks in Southern England (early June and late August). Tachet (1967) recorded the emergence of males before females (with peaks in July and September

respectively), and demonstrated that under conditions of a warmer environment and plentiful feeding the life cycle of P. conspersa could be considerably shortened.

It is possible that in the warmer south of England P. conspersa could under the right environmental conditions undergo two generations in a year. However at stations 1 and 4 the one main period of pupation each year, followed by the one main period of adult emergence, together with the absence of instar II larvae (and also instar III larvae at station 4) during June and July, all suggest that P. conspersa is univoltine. Hildrew and Townsend (1982) also found that P. conspersa had a one year life cycle in an iron rich stream in South East England, with a peak in adult emergence during June and July.

Reference to Figures 6.3, 6.4 and 6.5 shows that the mean numbers of larvae increased with instar from winter to summer at stations 1, 3 and 4, and no doubt this is partly accounted for by continued recruitment into the larval population during the year. This suggests that there may be some delayed hatching of eggs, and it was proposed by Edington and Hildrew (1981) that delayed egg hatching may have accounted for the presence of instar I larvae of P. conspersa throughout the year in an iron rich stream.

At station 1 there is a marked increase in the summer in instar V larvae, and it is believed that this coincides with the current at this riffle station falling to a velocity more suited for P. conspersa larvae, i.e. less than 20 cms^{-1} (Edington, 1968). It is interesting to note that the current velocity over much of station 1 was found to exceed 20 cm s^{-1} with a stream discharge in excess of 18 l s^{-1}, and reference to Figure 3.4 shows that the current velocity would have fallen below 20 cm s^{-1} from April to September in 1984 and from March onwards in 1985.

Thus in the summer the larvae are able to colonise station 1 rather than drifting through the riffle from the adit above station 1 (where there is a reservoir of larvae) to the pool (station 2). Indeed the mean numbers of instar V larvae at station 1 reached a maximum of 104 m^{-2} in June 1984. However in the winter the fast current probably prevents the long term establishment of larvae at station 1, and it is interesting to note that higher densities are then recorded at station 2 (see Figure 6.10).

It can be seen from Figure 6.9 that the larval densities at stations 3 and 4 are comparable, and yet these two populations are exposed to very different water quality conditions (viz. the elevated copper and hydrogen ion concentrations at station 3). The relatively low numbers of larvae at station 3 is rather surprising in view of the high densities at the upstream stations 1 and 2. However conditions at station 3 are hostile due to the additional physical impact of precipitation on the stream bottom. This precipitate accumulates on the nets of P. conspersa larvae (see Plate 6.3) and it may cause larvae to vacate their nets and be carried downstream. McKnight and Feder (1984) found that in an acidic metal enriched stream (pH = 3.5 to 4.0, filtrable concentrations of aluminium and iron were 4.0 and 0.7 mg l^{-1} respectively) there was a stable community, whilst further downstream (pH = 5.5 to 7.0, aluminium 0.1 mg l^{-1}, iron 0.2 mg l^{-1}) a thick flocculent precipitate of hydrous iron and aluminium oxides prevented the development of a stable community with both plants and invertebrates affected.

The larvae at station 4 are present within a much more diverse community than that found at the contaminated stations 1, 2 and 3 in the Darley Brook. Most of the mean larval densities at station 4 were between 5 m^{-2} and 30 m^{-2}, and these densities are similar to those recorded for the caddis P. montanus found in a metal uncontaminated stream in the English Lake District (Elliott, 1981).

PLATE 6.3. Prey catching nets of P. conspersa larvae covered by a precipitate at station 3.

When considering the total catches of larvae of P. conspersa, it was evident that the larvae at all three riffle stations were clumped, with larvae at station 1 (b = 1.65) more aggregated than at station 3 (b = 1.28) or station 4 (b = 1.31). Furthermore at station 1 the 'b' value is the highest recorded for total catches of caddis larvae in the literature. The values of 'b' for both separate larval instars and total catches of larvae at station 4 are similar to those recorded by Elliott (1981) in P. montanus, e.g. values of 1.31 and 1.27 were calculated for total catches of larvae of P. conspersa and P. montanus respectively.

Hildrew and Townsend (1976) also found that total larval numbers of P. conspersa were clumped, with the final instar larvae less aggregated than the other larval instars. Although in the present study instar V larvae were the least aggregated larval instar at station 4, it is interesting to note that this instar had the highest 'b' value of any of the larval instars at station 1 (see Table 6.3). Further work by Hildrew and Townsend (1980) has shown that the 'aggregative response' shown by P. conspersa larvae is due both to behaviour related to finding and remaining in patches of high prey density (until they become depleted) and to intraspecific encounters at net-spinning sites.

At stations 1 and 4 the values for 'b' for the pupae (1.64 and 1.44 respectively) were higher than for the final instar V larvae (b = 1.47 at station 1 and 1.17 at station 4). Although there are no published records of aggregation in P. conspersa pupae, Elliott (1981) has shown that in P. montanus, pupae (b = 1.62) were more clumped compared to instar V larvae (b = 1.17). In both species the aggregation of pupae could be due to the search and subsequent selection by instar V larvae of suitable pupation sites.

6.2. Macroinvertebrate community structure in the Darley Brook

6.2.1. Introduction

It is well established that metal contaminated streams and rivers generally support a reduced invertebrate community, with the elimination of non-tolerant species often accompanied by an increase in the densities of tolerant species due to lack of predation and competition, and to changes and simplifications in food chains. However the study by Brown (1977a) which contains some limited information on macroinvertebrates in the River Hayle, Cornwall, is the only published account on invertebrate communities in the metal contaminated waters of South West England.

A survey of the River Lynher (South West Water, 1982) found that at Rilla Mill there was a comparatively diverse macroinvertebrate community. By contrast three unnamed western tributaries in the Rilla Mill area, which were probably subject to metal contamination, contained severely restricted faunas. Although South West Water (1984) have recorded elevated copper levels in the lower reaches of the Darley Brook, they note that "without invertebrate and fish population data it is difficult to assess the effect of copper on the aquatic life of the system". Greer (1981) found that the Darley Brook was the only tributary of the River Lynher which was totally devoid of fish, further highlighting the need to study the biotic community in this stream including the macroinvertebrates.

There are only a few papers which deal with the impact of copper on the structure of lotic communities. An early account is by Butcher (1946) who noted that immediately downstream of copper works on the River Trent the invertebrate fauna was practically eliminated. Winner et al. (1975) studied the response of the macroinvertebrate fauna to a copper gradient in an experimentally polluted stream (Shayler Run) and

showed that the composition of the fauna differed depending on the copper concentration in the water, with Chironomidae dominant at the higher copper concentrations.

In a later study Winner et al. (1980) demonstrated that the insect community in two copper contaminated streams (Shayler Run and Elam's Run) showed a "predictable and graded response to heavy metal pollution", with Chironomidae dominant at the most polluted stations and Trichoptera more abundant at stations receiving intermediate copper pollution than at unpolluted stations. Recently (Sheehan & Winner, 1984) the density of macroinvertebrates in two copper contaminated streams (one of which was Shayler Run) was found to be inversely correlated with copper concentration, with Trichoptera and Chironomidae co-dominant at moderately polluted stations.

Darley Brook provides an excellent opportunity to study the invertebrate community in a stream subject to a significant and sustained input of copper, and to investigate how the community structure varies along the length of the Brook as the copper concentration in the water decreases. Comparisons can also be made with the invertebrate fauna of the control stream, a relatively clean tributary of the Darley Brook which is not contaminated with copper.

6.2.2. Materials and Methods

On 18.7.84 and 15.4.86 faunal surveys of the Darley Brook and its relatively clean tributary (station 4) were undertaken. Four riffle stations were selected along the length of the main channel of the Darley Brook and their positions are shown in Figures 2.5 and 2.6. Stations 1, D, F and X have all been described in chapter 2.

On both occasions a random sample of ten 0.075 m^2 sampling units was collected at each of the five stations using the same cylinder sampler and identical procedure to that previously outlined (see p.147). In the laboratory the contents of each sampling unit were sieved and hand sorted. Larvae of P. conspersa were separated into instars and then frozen for subsequent metal analysis, whilst all the other invertebrates were preserved in 70% alcohol for later identification.

To aid identification most of the invertebrates were examined under a binocular dissecting microscope. In the case of the Chironomidae, specimens were placed in gently boiling 10% KOH for 5 to 10 minutes and were then mounted in poly-vinyl lactophenol. The following keys were used in the identification of the invertebrates: Plecoptera (Hynes, 1977), Ephemeroptera (Macan, 1979a), cased Trichoptera (Hickin, 1967), caseless Trichoptera (Edington & Hildrew, 1981), Coleoptera (Galewski, 1976a; Nilsson, 1982a; Friday, 1986), Diptera (Macan, 1979b), Chironomidae (Wiederholm, 1983), Tricladida (Reynoldson, 1978), Hirudinea (Elliott & Mann, 1979) and Gastropoda (Macan, 1977).

In July 1984 an unfiltered acidified water sample was also collected in a 150 ml polythene bottle at each station for metal analysis by flame AAS.

6.2.3. Results

The copper concentrations measured in the water samples collected in July 1984 were at station 1 0.82 mg l^{-1}, at station D 0.26 mg l^{-1} and at station X 0.14 mg l^{-1}, whilst at stations 4 and F the concentration was below the detection limit of flame AAS (i.e. < 0.04 mg l^{-1}).

In July 1984 (see Appendix 5E) a total of 26 genera was extracted and identified from the samples taken at the five riffle stations. On this occasion the mean number of invertebrates was 320 m^{-2}, 93 m^{-2}, 267 m^{-2}, 200 m^{-2} and 493 m^{-2} at stations 1, D, X, F and 4 respectively. In the second survey carried out in April 1986 (see Appendix 5F) a total of 34 genera were identified. In April 1986 significantly ($p < 0.05$) higher numbers of invertebrates were recorded at all the stations, with mean densities of 640 m^{-2}, 360 m^{-2}, 560 m^{-2}, 347 m^{-2} and 733 m^{-2} at stations 1, D, X, F and 4 respectively.

On both sampling occasions insects were the dominant invertebrates, accounting for 88% of all genera in July 1984 and 85% in April 1986. Table 6.6 expresses the number of individuals in each insect order as a percentage of the total number of invertebrates extracted at a station on a sampling occasion. Diptera were the most numerous insects at all the stations(with the exception of station 4 in April 1986 when the Plecoptera were dominant) due to the relatively high numbers of chironomid larvae. Although each chironomid genus occurred at all five stations there was no apparent pattern to their distribution. However in April 1986 the number of Brillia at station 1 (mean density was 11 m^{-2}) was much lower than at the other stations (e.g. the mean density at station D was 89 m^{-2}).

Plecoptera were only found in large numbers at station 4 with Chloroperla torrentium the most numerous of the 6 genera, accounting for 76% of the stoneflies in July 1984 and 56% in April 1986. On both

TABLE 6.6. Number of individuals in each insect order expressed as a percentage of the total number of invertebrates at each of the five sampling stations in July 1984 and April 1986.

18.7.84

ORDER	STATION 1	STATION D	STATION X	STATION F	STATION 4
PLECOPTERA	0.0	1.5	0.0	0.0	21.8
EPHEMEROPTERA	0.0	0.0	12.8	13.5	2.2
TRICHOPTERA	28.1	30.3	3.4	8.8	10.9
COLEOPTERA	32.3	16.7	0.0	0.0	0.0
DIPTERA	39.1	50.0	82.8	53.4	50.1

15.4.86

ORDER	STATION 1	STATION D	STATION X	STATION F	STATION 4
PLECOPTERA	0.0	0.4	0.7	0.0	40.6
EPHEMEROPTERA	0.0	0.0	13.4	32.4	0.5
TRICHOPTERA	15.9	7.9	4.3	12.9	28.4
COLEOPTERA	7.3	3.4	0.5	0.4	0.0
DIPTERA	74.9	79.4	77.1	48.4	20.8

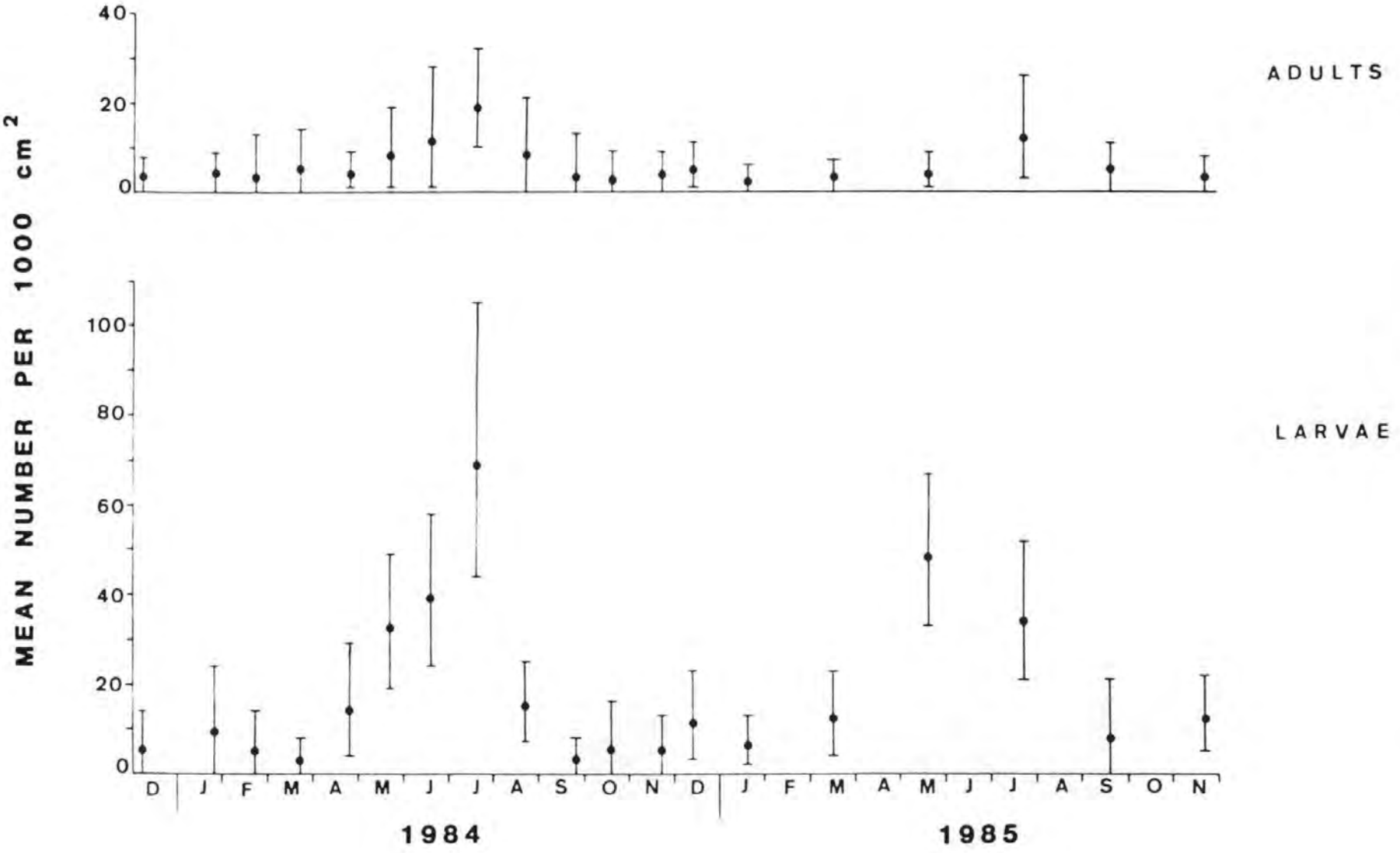
sampling occasions Plecoptera were absent from stations 1 and F. Only one species of Ephemeroptera was recorded in this study, namely Baetis rhodani. Whilst absent from stations 1 and D, this species occurred at stations X and F at mean densities of up to 75 m^{-2} and 111 m^{-2} respectively in April 1986 (at station F it was the dominant genus). In the control stream the numbers of B. rhodani were very low, e.g. in April 1986 a mean density of 4 m^{-2} was recorded.

The highest number of Coleoptera were recorded at station 1, and although no Coleoptera occurred in either survey at station 4 specimens of Elmis and Limnius have been found at this station on other occasions. At station 1 on both occasions two species of Agabus were identified, namely A. guttatus and A. biguttatus (A. bipustulatus has also been collected at this station), and in July 1984 specimens of Ilybius fuliginosus were taken at station 1.

The number of coleopteran larvae and adults occurring in the quantitative samples collected at station 1 from December 1983 to November 1985 are shown in Appendix 5G and 5H respectively, and it should be noted that both species and larval instars have been combined. After this data was transformed (log X + 1) it was possible to estimate the mean number of coleopteran larvae and adults per 1000 cm^2 (see Figure 6.11), with 95% confidence limits calculated by Method 2 previously described (see p.177).

Reference to Figure 6.11 shows that the mean numbers of coleopteran larvae at station 1 remained constant from December 1983 to March 1984, and then increased gradually to a maximum of 69 m^{-2} in July, when there was also a peak in the adult numbers (mean 19 m^{-2}). Numbers of larvae then decreased to a mean of 3 m^{-2} in September and remained constant over the winter. In 1985 a similar trend was observed with maximum mean densities of 48 m^{-2} in May for the larvae, and 12 m^{-2} in July for the adults.

FIGURE 6.11. Mean number (with 95% confidence limits) of coleopteran adults and larvae per 1000 cm^2 at station 1 from December 1983 to November 1985.

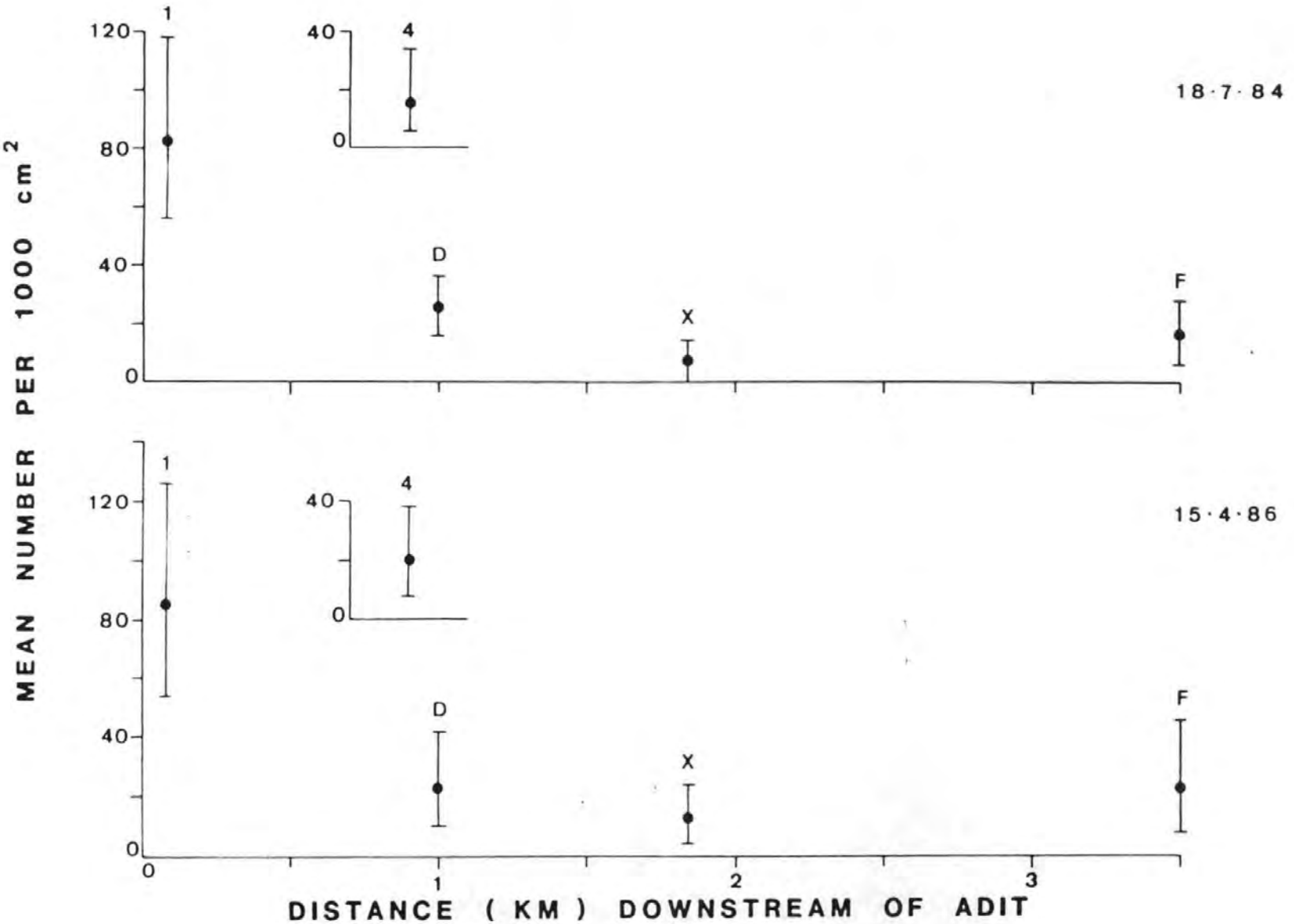

MEAN NUMBER PER 1000 cm²
ADULTS
LARVAE
40
20
0
100
80
60
40
20
0
D J F M A M J J A S O N D J F M A M J J A S O N
1984
1985

The densities of P. conspersa at stations 1 and 4 have already been described. Data from the faunal surveys allows us to further determine the numerical importance of this species at the five sampling stations, and to estimate its density along the length of the Darley Brook. In July 1984 P. conspersa was the only trichopteran species found at stations 1 and D where it was the dominant genus, and at station 1 in April 1986 it was the second most important genus after the chironomid Eukiefferiella sp.

In the lower reaches of the Darley Brook (i.e. stations X and F) the numerical importance of P. conspersa decreased on both sampling occasions compared to the numbers recorded upstream. In the control stream P. conspersa was one of six trichopteran genera that were collected in July 1984, and one of seven genera of this order collected in April 1986. Furthermore at station 4 P. conspersa accounted for 40% of the total number of trichopteran individuals in July 1984 and 8% in April 1986.

The larval counts of P. conspersa at the five riffle stations were logarithmically transformed (log X + 1), and the mean number of larvae per 1000 cm^2 was calculated (together with the 95% confidence limits calculated by Method 2; see p.177) to examine changes in the density of of this species along the length of the Darley Brook. The larval densities are shown in Figure 6.12 and it was evident that in July 1984 and April 1986 the highest mean densities were recorded at station 1 (82 m^{-2} and 84 m^{-2} respectively); with numbers falling sharply at station D (to means of 24 m^{-2} and 22 m^{-2} in July 1984 and April 1986 respectively). The numbers recorded in the control stream and at station F were similar to those at station D. The lowest mean densities were recorded at station X in July 1984 and April 1986 (6 m^{-2} and 12 m^{-2} respectively).

FIGURE 6.12. Mean number (with 95% confidence limits) of larvae of P. conspersa per 1000 cm^2 at stations 1, D, X, F and 4 in July 1984 and April 1986.


MEAN NUMBER PER 1000 cm²
120
80
40
0
40
0
1
4
D
X
F
18·7·84
15·4·86
0
1
2
3
DISTANCE (KM) DOWNSTREAM OF ADIT

Although it has been mentioned that in this study insects were the dominant group of invertebrates, it is worth noting the occurrence of two other groups. In July 1984 at station F the Hydracarina (water-mites) were the most numerically important group, accounting for 24% of all the invertebrates. In April 1986 the triclad Phagocata vitta was found at all five sampling stations, with mean densities ranging from 12 m^{-2} at stations 1 and F to 47 m^{-2} at station 4.

The data collected in each survey of the macroinvertebrate communities in the Darley Brook was processed, at the genus level of classification, using the Shannon-Weaver function H'

$$H' = - \sum_{i=1}^{s} pi \log_e pi$$

where 's' is the number of species (or genera in this study) and 'pi' is the proportion of the total number of individuals consisting of the ith species (genus). Hughes (1978) has shown that the time of year when communities are sampled can influence H', and so the two faunal surveys were treated separately. To obtain the best estimate of H' at a station the 10 sample units were combined to form one data set, it is however still possible to calculate the variance of H' using the equation (Poole, 1974):

$$\text{var } H' = \frac{\sum_{i=1}^{s} pi \log_e{}^2 pi - (\sum_{i=1}^{s} pi \log_e pi)^2}{N}$$

where N is the total number of individuals. Since the number of genera at each station is known, it is also possible to calculate the evenness J = H'/H' max. H' max assumes all the genera are equally common and is estimated by the formula H' max = $\log_e S$, where S is the total number of species (genera).

The number of genera together with the calculated values of the Shannon-Weaver function H' and evenness J for each station in July 1984 and in April 1986 are shown in Table 6.7, and some of this data is also presented graphically in Figure 6.13. It was evident, particularly in April 1986, that differences in the number of genera at the stations reflected similar differences in the more lengthily derived H' index.

In July 1984 the number of genera was constant (n = 11) at stations 1, D and X decreasing to 9 at station F, whilst the values of H' showed no obvious trend. At station 4 a total of 23 genera were recorded and H' was 2.668 $\pm$ 0.002. A t-test showed that the estimates of H' at station 4 and at station 1 (H = 1.976 $\pm$ 0.003) were significantly ($p < 0.001$) different.

Both the number of genera and the estimates of H' showed a clear trend in April 1986, with both indices increasing from station 1 to station X, and then falling slightly at station F. The value of H' at station 4 was 2.685 $\pm$ 0.002, and was significantly ($p < 0.001$) higher than the H' value of 2.280 $\pm$ 0.001 calculated for station 1.

An evenness J value of 1.00 would be obtained for a community in which there was an equal number of all genera. The communities at all stations had high values of J, with the lowest value of 0.78 at station X in April 1986 and the highest value of 0.95 at station F in July 1984.

TABLE 6.7. The number of genera (N) together with the values of the Shannon-Weaver function H' (± standard error) and evenness J calculated at the generic level at stations 1,D,X,F and 4 in July 1984 and April 1986.

	18.7.84			15.4.86		
	N	H' ± S.E.	J	N	H' ± S.E.	J
STATION 1	11	1.976 ± 0.003	0.82	12	2.280 ± 0.001	0.92
STATION D	11	2.118 ± 0.011	0.88	14	2.317 ± 0.002	0.88
STATION X	11	1.995 ± 0.003	0.83	22	2.412 ± 0.002	0.78
STATION F	9	2.085 ± 0.003	0.95	19	2.326 ± 0.004	0.79
STATION 4	23	2.668 ± 0.002	0.85	28	2.685 ± 0.002	0.81

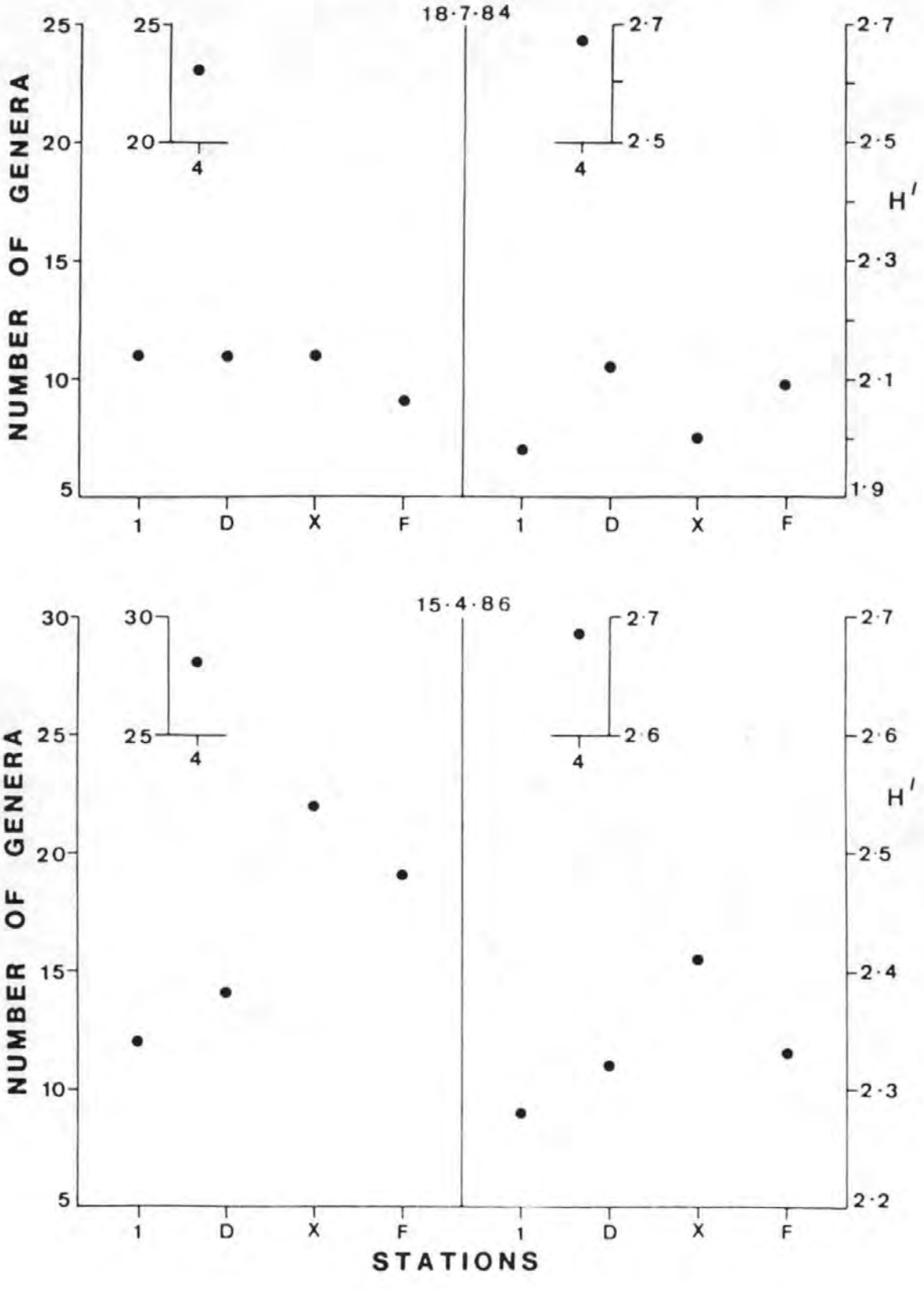


FIGURE 6.13. The number of genera together with the values of the Shannon-Weaver function H' calculated at the generic level at stations 1, D, X, F and 4 in July 1984 and April 1986.

6.2.4. Discussion

The benthic macroinvertebrate communities in the main channel of the Darley Brook and in the control stream are dominated by insects; these are usually prominent in soft waters and are almost invariably the dominant group of invertebrates in metal enriched or contaminated streams (Winner et al., 1975; 1980; Armitage, 1980; Burrows & Whitton, 1983).

In a number of studies chironomids have been found to be numerically important in streams with elevated metal concentrations. Winner et al. (1980) showed that in two streams subject to copper contamination only tubificid worms and chironomids were present at the most polluted stations and furthermore, the percentage of chironomids in a sample decreased in a predictable manner as the copper concentration in the water decreased (however no such pattern was evident in the present investigation). In a later study (Sheehan & Winner, 1984) which examined the macroinvertebrate communities in these same streams, and in another metal contaminated stream, chironomid larvae again dominated the benthic communities at the most severely polluted riffles.

The relative distributions of the chironomid genera at the five sampling stations showed no obvious pattern. Although Armitage and Blackburn (1985) were able to use chironomid associations to differentiate between sites with varying degrees of organic enrichment and zinc pollution, they noted that the use of genera for similar analyses was difficult since the pollution tolerance of different species in a single genus may vary considerably.

At all five stations significantly ($p < 0.05$) higher densities of invertebrates were recorded in the second faunal survey in April 1986. Both the number of genera and H' (Shannon-Weaver function) at each station were also higher in April 1986 than in July 1984. The higher

densities and diversities recorded in April were no doubt due to the larger numbers of younger aquatic stages occurring at this time of year, whilst in July pupation and emergence could reduce both the density and apparent diversity of the macroinvertebrate communities.

On both sampling occasions the value of H' at station 1 was significantly ($p < 0.001$) lower than at station 4, so reflecting the comparatively low number of genera found at station 1. The most striking feature of this reduced community is the numerical importance of P. conspersa which in July 1984 was the dominant genus at station 1. Chironomidae, Coleoptera and Trichoptera account for 39%, 32% and 28% respectively of the total number of invertebrates at station 1 in July 1984 (75%, 7% and 16% respectively in April 1986). Winner et al. (1980) studied the effects of copper on the insect communities in two streams and noted that Trichoptera were relatively and absolutely more abundant at sites receiving moderate copper pollution than at unpolluted sites. In a later study, Sheehan & Winner (1984) found Trichoptera to be co-dominant with Chironomidae at moderately polluted stations in these same streams and also in another metal enriched stream.

Apart from chironomid larvae, P. conspersa was the only invertebrate which occurred at all five stations in both faunal surveys, and so it is interesting to consider its relative densities along the length of the Darley Brook (see Figure 6.12). It has previously been mentioned (see p.145) that P. conspersa is characteristically found in small acid and cool moorland streams, and so conditions at stations 1 and 4 would naturally favour the occurrence of this species, although conditions further downstream in the Darley Brook may be less favourable. However the very high population density at station 1 is probably due to other factors such as reduced interspecific competition within a species-poor community. On the other hand it is interesting to note

that although the number of genera in April at stations X and F was double that recorded in July, the population density of P. conspersa was also higher in April at both stations.

Since P. conspersa is a numerically important species in the community at station 1, where it also occurs at its highest densities, it is interesting to consider the reported occurrence of this species in other metal rich waters. Armitage (1980) found that in streams in the Northern Pennines with high zinc levels P. conspersa was present in a reduced community. In their study of the effects of acidification in West Wales, Stoner et al. (1984) recorded the occurrence of P. conspersa in the impoverished fauna of streams with a low pH and elevated concentrations of aluminium. The detailed investigations of Hildrew and Townsend (1976; 1982) showed P. conspersa to be the important predator in an iron rich stream with a low faunal diversity. More locally Brown (1977a) recorded P. conspersa as one of only a few species in the copper rich waters of the River Hayle. Thus evidence from these studies, together with this investigation, clearly suggests that P. conspersa is a metal tolerant species.

In addition to the Chironomidae and Trichoptera, the other important group of invertebrates at station 1 were the Coleoptera. Three species of Agabus (A. guttatus, A. biguttatus and A. bipustulatus) and Ilybius fuliginosus have been identified at this station. Whilst members of the genus Agabus generally prefer stagnant small freshwater habitats, both A. guttatus and A. biguttatus are also known to be rheophilic (Galewski, 1976b). At station 1 larvae and adults were found throughout the year, and it was noted that the larvae were of different sizes. Several species of Agabus including A. guttatus are known to have three larval instars (Galewski, 1976a; Nilsson, 1982b), and in all three

species of Agabus found in this study other workers have demonstrated overwintering of these larval instars (e.g. Galewski, 1976a; 1976b).

Armitage (1980) found that in a zinc contaminated stream in the Northern Pennines there was a restricted macroinvertebrate community which included P. conspersa and three species of Agabus. It is of interest that two of these species, namely A. bipustulatus and A. guttatus, also occur at station 1 in the Darley Brook where there is an elevated copper concentration. Indeed all three species of Agabus and I. fuliginosus were restricted to stations 1 and D, where the copper concentration in the water in July 1984 was 0.82 mg l^{-1} and 0.26 mg l^{-1} respectively, and they were absent from the less copper contaminated stations.

Larvae of Agabus are predaceous, for example Galewski (1976a) found that A. guttatus and A. biguttatus consumed oligochaetes and larvae from a number of insect groups. If the density estimates of the coleopteran larvae and adults are combined, and added to the densities of P. conspersa larvae at station 1, then it is possible to calculate the mean number of macroinvertebrate predators per 1000 cm^2 at this station from December 1983 to November 1985 (see Figure 6.14). Maximum densities of 176 m^{-2} and 134 m^{-2} were recorded in June 1984 and May 1985 respectively, whilst minimum densities were recorded in September of both years (42 m^{-2} in 1984 and 32 m^{-2} in 1985). In another study on a reduced macroinvertebrate community in a metal contaminated stream (Hildrew & Townsend, 1982) the combined mean densities of the two major predators (P. conspersa and the neuropteran Sialis fuliginosa) reached a maximum of 596 m^{-2} in August and a minimum of 96 m^{-2} in February.

The highest number of invertebrate genera were recorded at station 4 with 23 collected in July 1984 and 28 in April 1986. Population densities of invertebrates at this station were also the

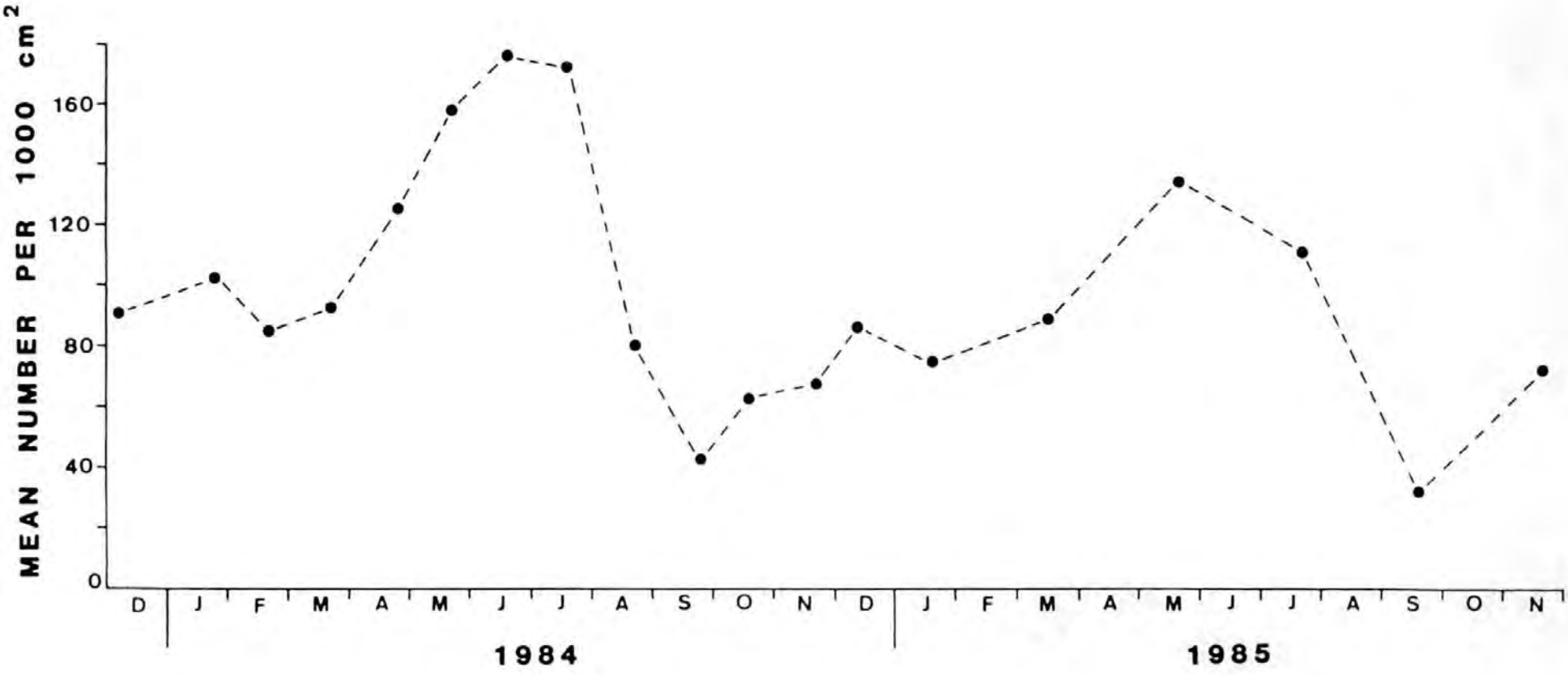


FIGURE 6.14. Mean number of macroinvertebrate predators (i.e. coleopteran larvae and adults, and larvae of P. conspersa) per 1000 cm^2 at station 1 from December 1983 to November 1985.

highest recorded at any of the five stations sampled, with mean values of 493 m^{-2} in July 1984 and 733 m^{-2} in April 1986. In a macroinvertebrate survey of the River Lynher carried out in September (South West Water, 1982) the number of taxa was shown to increase with distance downstream, and at Rilla Mill (which is just below the confluence of the Darley Brook with the River Lynher) the number of taxa was 18. In one of the tributaries of this river which received mine drainage only 12 taxa were recorded (and this compares with the 11 or 12 genera at station 1).

It is informative to consider whether the comparatively diverse community at station 4 affects the composition of the fauna at stations X and F, which are below the confluence of station 4 with the Darley Brook. Although plecopteran nymphs are found in large numbers at station 4, they are completely absent from station F and only 3 individuals were collected at station X in April 1986. This suggests that if plecopteran nymphs are carried by the current from station 4 into the main channel of the Darley Brook they may not be able to tolerate the copper concentration at station X (0.14 mg l^{-1}). The absence of stonefly nymphs from the Darley Brook is interesting since a number of workers (Hawkes, 1972; Hynes, 1974) have commented upon the occurrence of plecopteran nymphs in streams with high levels of zinc or lead.

Conditions at stations X and F are presumably favourable for the mayfly Baetis rhodani, since in April 1986 nymphs of this species accounted for 13% and 32% respectively of the total number of invertebrates at these stations. By contrast, in April 1986 B. rhodani nymphs only formed 0.5% of the invertebrates at station 4. The point of confluence of the control stream with the Darley Brook probably marks the upstream limit of the distribution of B. rhodani in the main channel, indeed this species is absent from stations 1 and D. The

large number of B. rhodani at station F (which is just prior to the confluence with the River Lynher) is perhaps partly accounted for by direct colonisation from the River Lynher.

Differences in the number of invertebrate genera at the stations usually reflected differences in the values of the Shannon-Weaver function H' (see Figure 6.13). In other studies on copper contaminated streams (Winner et al., 1975; Sheehan & Winner, 1984) the number of species reflected the same pattern of community response to copper stress as other indices, including the Shannon index.

In April 1986 the values of H' for the invertebrate communities in the middle and lower reaches of the Darley Brook (i.e. stations D, X and F) were higher than the value of 2.28 calculated at station 1. Analysis of water samples collected in July 1984 showed that there was a copper gradient along the Darley Brook, with a concentration of 0.82 mg l^{-1} at station 1 decreasing to < 0.04 mg l^{-1} at station F. Although the increased diversity of the communities at stations D, X and F is no doubt due in part to the decrease in copper, other physicochemical factors have been shown to influence community structure. For example Townsend et al. (1983) found that acid streams had low numbers of individuals and a low species richness compared to more basic streams, and that P. conspersa was one of the species characteristic of small acid cool streams. Sutcliffe and Carrick (1973) also found a close relationship between pH and the benthic faunas in mountain streams in the Lake District, with more acidic sites supporting an impoverished community which included P. conspersa.

Thus while the copper concentration in the water is clearly an important factor affecting the macroinvertebrate communities in the Darley Brook, other physicochemical variables such as pH (which increases with distance downstream) may also exert an influence on the community structure.

At station 1 the mean copper concentration is 0.86 mg l^{-1} and the values of H' were 1.98 in July 1984 and 2.28 in August 1986. At station F where the mean copper concentration is 0.029 mg l^{-1} values of H' were 2.09 and 2.33 in July 1984 and April 1986 respectively. Winner et al. (1975) recorded H' values of 1.20 and 1.50 in communities subject to copper concentrations of 0.12 mg l^{-1} and 0.02 mg l^{-1} respectively. In another copper contaminated stream (Sheehan & Winner, 1984) values of H' ranged from 0.50 (copper concentration in the water was 0.63 mg l^{-1}) to 1.80 (0.32 mg l^{-1} copper). Again, Chadwick et al. (1986) have studied the recovery of benthic invertebrate communities in a stream following improved metal mine wastewater treatment, and values of H' were on average 2.00, with copper concentrations of up to 0.36 mg l^{-1}.

CHAPTER 7

MEASUREMENT OF COPPER IN PLECTROCNEMIA CONSPERSA

7.1. Introduction

In the previous chapter it was shown that the elevated copper concentration in the headwaters of the Darley Brook resulted in a less diverse macroinvertebrate community compared to the fauna in an uncontaminated control stream. The influence of metals on invertebrates may further be investigated by measuring the concentration of metal in the animals. Indeed, analysis of the metal content of selected species of the aquatic fauna is invaluable in the quality assessment of surface water partly because of the difficulty of predicting biological availability of metals from physicochemical data. Metal concentrations in invertebrates are easily quantifiable so allowing comparisons to be made between populations at different stations and at the same station at different times (Burrows & Whitton, 1983).

Whilst noting that copper concentrations in marine and estuarine invertebrates are well documented, Stokes (1979) emphasizes the need for further investigation into copper concentrations in freshwater invertebrates. In both the Darley Brook and the control stream the faunal communities are dominated by insects, and it has recently been shown (Nehring, 1976; Burrows & Whitton, 1983) that metal analysis of insects serves as a useful means of monitoring heavy metal contamination in freshwater ecosystems. In view of this it is surprising that so little work has been undertaken on the metal concentrations of insects. No doubt this is due to their small size, and most workers have pooled a number of individuals to allow analysis by flame AAS. However this yields little or no information on metal levels in an individual, or on the variation associated with a particular mean concentration.

Since the caddis Plectrocnemia conspersa is one of the prominent insects in the uppermost reaches of the Darley Brook it is of interest to measure copper concentrations in this species. Only a few workers have investigated metal levels in Trichoptera. Harding et al. (1981) determined the concentrations of zinc, cadmium and lead in a number of invertebrates, including Rhyacophila dorsalis, taken from a metal contaminated stream in the River Derwent catchment. In a later study on this catchment (Burrows & Whitton, 1983) the concentrations of these same three metals were measured in five trichopteran species, and in a number of species from other insect orders. Smock (1983a) determined the concentrations of several metals (though not copper) in forty taxa of aquatic insects, including four species of caddis, taken from relatively metal uncontaminated tributaries. The study by Brown (1977a) on metal levels in macroinvertebrates in the River Hayle is noteworthy because not only is it one of the few studies of this type carried out in South West England, but it also provides the only record of copper concentrations in trichopteran larvae collected in the field (although different species were analysed at different sampling stations).

Using graphite furnace AAS it was hoped that it would be possible to measure copper concentrations in individuals of P. conspersa. Although this technique has been used to measure copper levels in pooled samples of aquatic insects (Namminga & Wilhm, 1977; Dodge & Theis, 1979) and in micro-organisms (Drapeau et al., 1983), it has not previously been used to measure copper concentrations in single specimens of aquatic insects.

A number of factors are known to influence metal concentrations in aquatic insects, for example Smock (1983b) found that the concentrations of several metals in a number of aquatic insects were influenced by size. Metals associated with material in an insect's digestive tract may account

for a significant proportion of the whole-body metal concentration (Elwood et al., 1976; Smock, 1983a). Clearly it would be important to examine the effect of such factors on copper concentrations in P. conspersa.

It has previously been suggested in this investigation that P. conspersa is a metal tolerant species, and that several studies on invertebrate communities in metal contaminated waters have provided corroborative evidence for this idea (see p.208). If larvae of P. conspersa are to be used as indicators of metal enriched conditions, it is necessary to understand the relationship between concentrations in the larvae and in the water. Brown (1977a) found some correlation between the copper concentration in 'free-living' Trichoptera and that in the water of the copper contaminated River Hayle.

There are relatively few studies on the variation over time of metal concentrations in aquatic invertebrates. Betzer and Pilson (1974) investigated temporal variation in copper concentrations in the whelk Busycon canaliculatum, whilst concentrations of zinc in the barnacle Balanus balanoides have been measured over a two year period by Walker & Foster (1979). Harding et al. (1981) examined how the concentrations of zinc, cadmium and lead varied over time in mayfly nymphs and some other unspecified invertebrates, and seasonal variation in zinc concentrations in the nymphs of the mayfly Ecdyonurus venosus was studied by Burrows and Whitton (1983).

To the author's knowledge there have been no investigations carried out to examine temporal variation in the copper concentrations of an aquatic insect. One of the aims of the present study was therefore to determine whether copper concentrations in P. conspersa larvae vary with time. Furthermore it is important to relate such changes to any temporal

variations in the immediate environment, especially the water and food.

At station 1 in the Darley Brook both chironomid and coleopteran larvae are likely to be potential prey items of P. conspersa (which is also cannibalistic). Sampling of these insect larvae allows us to study the passage of copper through a relatively simple food chain. Moreover if this sampling is carried out at different times of the year then it would be possible to examine whether temporal variation in the copper concentrations of the prey affect concentrations in the predator.

Most studies have invariably measured metal concentrations in just a single stage of the life cycle. In this investigation a number of larval instars (II, III, IV and V) of P. conspersa, together with the post-larval stages, have been collected during routine sampling. It was therefore possible to measure copper concentrations in several stages of the life cycle of this species, and so gain a more complete understanding of animal-metal relationships.

7.2. Materials and Methods

Larvae of P. conspersa which were collected in the field for metal analysis were each placed in a separate cell in a compartmentalised tray filled with water taken at the site of collection, and starved for 48 hours at 10°C to allow the guts to clear and to remove the contaminating effects of any ingested material. A starvation period of 48 hours was found to be sufficient since food generally passes quickly through a predator's intestine. The larvae were then separated into instars from head width measurements and frozen.

Each larva of P. conspersa was subsequently freeze dried (600 Pa pressure, -55°C, 24 hours) and weighed on a Cahn 29 automatic electrobalance (Cahn Instruments Inc., Cerritos, U.S.A.). Mason et al. (1983) have demonstrated that freeze drying provides constant dry weights and is advantageous over oven drying since specimens remain relatively intact. Individual larvae were then completely digested in 4 ml of Aristar grade nitric acid at 110°C for 4 hours, and made up to 5 ml with distilled deionised water.

Appendix 1E gives details of the programme devised to analyse the individual digests using an Instrumentation Laboratory Video 12 Absorption Spectrometer with Furnace Atomizer 655 (Instrumentation Laboratory Inc., Andover, U.S.A.).

Copper concentrations were measured in individual larvae of instars II, III, IV and V of P. conspersa collected from station 1 in the Darley Brook in March (representing spring), July (summer), September (autumn) 1984 and in January (winter) 1985. The concentrations of copper were also measured in separate larvae belonging to these four larval instars taken from station 4 (an uncontaminated tributary of the Darley Brook) in March and September 1984. Furthermore ten instar V larvae collected in

alternate months from May 1984 to July 1985 at stations 1 and 4 were individually anlaysed.

In order to investigate the fraction of the whole-body copper concentration that is associated with material in the larval gut, 50 larvae (consisting of instars III, IV and V) were sampled at station 1 in May 1985. Whilst 25 of these larvae were immediately frozen, the other 25 were starved for 48 hours at 10°C prior to freezing. All these larvae were then individually weighed, digested in nitric acid and analysed for their copper content.

Since it was known that P. conspersa occurred along the entire length of the Darley Brook, which showed a copper gradient decreasing from the source to the confluence with the River Lynher, it was decided to investigate copper concentrations of larvae along this gradient. Thus in July 1985 ten instar V larvae were collected at each of a number of stations along the Darley Brook, namely 1, D, X, F and 4 (see Figures 2.5 and 2.6 for the positions of these stations). The copper concentrations in each of these larvae was then determined using the procedure described above. An unfiltered acidified water sample was also taken at each of these stations.

In addition to metal analysis of the larval population from the control station 4 in the Darley Brook catchment, it was decided to measure the copper concentrations in P. conspersa larvae collected from a metal uncontaminated stream which did not drain a granitic metalliferous area. Relatively high densities of P. conspersa larvae have been recorded by South West Water in such a stream at Aylesbeare near Exeter, Devon. A shaded riffle section of this stream was selected for sampling (Nat. Grid Ref. SY 037918), and analysis by graphite furnace AAS of an unfiltered acidified water sample showed the copper concentration to be 0.004 mg l^{-1}.

Ten instar V larvae were collected from this stream on three occasions (May and November 1985, and March 1986), and were individually analysed for their copper concentration.

All the above procedures were carried out to investigate copper concentrations in the larvae of P. conspersa; however it was also of interest to study concentrations in the post-larval stages of this species. In July 1984 pupal cases were collected from station 1 in the Darley Brook and on return to the laboratory these cases were dissected, and any prepupae, pharate pupae and male and female pupae were removed. Furthermore some cases were placed in covered aquaria (details of which are given on p.150) to obtain male and female adults.

It was not necessary to starve any of the post-larval stages of P. conspersa, since Wiggins (1977) states that once a caddis larva is closed off within the pupal case, feeding has terminated and the gut becomes constricted. All the post-larval stages were therefore immediately frozen upon collection. They were subsequently treated in a similar manner to the larvae, with individual copper concentrations determined by graphite furnace AAS.

In order to determine the concentration of copper that is associated with the larval cuticle, instar V larvae were collected in July and August 1985 from station 1. These larvae were placed in separate cells in a compartmentalised tray filled with water and kept at 15°C. They were each fed final instar larvae of Chironomus riparius (at a rate of one per day) taken from a laboratory culture. Under these conditions the larvae eventually pupated; ten female pupae together with shed larval skins were thus obtained. All the material was individually analysed for its copper content.

It is likely that the copper concentration of P. conspersa larvae would be affected by the copper concentration associated not only with the

water, which has already been referred to, but also with the food. At station 1 coleopteran and chironomid larvae are likely to be the main prey items of P. conspersa. Thus the biomass and copper concentrations of these insect larvae were determined by taking ten random quantitative (0.075 m^2) sample units at station 1 in both January and July 1986. In the laboratory all the chironomid larvae extracted from a sampling unit were combined, and all the coleopteran larvae were also combined. Both sets of material were separately frozen, and subsequently freeze-dried, weighed and analysed by graphite furnace AAS.

7.3. Results

7.3.1. Larvae of P. conspersa

If copper concentrations in P. conspersa are to be compared with the copper levels recorded elsewhere in this investigation, and with the copper concentrations recorded in other aquatic invertebrates, then it is important to check the comparability between the flame AAS and graphite furnace AAS techniques. In February 1984, 30 instar V larvae were collected from station 1 to form 3 replicates of 10 larvae for copper analysis. The mean concentration (± standard error) recorded by graphite furnace AAS was 950 ± 58 $\mu g\ g^{-1}$ compared to 977 ± 33 $\mu g\ g^{-1}$ as measured by flame AAS. Thus there is close agreement between the copper concentrations recorded by the two techniques.

The copper concentrations and dry body weights of 73 larvae collected from stations 1 and 4 in September 1984 are given in Appendix 6A and are summarised in Table 7.1. Each larval instar of P. conspersa from station 1 had a significantly ($p < 0.001$) higher copper concentration than the same instar at station 4. Within each station there was a significant ($p < 0.001$) difference in the copper concentrations between the instars, with the lowest mean concentration in instar V larvae (258 and 70 $\mu g\ g^{-1}$ at stations 1 and 4 respectively) and the highest in instar II (3767 $\mu g\ g^{-1}$ at station 1 and 813 $\mu g\ g^{-1}$ at station 4).

When the copper concentration for each of the larvae was plotted against dry body weight for these samples, as in Figure 7.1, it can be seen that there was an exponential decrease in copper concentration with increasing body weight. It is interesting to note that this relationship was evident at both stations, even though the highest concentration recorded in instar II larvae from station 1 (4812 $\mu g\ g^{-1}$) was over 5 times greater than the maximum concentration measured in larvae of this same instar at station 4 (907 $\mu g\ g^{-1}$). This relationship was further examined using

TABLE 7.1. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in larval instars II to V of P. conspersa collected at stations 1 and 4 in September 1984, showing range, mean, standard error (S.E.) and number (n) of larvae which were individually analysed.

INSTAR	STATION 1				STATION 4			
	n	MEAN	S.E.	RANGE	n	MEAN	S.E.	RANGE
II COPPER	8	3767	278	2653-4812	5	813	37	705-907
WEIGHT	8	0.0470	0.0073	0.0172-0.0838	5	0.0550	0.0049	0.0431-0.0724
III COPPER	10	1806	171	1187-2666	10	344	18	258-420
WEIGHT	10	0.2491	0.0258	0.1519-0.3841	10	0.2974	0.0304	0.1563-0.4384
IV COPPER	10	740	104	400-1237	10	136	9	97-177
WEIGHT	10	0.9889	0.1369	0.3314-1.6199	10	1.5123	0.0929	1.1406-1.9997
V COPPER	10	258	33	122-424	10	70	8	38-113
WEIGHT	10	4.1165	0.6601	1.9447-7.3609	10	6.1102	0.9314	1.6262-10.5587

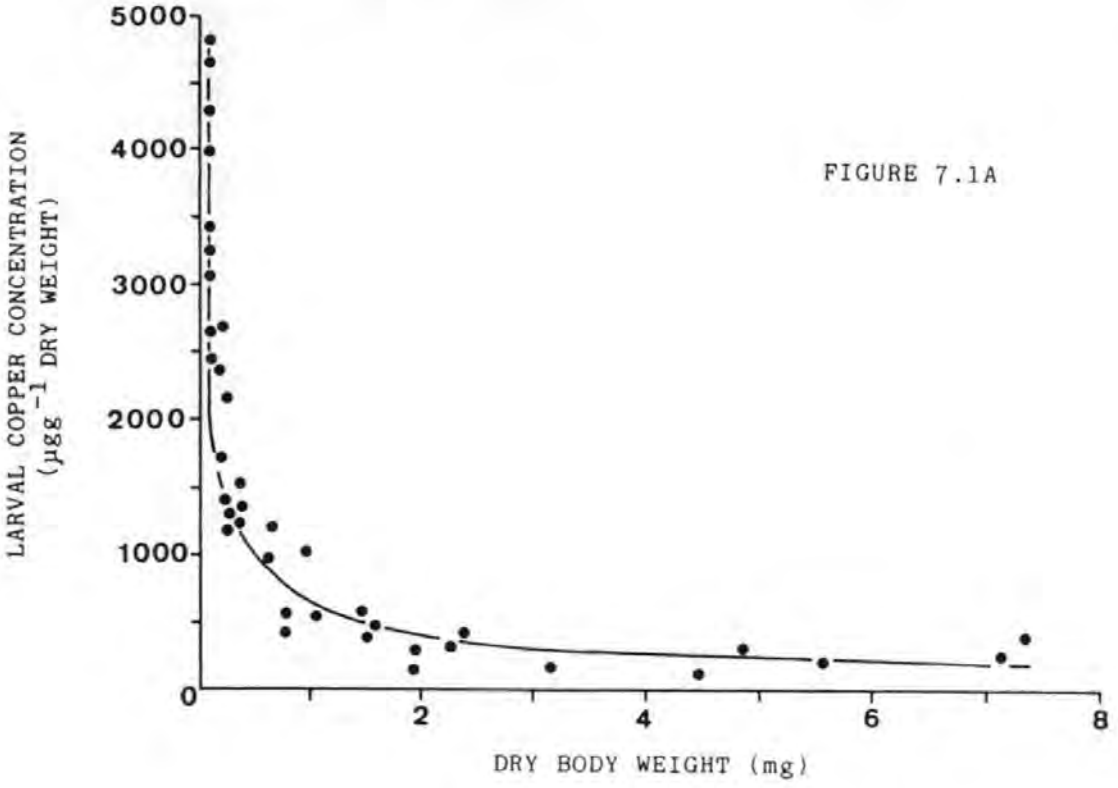


FIGURE 7.1A

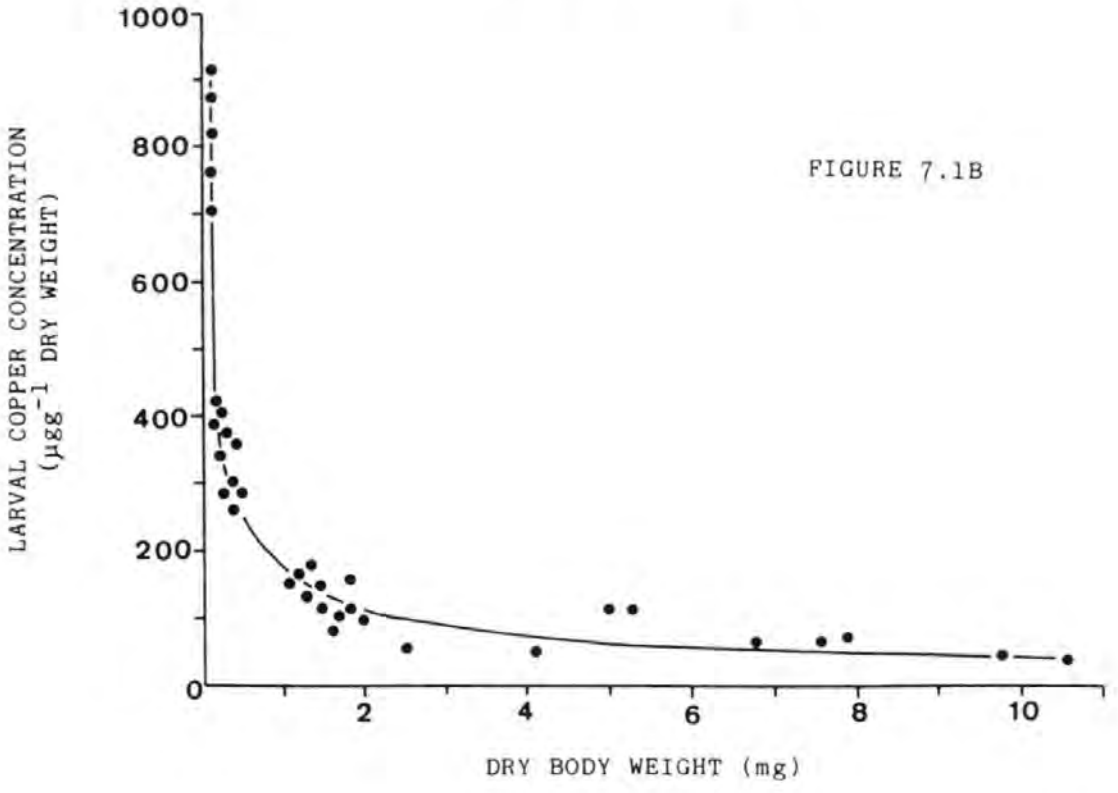


FIGURE 7.1B

FIGURE 7.1. Relationship between dry body weight (mg) and copper concentration (µg g^{-1}) in individual larvae (instars II to V) of P. conspersa collected in September 1984 at (A) station 1 and (B) station 4.

linear regression analysis, with both variables $\log_{10}$ transformed. The resulting equations are given in Table 7.2, and show a highly significant ($p < 0.001$) negative correlation between weight and concentration.

In addition to the above data for larvae collected in September 1984 at stations 1 and 4, larvae were also collected for metal analysis in March and July 1984 and in January 1985 at station 1, and in March 1984 at station 4. The individual copper concentrations and dry body weights of these larvae are given in Tables B to D (see Appendix 6) and are summarised in Table 7.3. Within a particular month at both stations the highest concentrations were recorded in the youngest larvae analysed and the lowest were measured in instar V larvae. Furthermore in any given month an exponential decrease in larval copper concentration with increasing body weight was evident at both stations 1 and 4.

From Table 7.3 it can be seen that copper concentrations in all the larval instars varied with time. In July 1984 the concentrations in the larvae at station 1 reached a maximum with mean values of 3348 $\mu g\ g^{-1}$ (instar III), 1072 $\mu g\ g^{-1}$ (IV) and 373 $\mu g\ g^{-1}$ (V). The lowest concentrations at station 1 were measured in March 1984 with mean levels of 1192, 492, 228 and 97 $\mu g\ g^{-1}$ in instars II, III, IV and V respectively. Although the data for station 4 is less complete, the copper concentrations in each larval instar in March 1984 were significantly ($p < 0.001$) lower than in the same instar in September 1984.

For each instar in both March and September 1984 the heaviest larvae were always recorded at station 4. This difference in weight was however only significant ($p < 0.01$) for instar IV larvae in September, when the mean weights were 0.9889 and 1.5123 mg at stations 1 and 4 respectively.

In addition to the temporal variation in copper concentrations, the weights of the larvae were also found to vary over time (see Table 7.3).

TABLE 7.2. Regression constants for the equation Y = a+bx, where Y = $\log_{10}$ larval copper concentration ($\mu g\ g^{-1}$) and X = $\log_{10}$ dry body weight (mg) for P. conspersa collected at stations 1 and 4 in September 1984.

STATION	NUMBER OF LARVAE	REGRESSION CONSTANTS		CORRELATION COEFFICIENT	SIGNIFICANCE LEVEL
		a	b	r	
1	38	2.78	-0.602	-0.933	$p < 0.001$
4	35	2.22	-0.537	-0.965	$p < 0.001$

TABLE 7.3. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in larval instars II to V of P. conspersa collected at stations 1 and 4 in March and September 1984, and at station 1 in July 1984 and January 1985. Standard errors (S.E.) and the number (n) of larvae which were individually analysed are also shown (N.A. not available for analysis).

	DATE		LARVAL INSTAR II n	II MEAN	II S.E.	III n	III MEAN	III S.E.	IV n	IV MEAN	IV S.E.	V n	V MEAN	V S.E.
	STATION 1													
1984	20/3	COPPER	5	1192	43	10	492	45	10	228	29	10	97	9
		WEIGHT	5	0.0305	0.0032	10	0.2066	0.0264	10	0.8617	0.1152	10	3.4494	0.4213
	18/7	COPPER		N.A.		10	3348	321	10	1072	108	10	373	33
		WEIGHT				10	0.3679	0.0635	10	1.6812	0.2460	10	8.5644	1.7002
	24/9	COPPER	8	3767	278	10	1806	171	10	740	104	10	258	33
		WEIGHT	8	0.0470	0.0073	10	0.2491	0.0258	10	0.9889	0.1369	10	4.1165	0.6601
1985	17/1	COPPER	10	2716	92	10	1171	60	10	397	76	10	132	21
		WEIGHT	10	0.0389	0.0039	10	0.2235	0.0197	10	0.8681	0.1150	10	2.7775	0.7332
	STATION 4													
1984	20/3	COPPER	5	273	38	10	98	12	10	42	6	10	21	2
		WEIGHT	5	0.0348	0.0034	10	0.2260	0.0248	10	1.0412	0.1590	10	3.8073	0.4934
	24/9	COPPER	5	813	37	10	344	18	10	136	9	10	70	8
		WEIGHT	5	0.0550	0.0049	10	0.2974	0.0304	10	1.5123	0.0929	10	6.1102	0.9314

At station 1 maximum weights for instars III, IV and V were recorded in July 1984, with the lowest weights measured in March 1984 for instars II, III and IV, and in January 1985 for instar V. In order to compare the copper content of a particular instar at different times it is therefore necessary to consider the amounts of copper, since weight and concentration have been shown to be significantly correlated. The amount of copper in these larvae was calculated from the concentration-weight data, and the resulting values are summarised in Table 7.4.

Reference to Table 7.4 shows that at both stations 1 and 4 the amount of copper progressively increased with instar (i.e. copper is cumulative with age), although at station 1 in January 1985 the mean amount of copper in instar IV larvae was greater than that measured in the final instar larvae. In both March and September 1984 each instar at station 1 contained a significantly ($p < 0.05$) higher amount of copper than the same instar in the corresponding month at station 4.

The amounts of copper were found to vary over time in a similar manner to that previously described for the larval copper concentrations, for example at station 1 the maximum amount of copper in instars III, IV and V was recorded in July 1984 (with mean amounts of 1.09, 1.75 and 2.93 μg respectively). Although the lowest amounts of copper in the larvae at station 1 were measured in March 1984 for instars II, III and IV (mean amounts of 0.04, 0.09 and 0.17 μg respectively), the minimum amount of copper in instar V larvae was recorded in January 1985 (mean amount was 0.31 μg). At station 4 the amount of copper recorded in each larval instar in September 1984 was significantly ($p < 0.01$) higher than in the same instar in March 1984.

In May 1985 two batches of 25 larvae (consisting of instars III, IV and V) were taken from station 1, with one batch unstarved and the second batch starved for 48 hours prior to being frozen. The individual copper

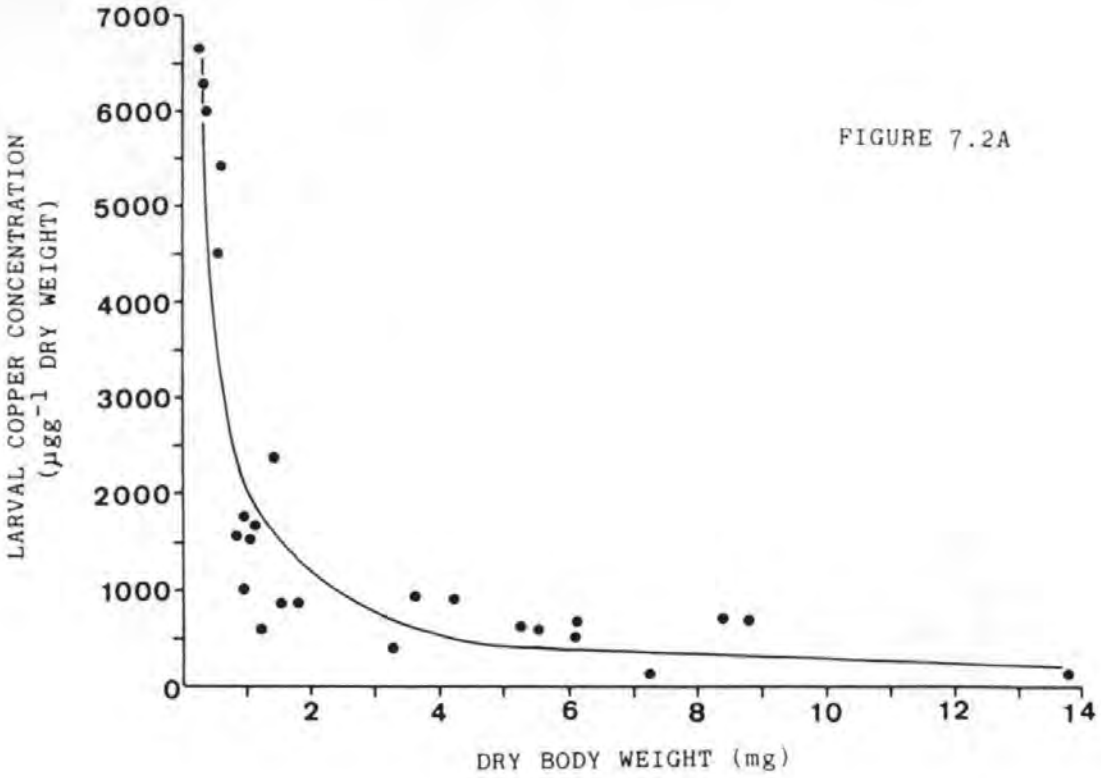
TABLE 7.4. Amount of copper (μg) in larval instars II to V of P. conspersa collected at stations 1 and 4 in March and September 1984, and at station 1 in July 1984 and January 1985. Standard errors (S.E.) and the number (n) of larvae which were individually analysed are also shown (N.A. not available for analysis).

		LARVAL INSTAR											
	DATE	II			III			IV			V		
		n	MEAN	S.E.	n	MEAN	S.E.	n	MEAN	S.E.	n	MEAN	S.E.
	STATION 1												
1984	20/3	5	0.04	0.004	10	0.09	0.01	10	0.17	0.01	10	0.32	0.04
	18/7	N.A.			10	1.09	0.13	10	1.75	0.25	10	2.93	0.47
	24/9	8	0.17	0.03	10	0.42	0.03	10	0.64	0.07	10	1.08	0.24
1985	17/1	10	0.11	0.01	10	0.26	0.02	10	0.35	0.09	10	0.31	0.07
	STATION 4												
1984	20/3	5	0.01	0.001	10	0.02	0.002	10	0.04	0.004	10	0.08	0.01
	24/9	5	0.04	0.004	10	0.10	0.01	10	0.20	0.01	10	0.40	0.06

concentrations and dry body weights of these larvae are given in Appendix 6E. Figure 7.2 shows that there was an exponential decrease in the copper concentration of both unstarved and starved larvae with increasing body weight. The copper concentrations in the unstarved larvae were significantly ($p < 0.01$) higher and showed more variation than the starved larvae (see Table 7.5). From this data we can calculate that on average 61% of the whole-body copper concentration in instar V larvae, 57% in instar IV and 51% in instar III was accounted for by material in the gut. It is also possible that these percentages include copper which was lost through continued excretion during the starvation period.

Figure 7.3 shows the mean concentration of copper in the 10 individually analysed instar V larvae collected in alternate months from May 1984 to July 1985 at stations 1 and 4. In each month the concentration of copper in the instar V larvae at station 1 was significantly ($p < 0.001$) higher than the concentration at station 4. Although there was a clear separation of the monthly mean concentrations at the two stations throughout the year, there was overlap between the standard error of the maximum concentration recorded for station 4 larvae in July 1984 and that of the minimum concentration recorded for station 1 larvae in January 1985. At both stations there was a clear seasonal variation in larval copper concentrations, with maxima of 373 and 99 $\mu g\ g^{-1}$ recorded in the same month of July 1984 and minima of 132 and 27 $\mu g\ g^{-1}$ recorded in the same month of January 1985 at stations 1 and 4 respectively.

The individual copper concentrations and dry body weights of these instar V larvae are given in Tables A and C-G (see Appendix 6), and are summarised in Table 7.6. Larvae from station 4 were heavier than station 1 larvae in every month (except in March and May 1985), although a series of t-tests showed that none of the differences in weight were significant at the 5% level. At both stations the weights in 1984 were greatest in July



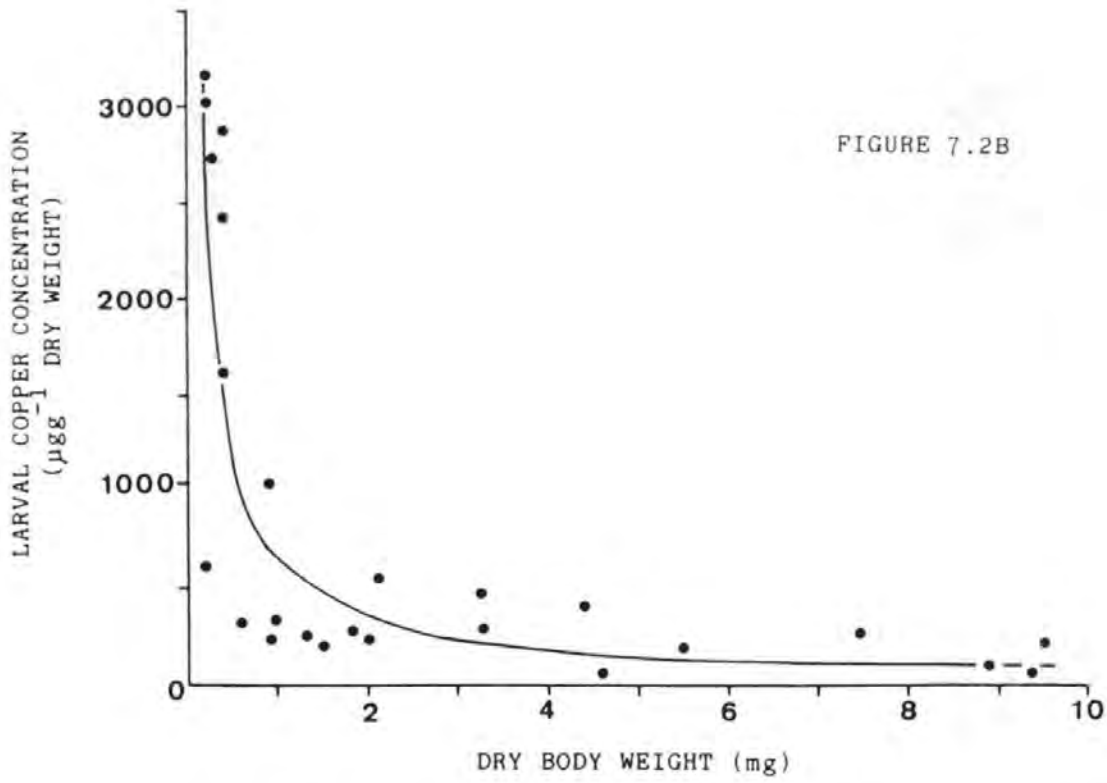


FIGURE 7.2. Relationship between dry body weight (mg) and copper concentration ($\mu g\ g^{-1}$) in individual larvae (instars III to V) of P. conspersa collected in May 1985 at station 1, and which were (A) unstarved (B) starved for 48 hours at 10°C.

TABLE 7.5. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in larval instars III to V of P. conspersa collected at station 1 in May 1985 and (a) unstarved (b) starved for 48 hours at 10°C, showing range, mean, standard error (S.E.) and number (n) of larvae which were individually analysed.

INSTAR	(a) UNSTARVED				(b) STARVED			
	n	MEAN	S.E.	RANGE	n	MEAN	S.E.	RANGE
III COPPER	5	5764	373	4523-6635	5	2840	126	2422-3155
WEIGHT	5	0.3956	0.0698	0.2133-0.5673	5	0.2906	0.0374	0.2104-0.3861
IV COPPER	10	1263	195	391-2383	10	539	145	192-1617
WEIGHT	10	1.4319	0.2210	0.9444-3.2597	10	1.0952	0.2038	0.1883-2.1356
V COPPER	10	594	87	119-929	10	229	45	49-473
WEIGHT	10	6.9274	0.9246	3.6544-13.7794	10	5.8190	0.8866	1.8774-9.5322

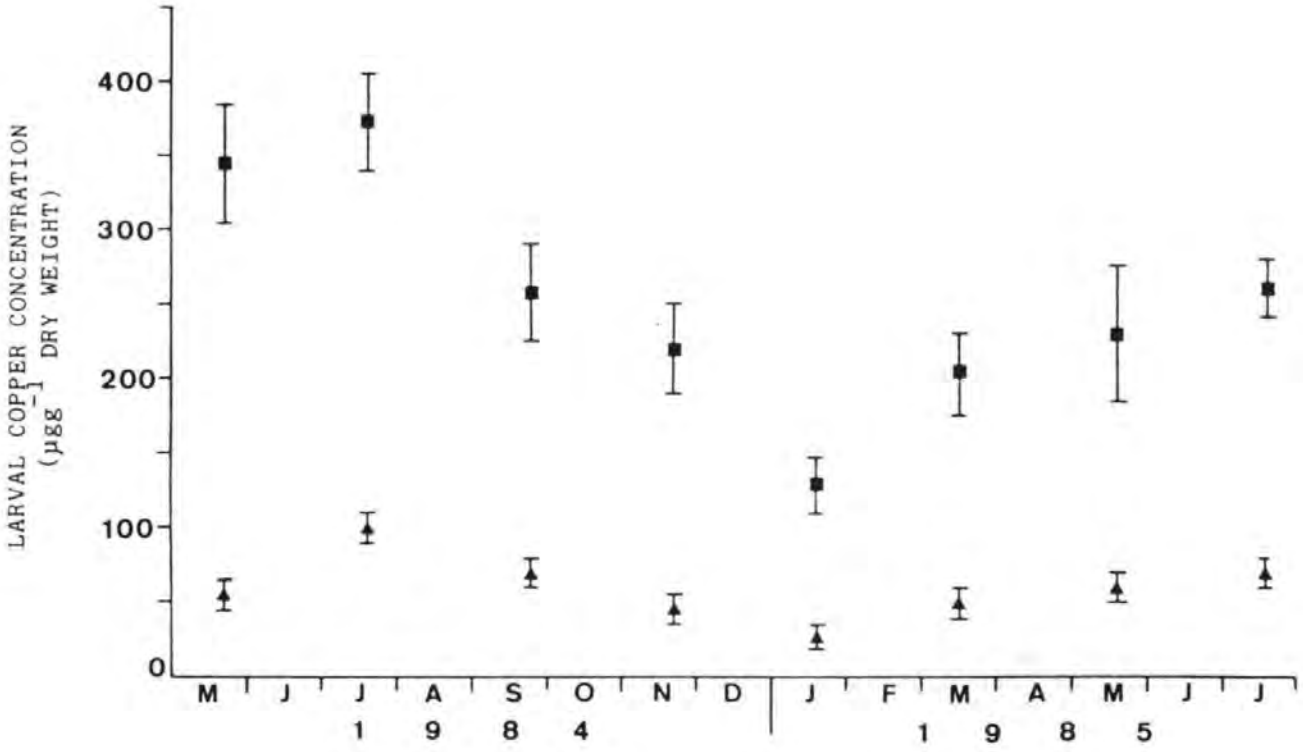


FIGURE 7.3. Mean copper concentration (μg g^{-1}) in ten individually analysed instar V larvae of P. conspersa collected in alternate months from May 1984 to July 1985 at station 1 = ■ and station 4 = ▲ (vertical bars show standard errors).

TABLE 7.6. Mean concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in ten individually analysed instar V larvae of P. conspersa collected at stations 1 and 4 in alternate months from May 1984 to July 1985. Standard errors (S.E.) are also shown.

DATE		STATION 1		STATION 4	
		MEAN	S.E.	MEAN	S.E.
1984					
21/5	COPPER	344	39	55	7
	WEIGHT	5.9086	0.9939	7.5584	1.4997
18/7	COPPER	373	33	99	9
	WEIGHT	8.5644	1.7002	11.9951	2.0994
24/9	COPPER	258	33	70	8
	WEIGHT	4.1165	0.6601	6.1102	0.9314
21/11	COPPER	222	31	43	8
	WEIGHT	3.4876	0.5673	5.1335	0.6857
1985					
17/1	COPPER	132	21	27	5
	WEIGHT	2.7775	0.7332	3.1297	0.4913
15/3	COPPER	203	27	51	9
	WEIGHT	4.3139	0.8713	3.4623	0.4143
17/5	COPPER	229	45	59	8
	WEIGHT	5.8190	0.8866	5.5604	0.7093
18/7	COPPER	259	23	70	7
	WEIGHT	5.0625	0.8624	6.0136	0.6067

and smallest in November. In 1985 the weights at both stations were at a minimum in January and reached a maximum in May at station 1 and July at station 4.

Since there is temporal variation in weight it is again useful to convert concentrations to amounts of copper so that a more complete comparison may be made of the copper relationships of instar V larvae over time. The mean amounts of copper in final instar larvae at stations 1 and 4 are shown in Figure 7.4. At both stations the maximum amount of copper in each year was recorded in July, and the minimum mean amounts were measured in January 1985.

It is interesting to note that the calculation of amounts of copper results in significant overlap between the annual range of mean values for station 1 larvae with the annual range for larvae from station 4 (see Figure 7.4). However in any given month there was no overlap between the amounts of copper in larvae from stations 1 and 4. Furthermore instar V larvae from station 1 contained significantly ($p < 0.01$) higher amounts of copper than final instar larvae at station 4 in any given month.

In July 1985 ten instar V larvae were collected at station 4 and stations 1, D, X and F along the Darley Brook. Figure 7.5 shows the relationship between the mean copper concentration in the larvae and that in the water, whilst the individual larval copper concentrations and weights are given in Appendix 6G. There was a positive linear relationship between the concentration of copper in the larvae and that in the water upto a concentration of 0.32 mg l^{-1} (station D). However the increase was less steep when extended to the mean concentration recorded in the larvae from station 1, where the concentration of copper in the water was 0.74 mg l^{-1}.

Ten instar V larvae were collected from the Aylesbeare stream in May and November 1985 and in March 1986. The copper concentrations and dry

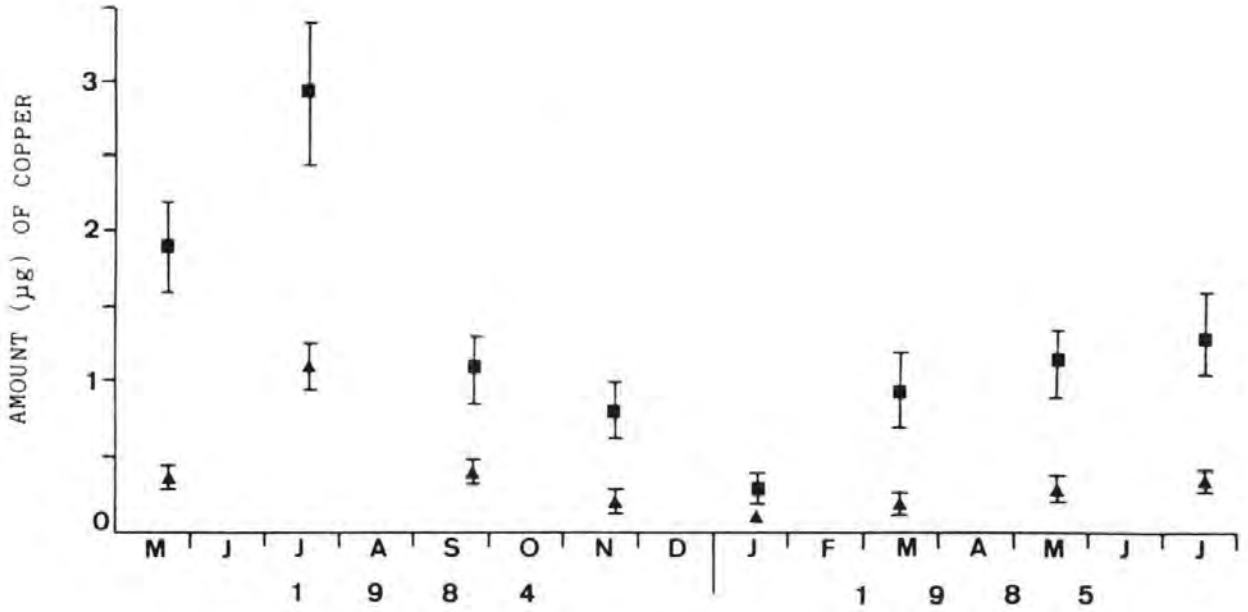


FIGURE 7.4. Mean amount of copper (µg) in ten individually analysed instar V larvae of P. conspersa collected in alternate months from May 1984 to July 1985 at station 1 = ■ and station 4 = ▲ (vertical bars show standard errors).

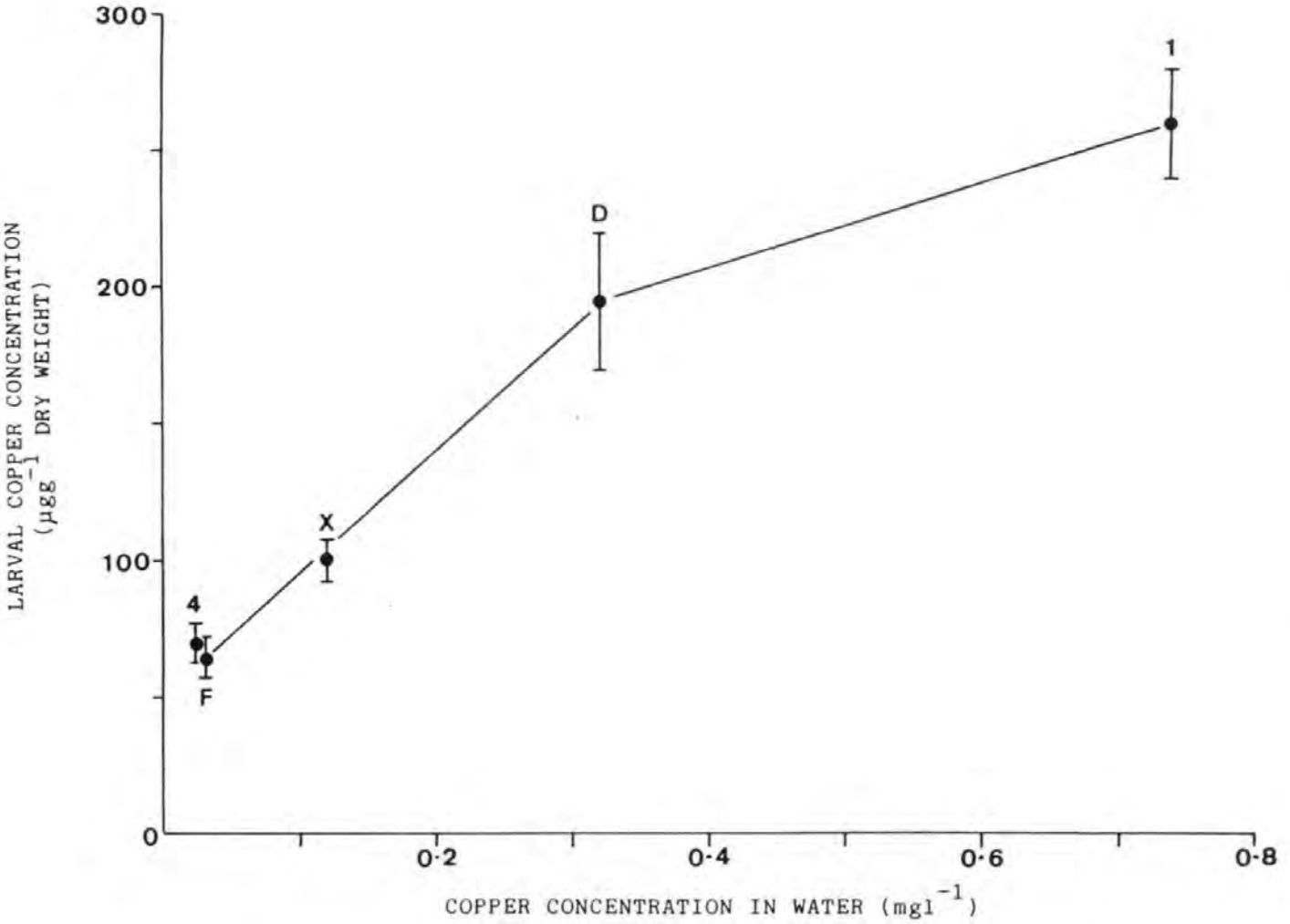


FIGURE 7.5. Relationship between the copper concentration (mg l^{-1}) in the water and the mean copper concentration (μg g^{-1}) in ten individually analysed instar V larvae of P. conspersa collected in July 1985 at station 4 and at stations 1, D, X and F along the Darley Brook (vertical bars show standard errors).

body weights of these larvae are detailed in Appendix 6H and summarised in Table 7.7. Both the concentration and amount of copper in the Aylesbeare stream larvae was found to vary on the three sampling occasions, e.g. mean concentrations of 29, 18 and 33 $\mu g\ g^{-1}$ were recorded in May, November and March respectively. Thus copper was measurable in larvae of P. conspersa living in a stream where the concentration of copper in the water was only 0.004 mg l^{-1}, and the levels of copper in these larvae varied with time.

TABLE 7.7. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in instar V larvae of P. conspersa collected from Aylesbeare stream in May and November 1985 and in March 1986, showing range, mean, standard error (S.E.) and number (n) of larvae which were individually analysed.

	DATE		n	MEAN	S.E.	RANGE
1985						
	16/5	COPPER	10	29	3	17-43
		WEIGHT	10	7.0677	1.0131	2.8310-12.9540
	19/11	COPPER	10	18	2	10-25
		WEIGHT	10	4.1012	0.7072	1.6135-9.5848
1986						
	11/3	COPPER	10	33	4	20-54
		WEIGHT	10	12.0903	1.6565	4.6809-20.5129

7.3.2. Post-larval stages of P. conspersa

In July 1984, 5 pharate pupae, 7 male pupae and 7 female pupae were obtained from pupal cases collected at station 1. Furthermore 10 male and 6 female adults emerged from cases placed in the aquaria in the laboratory. The individual weights and copper concentrations of these specimens (together with similar data for the instar V larvae also collected at station 1 in July 1984) are shown in Appendix 6I, and the concentrations are summarised in Table 7.8.

It is evident from Table 7.8 that there was a significant ($p < 0.001$) difference between the copper concentrations in the instar V larvae and in the pharate pupae, with mean concentrations of 373 and 146 $\mu g\ g^{-1}$ respectively. Although the mean concentration recorded in the pupae (males and females combined) was 106 $\mu g\ g^{-1}$, this was not significantly different (at the $p = 0.05$ level) from the mean concentration measured in the pharate pupae. Furthermore the concentrations recorded in the adults (mean of 103 $\mu g\ g^{-1}$ for males and females combined) were not significantly different from the concentrations in the pharate pupae or pupae.

Male pupae were found to contain higher concentrations of copper than female pupae, with mean concentrations of 122 and 90 $\mu g\ g^{-1}$ respectively. Similarly, higher concentrations were also recorded in male adults (mean concentration was 107 $\mu g\ g^{-1}$) compared to female adults (mean was 95 $\mu g\ g^{-1}$). However both these sex differences in concentration were not significant at the 5% level.

There were significant sex differences between the weights of the post-larval stages. Thus female pupae (mean weight was 14.3660 mg) and adults (mean was 9.4541 mg) were significantly ($p < 0.05$) heavier than the corresponding male instars (mean weights of 8.4292 and 4.8187 mg for pupae and adults respectively).

TABLE 7.8. Concentration of copper ($\mu g\ g^{-1}$) in instar V larvae, pharate pupae, pupae and adults of P. conspersa collected at station 1 in July 1984, showing range, mean, standard error (S.E.) and number (n) of specimens which were individually analysed.

INSTAR		n	MEAN	S.E.	RANGE
LARVAE (V)		10	373	33	247-525
PHARATE PUPAE		5	146	18	98-210
PUPAE	TOTAL	14	106	13	57-209
	MALE	7	122	22	59-209
	FEMALE	7	90	10	57-124
ADULTS	TOTAL	16	103	11	23-171
	MALE	10	107	17	23-171
	FEMALE	6	95	11	57-132

Since there are these significant differences in weight, it is important to consider amounts as well as concentrations of copper. Thus the amount of copper in all these post-larval stages (and in the instar V larvae) was calcualted, and this data is summarised in Table 7.9. There was a significant ($p < 0.01$) decrease in the amount of copper between instar V larvae and pharate pupae, with the pharate pupae (mean amount of copper was 1.25 µg) containing on average only 43% of the amount of copper measured in the instar V larvae (mean 2.93 µg). The amount of copper in the pharate pupae and in the pupae (mean amount was 1.15 µg for male and female pupae combined) was not significantly different at the 5% level. However there was a significant ($p < 0.01$) decrease in the amount of copper between the pupae and the adults, with the adults (males and females combined) containing only 56% of the amount of copper in the pupae.

Reference to Table 7.9 also shows that female pupae contained higher amounts of copper than male pupae, with mean values of 1.28 and 1.02 µg respectively; although this difference was not significant at the $p = 0.05$ level. However there was a significant difference ($p < 0.05$) between the higher amounts of copper recorded in the adult females (mean 0.85 µg) and that in the adult males (mean 0.51 µg).

Seven prepupae and ten instar V larvae were collected from station 1 in May 1985, and were subsequently starved (in the case of the larvae), weighed and individually analysed (see Appendix 6E and 6J). The results, presented in Table 7.10, show that there was a significant ($p < 0.05$) difference in the copper concentrations of these two instars, with prepupae containing on average only 45% of the copper concentration recorded in instar V larvae (mean concentrations were 103 and 229 µg g^{-1} respectively).

In July and August 1985 instar V larvae from station 1 were subjected to a set of laboratory conditions designed to encourage these individuals

TABLE 7.9. Amount of copper (μg) in instar V larvae, pharate pupae, pupae and adults of P. conspersa collected at station 1 in July 1984, showing range, mean, standard error (S.E.) and number (n) of specimens which were individually analysed.

INSTAR		n	MEAN	S.E.	RANGE
LARVAE (V)		10	2.93	0.47	1.39-5.91
PHARATE PUPAE		5	1.25	0.11	1.06-1.63
PUPAE	TOTAL	14	1.15	0.13	0.38-2.03
	MALE	7	1.02	0.17	0.38-1.45
	FEMALE	7	1.28	0.19	0.50-2.03
ADULTS	TOTAL	16	0.64	0.07	0.10-1.20
	MALE	10	0.51	0.08	0.10-0.88
	FEMALE	6	0.85	0.08	0.62-1.20

TABLE 7.10. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in instar V larvae and prepupae of P. conspersa collected at station 1 in May 1985, showing range, mean, standard error (S.E.) and number (n) of specimens which were individually analysed.

INSTAR		n	MEAN	S.E.	RANGE
LARVAE (V)	COPPER	10	229	45	49-473
	WEIGHT	10	5.8190	0.8866	1.8774-9.5322
PREPUPAE	COPPER	7	103	6	73-126
	WEIGHT	7	6.8456	0.4385	5.2149-8.2937

to pupate, and so obtain the pupa and shed larval skin. As female pupae were the more numerous, the experiment was terminated when 10 female pupae had been collected. The individual weights and copper concentrations of these pupae (females only) and the shed larval skins are given in Appendix 6K, and are summarised in Table 7.11.

It can be seen from Table 7.11 that the concentrations of copper in the female pupae were significantly ($p < 0.001$) less than the concentrations in the shed larval skins, with mean values of 70 and 534 $\mu g\ g^{-1}$ respectively. However, the weights of these two fractions were significantly ($p < 0.05$) different, and so Table 7.12 shows the amounts of copper in the specimens.

The shed final instar larval skin contained quantitatively important amounts of copper, with a mean value of 0.23 µg. By comparison the mean amount of copper in the female pupae was 0.56 µg. The variation about both these mean values was very small.

TABLE 7.11. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in female pupae and shed larval skins of P. conspersa collected as instar V larvae at station 1 in July and August 1985, showing range, mean, standard error (S.E.) and number (n) of specimens which were individually analysed.

SPECIMEN		n	MEAN	S.E.	RANGE
PUPAE	COPPER	10	70	3	57-82
	WEIGHT	10	7.9571	0.5525	5.5257-11.6397
SHED LARVAL SKINS	COPPER	10	534	13	480-608
	WEIGHT	10	0.4310	0.0264	0.3131-0.5883

TABLE 7.12. Amount of copper (µg) in female pupae and shed larval skins in ten individuals of P. conspersa collected as instar V larvae at station 1 in July and August 1985, together with an overall mean and standard error (S.E.).

INDIVIDUAL	AMOUNT OF COPPER (µg)	
	PUPA	SHED LARVAL SKIN
1	0.56	0.26
2	0.91	0.33
3	0.51	0.25
4	0.44	0.18
5	0.57	0.21
6	0.52	0.26
7	0.38	0.22
8	0.52	0.19
9	0.48	0.18
10	0.66	0.20
MEAN ± S.E.	0.56 ± 0.05	0.23 ± 0.01

7.3.3. Macroinvertebrate prey

An examination of the gut contents of instar V larvae of P. conspersa taken from station 1 in November 1984 and in March and July 1985, showed that coleopteran and chironomid larvae were the main prey items, with a few larvae of P. conspersa also consumed.

The copper concentrations and weights of both of these prey items in 10 sample units collected at station 1 in January and July 1986 are shown in Appendix 6L, and are summarised in Table 7.13. Both the dry weight and copper concentration of chironomid larvae in a sample unit were/was significantly ($p < 0.001$) higher in July than in January, e.g. mean concentrations of 1054 and 407 $\mu g\ g^{-1}$ were recorded in July and January respectively. Similarly, significantly ($p < 0.05$) higher weights and copper concentrations were recorded in the coleopteran larvae in the sample units taken in July, e.g. a mean concentration of 356 $\mu g\ g^{-1}$ was measured in July compared to a mean of 191 $\mu g\ g^{-1}$ in January.

In Table 7.14 the data for the coleopteran and chironomid larvae is combined to show the total prey biomass and total concentration of copper in each sample unit. In July the total prey biomass (mean of 27.5447 mg/sample unit) was significantly ($p < 0.01$) higher than that recorded in January (mean of 11.9281 mg/sample unit). Similarly the total copper concentration in July (mean of 1410 $\mu g\ g^{-1}$/sample unit) was significantly ($p < 0.001$) higher than in January (mean of 597 $\mu g\ g^{-1}$/sample unit).

TABLE 7.13. Mean concentration of copper ($\mu g\ g^{-1}$) and dry weight (mg) of chironomid and coleopteran larvae per sample unit ($0.075\ m^2$) collected at station 1 in January and July 1986 (standard errors are shown in brackets).

		10.1.86	14.7.86
CHIRONOMIDAE	COPPER	407 (55)	1054 (93)
	WEIGHT	2.3152 (0.1500)	7.4467 (0.9330)
COLEOPTERA	COPPER	191 (44)	356 (47)
	WEIGHT	9.6129 (2.1243)	20.0780 (2.9826)

TABLE 7.14. Total prey biomass (mg) and total concentration of copper ($\mu g\ g^{-1}$) in each of ten sample units collected at station 1 in January and July 1986, together with an overall mean and standard error (S.E.).

SAMPLE UNIT	TOTAL PREY BIOMASS (mg)		TOTAL CONCENTRATION OF COPPER ($\mu g\ g^{-1}$)	
	10.1.86	14.7.86	10.1.86	14.7.86
1	5.5827	42.8788	566	1049
2	21.0926	26.8380	630	2026
3	1.7268	37.8855	434	1068
4	16.0930	5.4196	667	1888
5	16.4557	36.8328	816	1349
6	10.4729	38.1213	407	1014
7	1.8156	19.8792	765	1661
8	18.1998	17.1070	445	1403
9	17.7616	24.9484	672	1533
10	10.0804	25.5368	571	1109
MEAN ± S.E.	11.9281 ± 2.2294	27.5447 ± 3.6647	597 ± 44	1410 ± 115

7.4. Discussion

The copper concentrations in all the larval instars of P. conspersa at station 1 have been consistently high during this investigation, reflecting the elevated copper concentration in the water at this station (mean of 0.86 mg l^{-1}). By contrast the larvae at station 4, where the copper concentration in the water is 0.025 mg l^{-1}, contained significantly lower copper concentrations than the station 1 larvae.

Copper was measurable in instar V larvae taken from stations 4 and F, and from the Aylesbeare stream, where the copper concentration in the water is below the detection limit of flame AAS. It is interesting to note that in May 1985 the concentration of copper in the Aylesbeare stream larvae (mean of 29 μg g^{-1}; see Table 7.7) was significantly ($p < 0.01$) less than the concentration in instar V larvae from station 4 (mean of 59 μg g^{-1}; see Table 7.6), and that the concentration of copper in the water in these two streams was 0.004 and 0.025 mg l^{-1} respectively.

The collection of larvae along the Darley Brook showed there to be a positive linear relationship between the copper concentration in the larvae and that in the water, upto a concentration of 0.32 mg l^{-1} (see Figure 7.5). This relationship was further examined by collecting larvae of P. conspersa from Porthtowan stream, Cornwall (Nat. Grid Ref. SW 695475). This stream originates as groundwater from several disused mine shafts, and analysis of an unfiltered acidified water sample showed the copper concentration to be 0.24 mg l^{-1}. In this stream P. conspersa is a prominent species in an invertebrate community which is more diverse than at station 1. In May 1985 ten instar V larvae and water samples were collected at stations 1 and 4, Porthtowan stream (see Appendix 6M) and Aylesbeare stream. What is interesting about the analysis of these larvae (see Figure 7.6) was that it showed that at low concentrations (i.e. < 0.025 mg l^{-1}) the copper

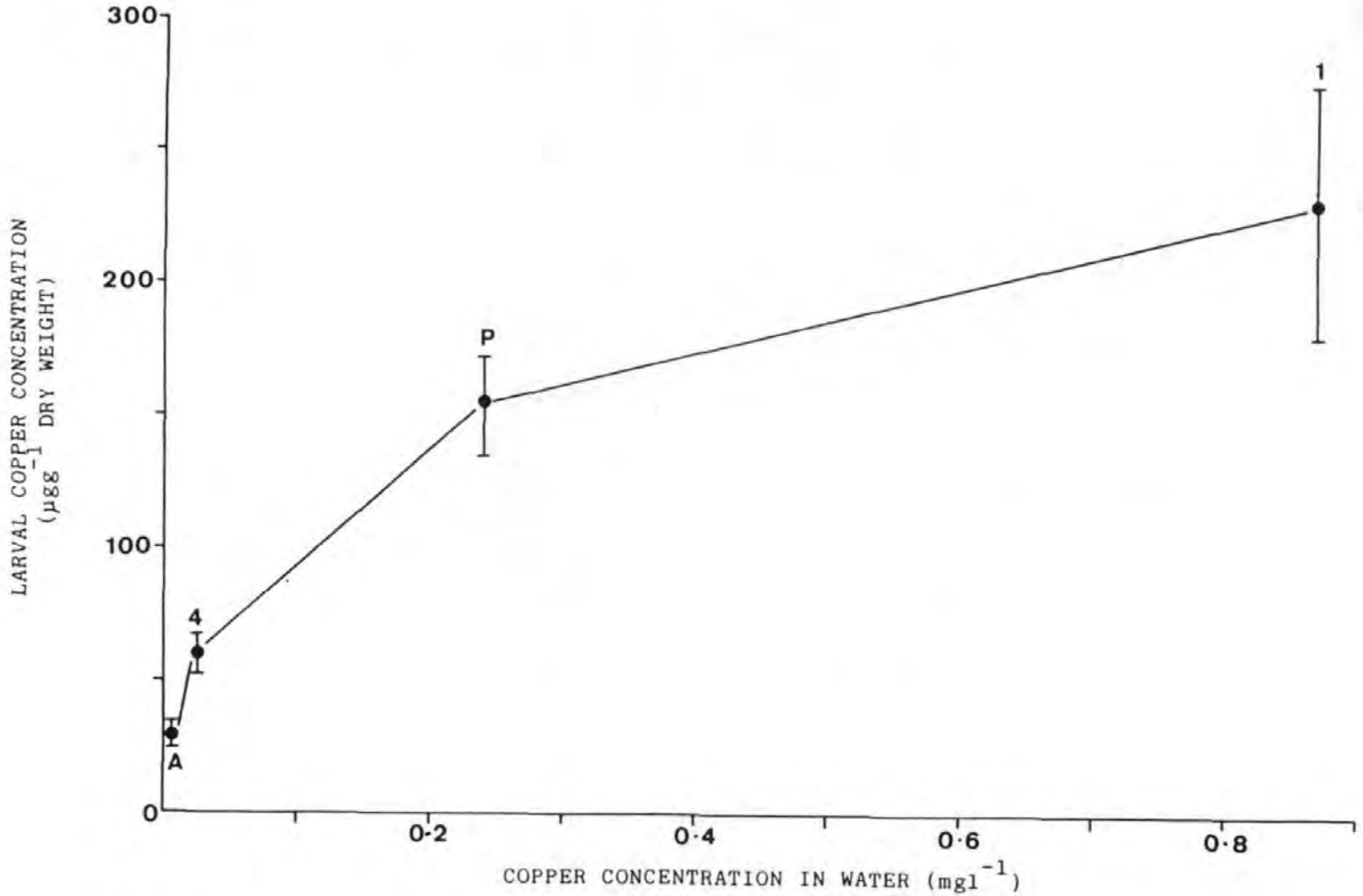


FIGURE 7.6. Relationship between the copper concentration (mg l^{-1}) in the water and the mean copper concentration (µg g^{-1}) in ten individually analysed instar V larvae of P. conspersa collected in May 1985 at stations 1 and 4, Aylesbeare (A) stream and Porthtowan (P) stream (vertical bars show standard errors).

concentration in the larvae increases rapidly with copper in the water; however this increase is less steep between 0.025 and 0.24 mg l^{-1} and even less steep again between 0.24 and 0.87 mg l^{-1}.

Brown (1977a) found an almost proportional increase in the copper concentration of 'free-living' Trichoptera with increasing 'total' copper in the waters of the River Hayle catchment, with a concentration of 1000 μg g^{-1} measured in larvae of the genus Plectrocnemia taken from a site with a 'total' copper concentration of 0.20 mg l^{-1} in the water. However in this same study, Plectrocnemia larvae taken from an adit where the 'total' copper concentration was 0.60 mg l^{-1} contained markedly less copper (488 μg g^{-1}) than was expected from regression analysis. Although excluded from the regression analysis, the levels in the latter group of larvae now appear to be significant in view of the concentrations recorded in larvae taken from station 1 during the present study, which were also less than might be expected from the positive linear relationship found at those stations with much lower copper concentrations (see Figures 7.5 and 7.6). It is possible that excretion of copper may be occurring at a greater rate in larvae exposed to high copper concentrations.

Graphite furnace AAS has enabled analysis of separate specimens of the larval and post-larval stages of P. conspersa. It has even been possible to analyse very small larvae, indeed the smallest larvae analysed (instar II) contained the highest copper concentrations (see Table 7.3). This technique has also facilitated (with a comparatively small number of larvae) an examination of the relationship between larval copper concentration and dry body weight. An exponential decrease in copper concentration with increasing body weight (such as that shown in Figure 7.1) has been observed at both stations 1 and 4 on every occasion when a number of larval instars have been analysed.

The observed relationship between copper concentration and dry body weight in larvae of P. conspersa may be explained by a surface to volume phenomenon. The size of the larvae may also influence concentration by exerting morphological constraints on the size of prey consumed. Thus smaller larvae can only ingest small prey items which have high copper concentrations, whilst larger larvae can consume a wider size range of prey including less copper rich individuals.

Smock (1983b) has also studied the relationship between metal concentration and size (body weight) in aquatic insects, but using the technique of neutron activation analysis. Most metals (cobalt, chromium, iron, antimony and scandium) showed an exponential decrease in body concentration with increasing size, and he suggests that for these metals surface adsorption is an important mode of metal accumulation. For the other metals (potassium, manganese and sodium) size had no significant effect, or at most there was a slight increase in concentration with increasing size, leading Smock to suggest that in these metals absorption was the more important factor determining metal concentration.

In a recent study the concentrations of cadmium, lead and zinc were measured in individuals of a number of forest litter arthropods (Straalen & Wensem, 1986). There was found to be an almost linear positive relationship between the concentration of lead and dry weight (i.e. larger individuals contained more lead, regardless of species), and this is in contrast to the concentration-weight relationships found either in the present study or by Smock (1983b). For zinc and cadmium there was no clear linear relationship, and concentrations did not rise with increasing body weight.

The single most important fraction of copper in P. conspersa larvae is that associated with material in the gut, for example it is estimated

that this fraction accounts for on average 61% of the whole-body copper concentration in instar V larvae (see Table 7.5); however it should be noted that excreted material may also contribute to this 61%. Elwood et al. (1976) measured 30 elements (though not copper) both before and after gut evacuation in the aquatic cranefly Tipula sp., and found that the concentrations of most elements were significantly ($p < 0.01$) lower after gut evacuation mainly due to defecation. Similarly Smock (1983a) has demonstrated that metals associated with the gut contents of aquatic insects are often the major fraction of the whole-body concentration.

Unstarved larvae as well as starved larvae of P. conspersa showed an exponential decrease in copper concentration with increasing body weight (see Figure 7.2A). In a study on the nymphs of the mayfly Stenonema modestum (Smock, 1983b) the exponential decrease in the concentration of several metals with increasing size was also shown not to change after evacuation of the digestive tracts.

It is possible to quantitatively differentiate between the various copper fractions in P. conspersa larvae. Ingested material has been shown in this investigation to account for approximately 61% of the whole-body copper concentration of instar V larvae. In July 1985 the mean amount of copper in final instar larvae collected at station 1 was 1.31 µg (see Figure 7.4), and when these larvae pupated under laboratory conditions the ecdysed larval skins were found to contain on average 0.23 µg of copper (see Table 7.12). Therefore it can be estimated that 18% of the copper in instar V larvae is associated with the cuticle.

The adults of P. conspersa are non-aquatic, and so it is reasonable to assume that the copper in these individuals is internally absorbed in the body tissues. From Table 7.9 it can be seen that the mean amount of copper in adults (males and females combined) collected as pupae from

station 1 in July 1984 was 0.64 µg, and that in this same month the mean amount of copper in instar V larvae was 2.93 µg. Thus it can be calculated that 22% of the copper in final instar larvae is internally absorbed.

A summary of these calculations shows that ingested material (possibly together with excreted material) accounts for 61% of the whole-body copper content, 18% is associated with the cuticle and 22% is absorbed in the tissues. By comparison Smock (1983b) found that 52% of the whole-body content of chromium in the nymphs of two mayfly species was associated with gut material, 33% was surface adsorbed and 15% was absorbed in the body.

The concentrations of copper in larval instars II, III, IV and V of P. conspersa collected at stations 1 and 4 were found to vary with time (see Table 7.3), as was the amount of copper in these larvae (see Table 7.4). Further investigation of the instar V larvae which were collected every two months at stations 1 and 4, showed that at both stations there was a clear seasonality, with a summer maximum and winter minimum in larval copper concentrations (see Figure 7.3). The amounts of copper in these final instar larvae showed a similar temporal variation (see Figure 7.4). Even the copper concentrations in the instar V larvae collected from the Aylesbeare stream were found to vary with time (see Table 7.7).

This temporal variation has important implications for the potential use of P. conspersa as an indicator of metal enriched conditions. In a way it may be of advantage to compare concentrations (rather than amounts) of copper in the larvae since, as Figure 7.3 shows, there was minimal overlap (i.e. the standard error for July 1984 at station 4 overlapped with that recorded in January 1985 at station 1) between the concentrations measured in the two larval populations.

Of course, if the metal concentrations in larvae collected from different streams are to be compared, the larvae should if possible be

sampled at the same time. Comparisons can also only be made between concentrations in the same larval instar, since there are significant differences between the concentrations in different instars. It is suggested that in this work on P. conspersa metal analysis is perhaps best carried out on either instar IV or V larvae, as they were present throughout the year at stations 1 and 4 (see Figures 6.3 and 6.5 respectively) and are of a reasonable size for the purposes of sampling, extraction and subsequent processing.

Only a few workers have investigated temporal variation in metal concentrations in aquatic invertebrates. Copper concentrations in the digestive gland, gut and blood of the whelk Busycon canaliculatum were shown by Betzer & Pilson (1974) to increase in early summer, due to the commencement of feeding, and reached a maximum in mid-summer. In a study on metal levels in the barnacle Balanus balanoides (Walker & Foster, 1979), zinc concentrations increased during periods of growth, remained at a high level over winter, and decreased when eggs were produced since zinc was shown to accumulate in the ovaries.

Harding et al. (1981) found that the concentrations of zinc, cadmium, and to a lesser extent lead, in mayfly nymphs and some other unnamed aquatic invertebrates were highest in August, suggesting that the higher water temperature may cause an increase in metal uptake. Although zinc concentrations in nymphs of Ecdyonurus venosus were higher in the summer, the amounts of zinc were similar in summer and winter (Burrows & Whitton, 1983).

At stations 1 and 4 the copper concentration in instar V larvae of P. conspersa reached a maximum of 373 and 99 $\mu g\ g^{-1}$ respectively in July 1984, and was at a minimum of 132 and 27 $\mu g\ g^{-1}$ respectively in January 1985 (see Table 7.6 and Figure 7.3). It is suggested that the summer

maximum could be due to more prey being consumed and to an increase in the copper concentration of the prey itself. An examination of the gut contents of final instar larvae collected at station 1 at different times of the year showed that coleopteran and chironomid larvae were always the main prey item.

To test the above hypothesis, coleopteran and chironomid larvae were quantitatively sampled at station 1 in January and July 1986. The mean biomass of chironomid larvae was significantly higher in July (100 mg m^{-2}) than in January (31 mg m^{-2}). In a study on P. conspersa in a species poor, iron rich stream (Townsend & Hildrew, 1979b; Hildrew & Townsend, 1982) a maximum mean biomass of chironomid larvae was recorded in August (785 mg m^{-2}) due to recruitment, and a minimum in February (19 mg m^{-2}). In this same study chironomid and stonefly larvae were the major prey items in the diet of P. conspersa, reaching a combined maximum mean biomass of 875 mg m^{-2} in August. By comparison the total (i.e. coleopteran and chironomid larvae) mean prey biomass at station 1 in the Darley Brook (see Table 7.14) was higher in July (367 mg m^{-2}) than in January (159 mg m^{-2}).

Although Hildrew and Townsend (1982) were unable to demonstrate a clear functional response in P. conspersa, their data suggests that predator consumption rates are generally higher in patches of high prey density. In the present study it has been shown that both the biomass and density of the major prey items of P. conspersa at station 1 are higher in the summer than in the winter. Thus larvae of P. conspersa will consume more prey in the summer which, even if the copper concentrations of the prey were constant throughout the year, would probably lead to a summer increase in the copper concentration of the predator.

If the copper concentrations of the prey of P. conspersa varied over time, it is possible that these variations might be reflected in the copper

concentrations measured in the predator. The concentrations of copper in both the coleopteran and chironomid larvae at station 1 was significantly higher in July 1986 than in January 1986 (see Table 7.13), and interestingly the copper concentration of instar V larvae of P. conspersa at this station was at a maximum in summer (July 1984) and a minimum in winter (January 1985). It should however be noted that other factors such as water temperature and metabolic rate may influence the copper concentration of P. conspersa larvae.

The above evidence would appear to support the hypothesis proposed earlier on in this discussion to explain the seasonal variation in the copper concentration of instar V larvae of P. conspersa at station 1. Thus in the summer the predator is not only consuming more prey (partly due to increased prey biomass and density), but the copper concentration of the prey itself is higher. In the winter the lower biomass, density and copper concentration of the prey may contribute to lower concentrations in the predator.

It has been shown in this investigation (see Table 7.10) that there is a significant difference between the copper concentration of instar V larvae and prepupae, with mean concentrations of 229 and 103 $\mu g\ g^{-1}$ respectively. A prepupa is morphologically similar to a final instar larva, with the exception that it is within a pupal case which is held together with silk secreted by the final instar larva. However as explained on page 325 no copper was found to be associated with the larval silk glands. It may be more likely that a change in the nature and level of metabolic activities, including excretion, accounts for the reduction in copper in the prepupal stage. Clearly the significant difference between the copper concentrations of the final instar larvae and the prepupae needs to be further examined.

Although there was no significant difference between the concentrations of copper in the pupae and adults, when the amounts of copper were calculated in these instars (see Table 7.9) there was a significant decrease between the pupae (mean amount for males and females combined was 1.15 μg) and the adults (mean amount was 0.64 μg). This decrease may be partly due to the loss of copper associated with the shed pupal skin. There is also the possibility that there is some excretion of copper during pupation. In another study (Rossaro et al., 1986) on metal concentrations in the larvae and adults of a freshwater insect, Chironomus riparius, the mean concentrations of mercury in the larvae, pupal exuviae and adults were 69, 58, and 20 μg g^{-1} respectively, and thus in this species a significant amount of mercury was associated with the shed pupal skin.

CHAPTER 8

TOXICITY OF COPPER TO LARVAE OF PLECTROCNEMIA CONSPERSA

8.1. Introduction

Whilst there is now a considerable body of literature on the toxicity of copper to fish, comparable work on freshwater macroinvertebrates has received less attention, and it is only recently that interest in this group of organisms has increased. However there are still relatively few studies on the toxicity of copper to aquatic insects, which is surprising since they are frequently the dominant group of invertebrates in metal enriched streams.

Sprague et al. (1965) found that mayfly nymphs and the salmon Salmo salar had comparable copper and zinc sensitivities, whereas trichopteran larvae and some chironomid larvae were at least 1.5 times more resistant to these metals. Several workers have investigated the toxicity of copper to different insect orders (Warnick & Bell, 1969; Rehwoldt et al., 1973; Nehring, 1976). Nebeker et al. (1984a) investigated the sensitivities of different larval instars of the Chironomidae Chironomus tentans to copper, and found reduced sensitivity with development.

The use of Trichoptera in toxicological work has been limited, although in a recent investigation Nebeker et al. (1984b) studied the effects of copper, nickel and zinc on the life cycle of Clistoronia magnifica. Abel and Green (1981) have shown that Limnephilus sp. can withstand high levels of zinc (e.g. up to 100 mg l^{-1}), although feeding rates in this caddis are reduced by much lower zinc concentrations. In a study on the toxicity of a number of metals to three insect species (Warnick & Bell, 1969), larvae of the net-spinning caddis Hydropsyche betteni lived beyond the 96 hour test period even at copper concentrations

of up to 64 mg l^{-1}. Copper enhancement has been shown to change the symmetry, size and regularity of hydropsychid nets (Besch et al., 1979; Petersen & Petersen, 1983). There are however no published records on the toxicity of copper to larvae of Plectrocnemia conspersa.

The larvae of P. conspersa at station 1 in the Darley Brook are constantly subjected to an elevated copper concentration (mean 0.86 mg l^{-1}), and it is therefore of interest to determine what concentration of copper is necessary to cause the deaths of individuals from this population. One way of determining the lethal concentration (LC) is by means of a static toxicity test, whereby larvae in test chambers are exposed to a range of experimental dilutions and are maintained under these conditions for the duration of the test. Such tests are usually limited to 96 hours because of a possible decrease in the dissolved oxygen content and metal concentration of the dilutions, and a possible increase in metabolic wastes within the test chamber. However these problems may be overcome by the periodic renewal of the test dilutions, although this itself may stress the test organisms.

Stephenson (1983) showed that copper was 4 to 6 times more toxic to Gammarus pulex in soft water than in hard water. In addition in their review on acidification and the toxicity of metals to aquatic organisms, Campbell and Stokes (1985) noted that in several studies on algae, invertebrates and fish the toxicity of copper decreases with decreasing pH. Consequently hardness and pH are just two of the many factors which may influence the toxicity of copper to an organism, and therefore in toxicity tests it is necessary to carefully control the physicochemical environment.

A criticism that has been made of toxicity tests carried out in the laboratory is that test organisms may be subject to conditions which are dissimilar from those that would be experienced in the field

situation. Consequently in this study larvae of P. conspersa were taken from a clean control stream and transferred to a metal contaminated stream, where they were observed for several weeks. Although the introduction of caged fish into suspect waters has been reported in the literature (e.g. Herbert et al., 1965; Uthe et al., 1973; Stoner & Gee, 1985), to the author's knowledge there are no published accounts of similar work on aquatic insects.

The transfer experiment undertaken here represented a long term toxicity test carried out in the field, and would allow us to determine whether the species itself, or just the population living in the metal contaminated stream, was metal tolerant. Furthermore, if the larvae introduced into the copper rich stream did survive, then it would be desirable to investigate the patterns of tolerance, including any changes in the copper concentration of the larvae.

8.2. Transfer Experiment

8.2.1. Materials and Methods

The instar V larvae of P. conspersa used in this experiment were taken from two populations:

Population A from Aylesbeare stream near Exeter, Devon (see p.219 for site description). In this relatively clean stream (copper concentration is 0.004 mg l^{-1}) P. conspersa larvae occur in significant numbers.

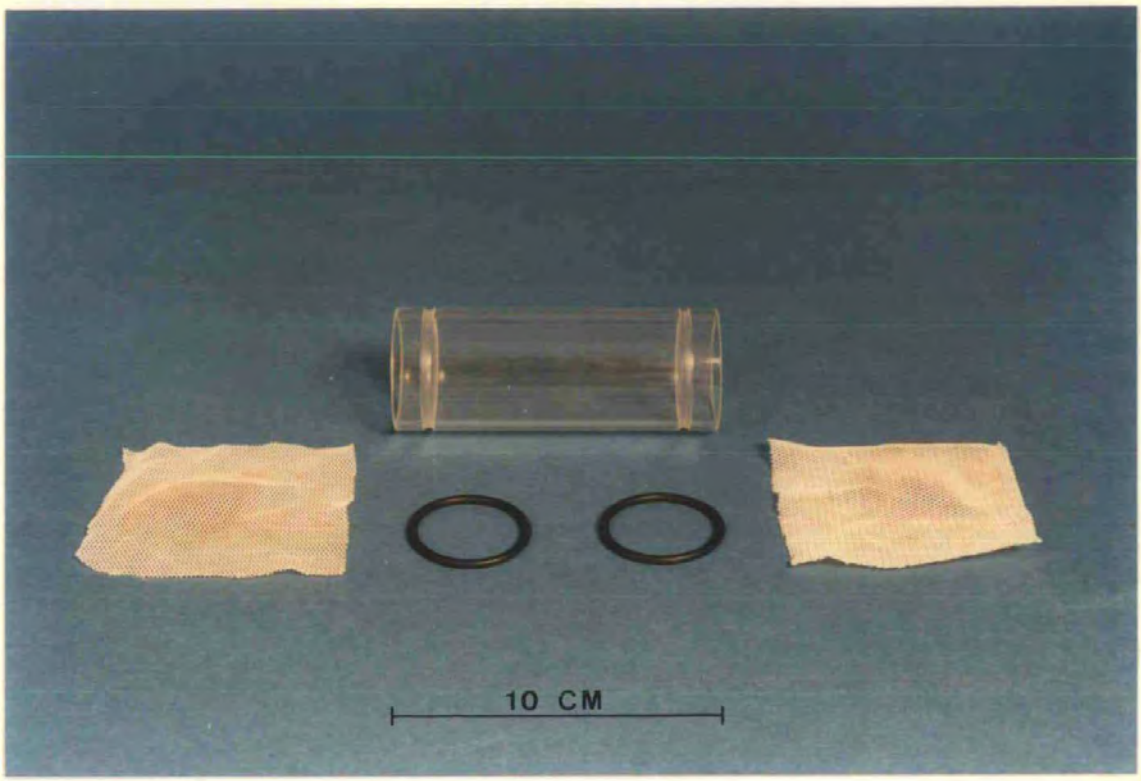
Population B (the control) from station 2 in the Darley Brook, where the mean copper concentration in the water is 0.79 mg l^{-1} and high densities of P. conspersa have been recorded during this investigation.

On 28.5.85, 140 instar V larvae were collected from each stream. Since the larvae are characteristically aggressive and cannibalistic, the 280 individuals had to be confined separately under experimental conditions. Each larva was enclosed in a specially prepared perspex tube (see Plate 8.1) 10 cm long, 3 cm in diameter with a groove 1 cm from each end to take a rubber O-ring which retained some netting (mesh 1 mm) to allow free movement of water, whilst preventing escape of the larva.

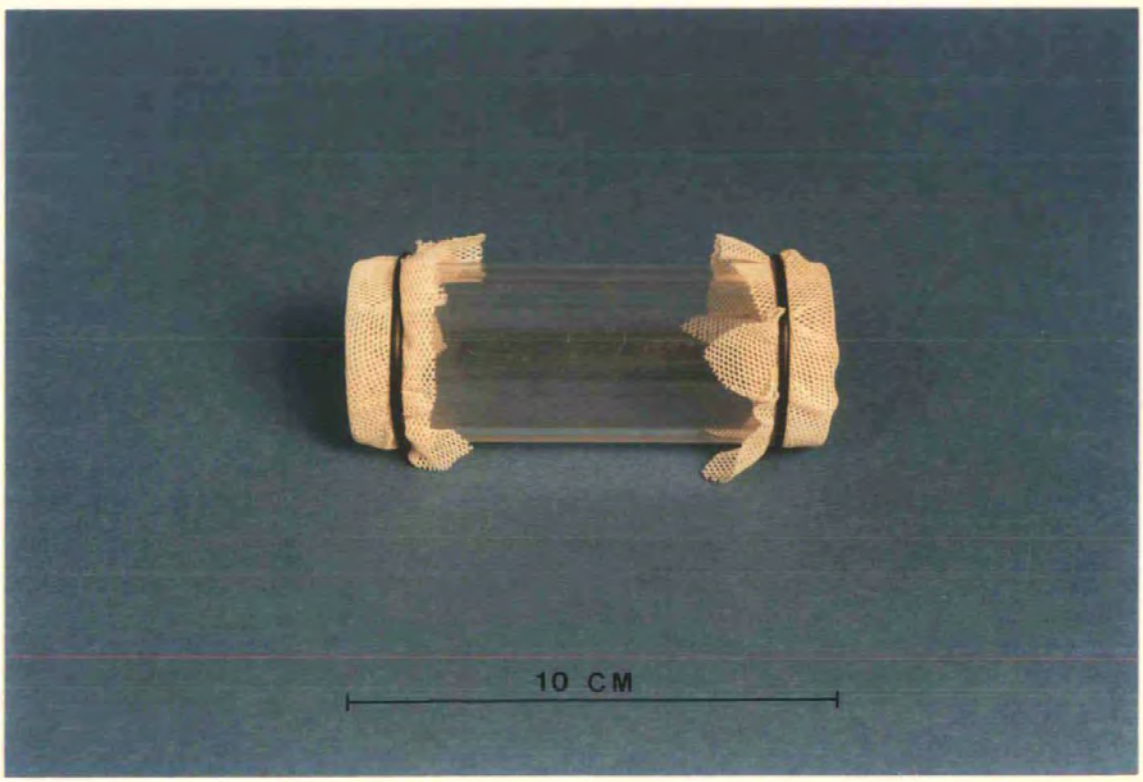
Although the perforated ends of the tubes theoretically allowed food organisms such as chironomid larvae in the surrounding water at station 2 to pass through, there was no guarantee that even if this occurred, a sufficient supply of food would be available. Furthermore, since the objective of the transfer experiment was long term exposure, half of the larvae in each population (treatment F) was provided with food on a regular basis. For this a laboratory culture of final instar larvae of Chironomus riparius was used.

Each population was thus divided into two treatments (each of 70 tubed larvae), one lot fed (F) and the other lot unfed (U). For

PLATE 8.1. Perspex tube used for enclosing a final instar larva of P. conspersa during the transfer experiment
A). component parts B). complete.



A



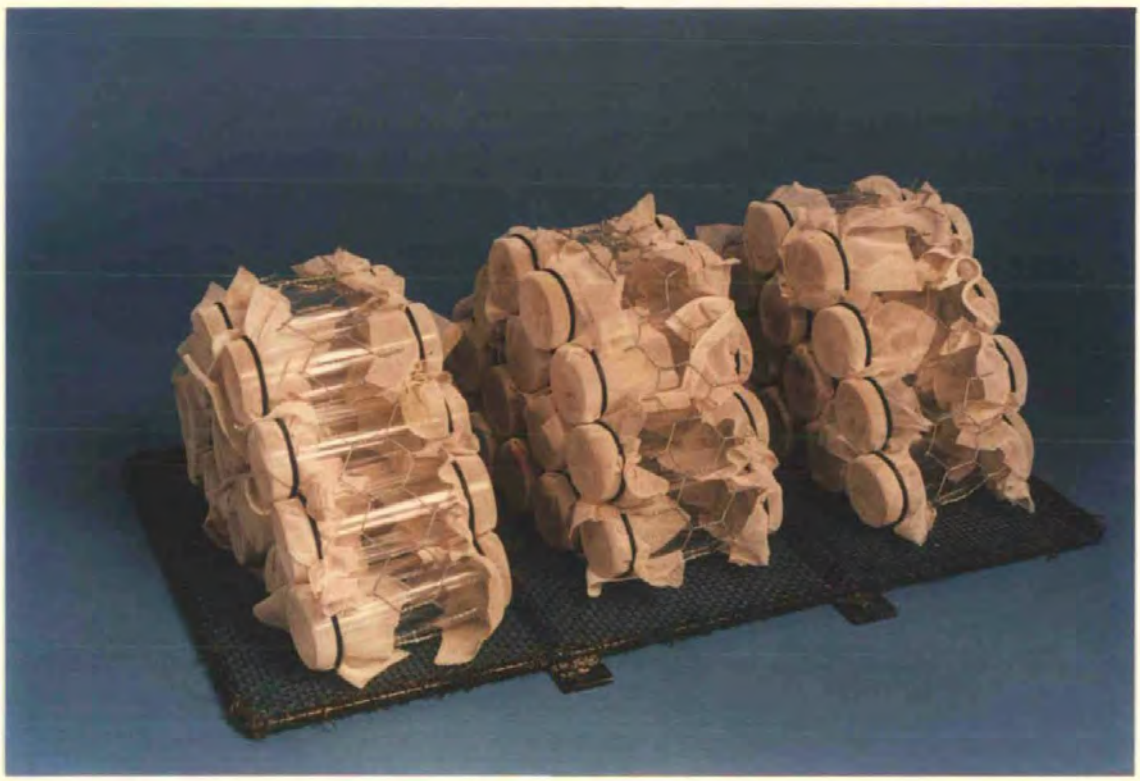
B

ease of handling each treatment consisted of 5 batches of 14 tubes, which enabled examination of the larvae at regular intervals over the experimental period. These batches of tubes were tied on to racks (see Plate 8.2) which were submerged at station 2 in such a way that they were concealed (to avoid vandalism) and at the same time exposed to sufficient flow of water.

For the duration of the experiment all the larvae were examined, and fed where necessary twice a week (on Tuesday and Friday). Feeding of treatment F larvae was at a rate of one final instar chironomid larva per day. The number of dead larvae was recorded and these individuals were then discarded. Field notes were also made on the condition of the live larvae, e.g. whether they had spun a feeding net or pupated.

Every 7 days, 10 live larvae were removed from each of the 4 treatments. On return to the laboratory these larvae were starved for 48 hours at 10°C and then frozen. They were subsequently individually analysed for their copper concentration using graphite furnace AAS (see Appendix 1E). At the start of the experiment an additional 10 instar V larvae were collected from both the Aylesbeare stream and from station 2 in the Darley Brook, and these individuals were analysed to establish the 'background' copper concentration of both populations.

PLATE 8.2. Batches of perspex tubes mounted on a rack and used for containing final instar larvae of P. conspersa during the transfer experiment.



8.2.2. Results

Since larvae were examined every few days, their condition was kept under close scrutiny and although there were some mortalities, the larvae seemed to be generally healthy and constructed nets for feeding. A number of larvae proceeded to construct pupal cases (silk only), and since this number increased steadily with time, it was decided to curtail the experiment after 35 days (treatment U) and 42 days (treatment F).

The number of post-larval stages of P. conspersa in each treatment, together with the number of larvae which had escaped is shown on a weekly basis in Appendix 7A. Furthermore this appendix details the number of larvae which were removed each week for copper analysis, and the number of larvae remaining.

The cumulative number of larval deaths in each treatment over the experimental period is shown in Figure 8.1 (see Appendix 7A for the weekly larval mortalities). After 35 days a total of 16% of the population A (U) larvae had died compared to 10% of the population B (U) larvae. Of the larvae that were fed 14% from the Aylesbeare stream and 10% from the Darley Brook had died by the end of the 42 day experimental period.

A Mann-Whitney U-test showed that there was no significant difference (at the $p = 0.05$ level) between the number of deaths that occurred in the fed larvae from the Aylesbeare stream and the Darley Brook ($U = 25.5$). There was also no significant difference between the number of deaths in the unfed larvae from the two streams ($U = 12.5$).

As previously explained, 10 larvae were removed each week for copper analysis from each of the 4 treatments. In the case of treatment F, this left 6 larvae and 9 larvae from populations A and B respectively for final removal after 42 days. Copper concentrations were subsequently

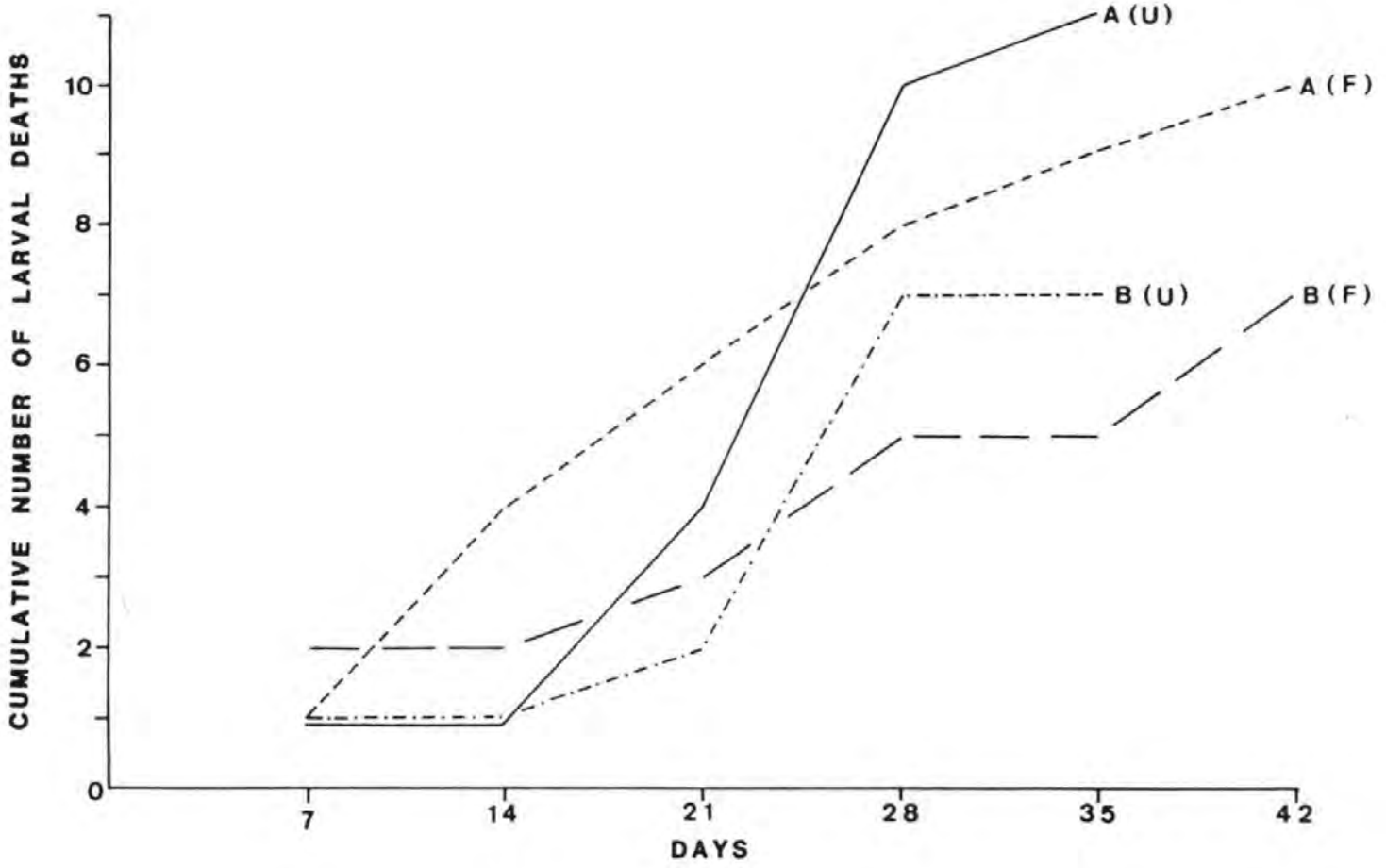


FIGURE 8.1. Cumulative number of deaths occurring over time in the fed (F) and unfed (U) treatments of the Aylesbeare stream (population A) and Darley Brook (population B) larvae of P. conspersa.

measured in 6 larvae selected at random from each of the larval samples frozen at the 7 day intervals during this experiment. However 10 individuals were analysed to establish the 'background' levels of the two larval populations, and for the population B (F) larvae removed after 7 days.

The individual weights and copper concentrations of all of these larvae are given in Appendix 7B (see Appendix 6E and 6H for 'background' concentrations), and the results are summarised in Table 8.1. At the start of the experiment the Darley Brook larvae had a significantly ($p < 0.001$) higher copper concentration than the Aylesbeare stream larvae, with mean concentrations of 229 and 29 $\mu g\ g^{-1}$ respectively. By the end of the experiment the mean concentration of copper in the population A (Aylesbeare stream) larvae had increased to 52 $\mu g\ g^{-1}$ (F treatment) and 131 $\mu g\ g^{-1}$ (U treatment). In the population B (Darley Brook) larvae, the mean copper concentrations of the F and U treatments were 90 and 318 $\mu g\ g^{-1}$ respectively at the end of the experimental period.

Reference to Table 8.1 also shows that there was a marked overall increase in the weights of the Darley Brook larvae that were fed, with a mean weight of 5.8190 mg at the start and 13.2814 mg at the end of the experiment. Similarly the mean weight of the fed Aylesbeare stream larvae increased from 7.0677 mg to 14.5765 mg over the experimental period. By contrast, the weight of the unfed larvae from both populations had decreased by the end of the experiment, e.g. the mean weight of the population B (U) larvae decreased from 5.8190 mg to 3.3308 mg after 35 days. The weight change in the population A (U) larvae was more variable, but with an overall decrease from 7.0677 mg to 3.7999 mg during the period of exposure.

Weight changes make it difficult to compare the two populations of larvae on the basis of copper concentrations, since in the previous

TABLE 8.1. Changes over time in mean concentrations C ($\mu g\ g^{-1}$), weights W (mg) and amounts A (μg) of copper in samples of individually analysed instar V larvae of *P. conspersa* in the fed (F) and unfed (U) treatments of the Aylesbeare stream (population A) and Darley Brook (population B) larvae. Standard errors are shown in brackets. n = 6 except for asterisked samples where n = 10.

TIME		POPULATION A (AYLESBEARE STREAM)		POPULATION B (DARLEY BROOK)	
0 DAYS (28.5.85)	C	29 (3) *		229 (45) *	
	W	7.0677 (1.0131) *		5.8190 (0.8866) *	
	A	0.18 (0.01) *		1.13 (0.21) *	
		F	U	F	U
7 (4.6.85)	C	56 (4)	28 (4)	186 (30) *	293 (35)
	W	6.6007 (1.2884)	9.0084 (1.1468)	7.5701 (0.8475)*	4.9069 (1.3719)
	A	0.35 (0.04)	0.23 (0.01)	1.24 (0.11)*	1.26 (0.20)
14 (11.6.85)	C	63 (4)	51 (14)	227 (24)	312 (34)
	W	6.9724 (1.4230)	5.9210 (1.2703)	5.7035 (0.4626)	4.4852 (1.4733)
	A	0.42 (0.06)	0.23 (0.01)	1.24 (0.02)	1.23 (0.28)
21 (18.6.85)	C	53 (5)	40 (5)	143 (35)	299 (31)
	W	7.6066 (0.9449)	7.9897 (1.1778)	9.5173 (0.8883)	4.4959 (1.0991)
	A	0.38 (0.03)	0.29 (0.03)	1.22 (0.15)	1.18 (0.12)
28 (25.6.85)	C	42 (4)	69 (5)	118 (24)	320 (51)
	W	12.2874 (1.3695)	6.2623 (1.2318)	10.7507 (1.1135)	4.2732 (1.3037)
	A	0.49 (0.04)	0.41 (0.08)	1.15 (0.12)	1.09 (0.18)
35 (2.7.85)	C	61 (6)	131 (8)	87 (11)	318 (47)
	W	12.1468 (0.6590)	3.7999 (0.8047)	12.9661 (1.4540)	3.3308 (0.4467)
	A	0.74 (0.07)	0.47 (0.08)	1.06 (0.07)	1.08 (0.24)
42 (9.7.85)	C	52 (6)		90 (11)	
	W	14.5765 (1.4603)		13.2814 (1.8687)	
	A	0.73 (0.03)		1.10 (0.07)	

chapter it was shown that there is a significant negative correlation between these two variables (see Table 7.2). Therefore the amounts of copper in the larvae were calculated and this data is also presented in Table 8.1.

Figure 8.2 shows changes in the amounts of larval copper in the four treatments over the experimental period. At the start of the experiment larvae taken from the Darley Brook contained over 6 times as much copper as the Aylesbeare stream larvae, with mean amounts of 1.13 and 0.18 μg respectively.

There was a gradual increase in the amount of copper in the Aylesbeare stream F treatment larvae, from a mean of 0.18 μg at the start to 0.73 μg at the end of the 42 day period. A series of t-tests showed that the amount of copper at each 7 day period (e.g. day 7, day 14) was significantly ($p < 0.05$) higher than the initial background amount (i.e. day 0). In the population B (F) larvae, which acted as a control treatment, there was no significant difference at the 5% level in the amounts of copper throughout the experiment.

The amount of copper in the Aylesbeare stream U treatment larvae also increased during the experiment to a mean of 0.47 μg after 35 days. At each 7 day period the amount of copper in these unfed larvae was significantly ($p < 0.05$) higher than at the start of the experiment. By contrast, the amount of copper in the population B (U) larvae did not significantly change during the 35 day experimental period.

Within population A, the amount of copper in the F treatment larvae was significantly ($p < 0.05$) higher than that measured in the U treatment larvae at each 7 day period (with the exception of day 28 when there was no significant difference). Thus after 35 days the mean amount of copper in the F and U treatment larvae was 0.74 and

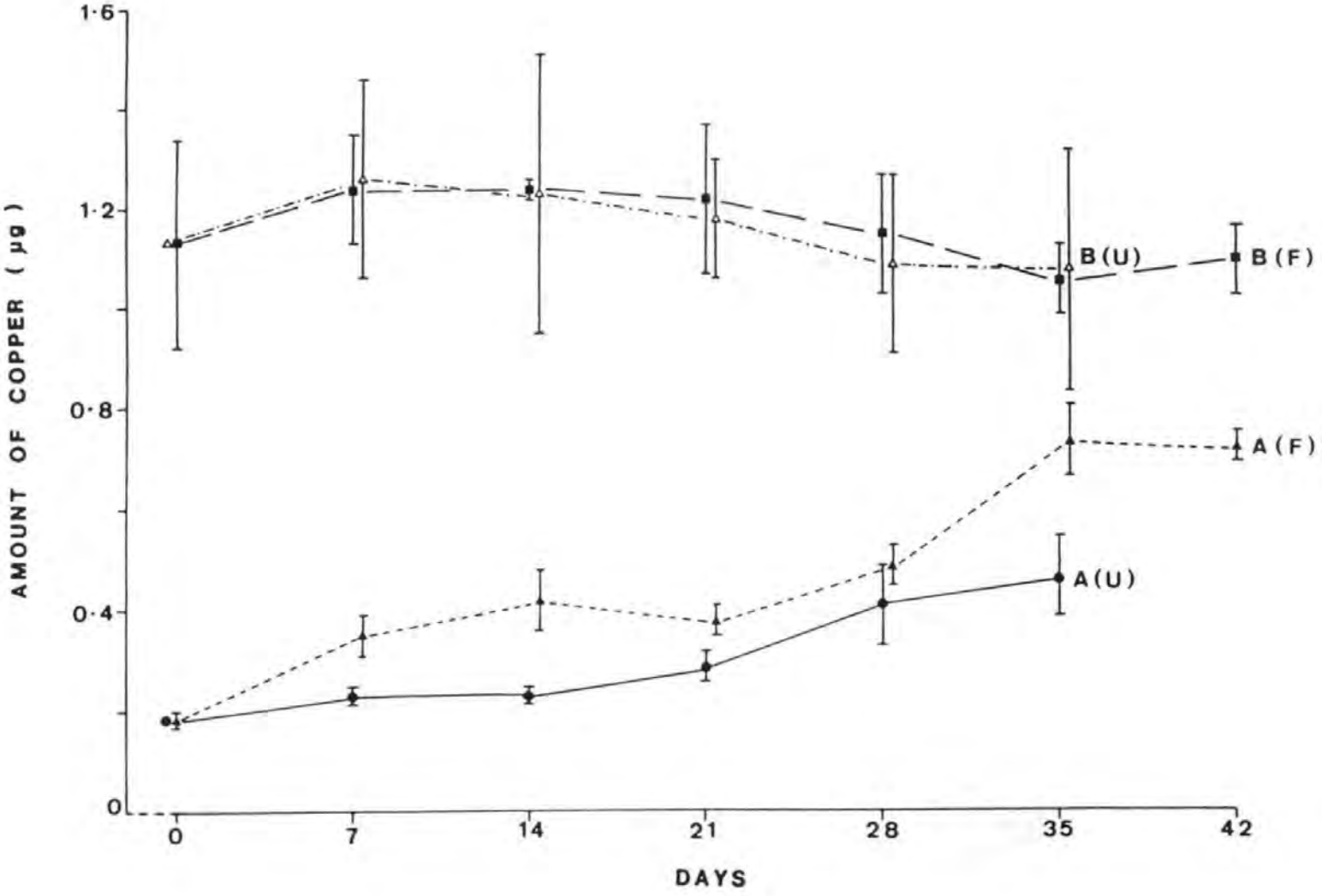


FIGURE 8.2. Changes over time in the amount of copper (µg) in samples of individually analysed instar V larvae of P. conspersa in the fed (F) and unfed (U) treatments of the Aylesbeare stream (population A) and Darley Brook (population B) larvae. Vertical bars show standard errors.

0.47 μg respectively. In the population B larvae, there was at no time any significant difference (at the $p = 0.05$ level) between the amounts of copper in the two treatments.

One further observation evident from Figure 8.2 is that the variation about the mean amounts of copper was generally greater for the population B larvae compared to the population A larvae, and that the largest variations were observed for the Darley Brook unfed larvae.

8.2.3. Discussion

The transfer experiment conducted in this study provides information on the effects of long-term exposure (upto 42 days) to copper of larvae from a metal uncontaminated stream, and on the uptake of copper by these larvae.

Most of the Aylesbeare stream larvae, including the unfed individuals, were in a healthy condition throughout the experiment. Although the weight of the unfed Darley Brook larvae gradually declined over the experimental period, the change in weight of the Aylesbeare stream unfed larvae was more variable (see Table 8.1). This variability might reflect occasional feeding by these larvae, but the decline in weight towards the end of the experiment does suggest lack of food.

The number of mortalities occurring in the Aylesbeare stream larvae (16% in the unfed and 14% in the fed treatments) was not significantly different from the number of deaths recorded in the 'control' Darley Brook larvae (10% in both treatments). This suggests that P. conspersa as a species is metal tolerant, since larvae taken from the Aylesbeare stream have no selective pressure upon them to be metal tolerant and yet they can survive in a stream with a high copper concentration.

It has previously been mentioned (see p.208) that a number of workers have recorded the presence of P. conspersa in streams subject to elevated concentrations of iron, copper, zinc and aluminium (Hildrew & Townsend, 1976; Brown, 1977a; Armitage, 1980; Stoner et al., 1984). These studies corroborate the suggestion made here that P. conspersa is a metal tolerant species, and consequently it is unlikely that copper tolerance in the population in the headwaters of the Darley Brook is a form of tolerance requiring genetic reinforcement within that population.

Although the majority of the Aylesbeare stream larvae appeared to be unaffected by the high copper concentration, for example they carried out net-spinning activity and some pupated, it is possible that there may still be some sub-lethal effects on these larvae. Brown (1976) found that a copper concentration of 0.10 mg l^{-1} significantly inhibited the growth of individuals of Asellus meridianus taken from streams where the maximum copper concentration in the water was 0.05 mg l^{-1}. Growth of the snail Physa integra and the amphipod Gammarus pseudolimnaeus was stopped during a six week exposure to a copper concentration of only 0.015 mg l^{-1} (Arthur & Leonard, 1970). Copper has also been shown to cause anomalies in hydropsychid nets (Besch et al., 1979; Petersen & Petersen, 1983), either by affecting the secretion of silk or the spinning process itself.

The steady increase in the amount of copper in the unfed Aylesbeare stream larvae (see Figure 8.2) is probably due to the uptake of copper from the water. After 7 days there was a 1.3 times increase in the amount of copper, which is less than the increase of 3.8 recorded in the amount of copper in a non-tolerant population of A. meridianus individuals which were unfed and exposed to a copper solution of 0.5 mg l^{-1} over an 8 day period (Brown, 1977b). One possible explanation is that P. conspersa is able to excrete copper at a greater rate than A. meridianus.

In most aquatic animals the absorption of metals from solution involves diffusion of the metal along gradients created by adsorption at the surface, and binding by constituents of the surface cells, body fluids and internal organs (Bryan, 1976). Absorption is affected by a number of factors including the chemical form of the metal, the presence of other metals or of complexing agents, hardness, temperature, pH and size of the animal. Dodge and Theis (1979) demonstrated that

free cupric ions and weakly complexed copper are readily absorbed by larvae of Chironomus tentans, and that this is largely a non-biological process. At station 2 it is estimated (see Table 3.6) that on average 14% of the copper is present as the Cu^{2+} ion.

Throughout most of the experiment the Aylesbeare stream fed larvae were found to contain significantly more copper than the unfed larvae from this population, and no doubt this is partly because in the population A (F) larvae the supply of Chironomus riparius in the experiment provided an additional source of copper. Indeed from the difference between the amounts of copper in the two treatments it is calculated that approximately 30% of the copper in the fed larvae could have been derived from the food.

After 7 days the mean amounts of copper in the fed and unfed Aylesbeare stream larvae were 0.35 and 0.23 μg respectively, i.e. the F treatment larvae contained 1.5 times as much copper as the U treatment larvae. By comparison, non-tolerant individuals of A. meridianus fed on copper-enriched elm leaves over an 8 day period accumulated over 4 times as much copper as individuals just kept in a copper solution (Brown, 1977b). The more pronounced accumulation in A. meridianus is probably the result of the much higher copper concentration of the food provided for these individuals, since the initial copper concentration of the elm leaves was 2600 μg g^{-1} compared to a concentration of 54 μg g^{-1} recorded for the laboratory culture of C. riparius fed to the P. conspersa larvae.

No significant difference was found between the amounts of copper in the two treatments of the Darley Brook larvae. The copper concentration of the C. riparius larvae from the laboratory culture (54 μg g^{-1}) is less than that measured in chironomid larvae taken from station 1 (407 μg g^{-1} in January and 1054 μg g^{-1} in July 1986). Thus

the F treatment larvae, starting from a relatively high body copper concentration, are being provided with a less copper rich food than that which they had previously been feeding on in the Darley Brook, and so they are unlikely to accumulate significantly more copper than the U treatment larvae.

8.3. Acute Toxicity Test

8.3.1. Materials and Methods

A total of 96 instar V larvae were collected from station 1 in the Darley Brook on 13.9.86, and placed in separate cells in compartmentalised trays filled with stream water from this station. These circular perspex compartmentalised trays, which had been used in routine sampling (see Plate 6.2), also proved to be ideal for the storage of live larvae and so they were employed in the toxicity experiment. Thus on return to the laboratory the trays were attached to an extended platform on a mechanical shaker (see Plate 8.3), with the r.p.m. at a low setting to provide a gentle water movement within each cell and also to facilitate aeration. The larvae were acclimated for 48 hours to conditions similar to those experienced in the field, namely a constant water temperature of 10°C and a photoperiod of 12 hours light and 12 hours dark.

On 15.9.86 unfiltered non-acidified water was collected from station 1 in 1000 ml polythene bottles. Previous analysis of water taken from this station (see Table 3.3) showed it to have a mean copper concentration of 0.86 mg l^{-1}, to be 'soft' in nature (mean hardness 8.04 mg l^{-1}) and acidic (mean pH 5.5).

A stock solution of 5000 mg l^{-1} copper was prepared by dissolving 19.0116 g of Analar grade copper nitrate in 1000 ml of station 1 stream water. This stock solution was then diluted with the stream water to give the following geometric progression of copper concentrations: 5000, 4000, 3200, 2500 and 2000 mg l^{-1} copper. The pH of each of these dilutions and of the control (i.e. stream water alone) was adjusted to pH 5.0 using a buffer prepared by adding 0.01 M acetic acid to 0.01 M sodium acetate.

PLATE 8.3. Experimental set-up used for the acute toxicity test, showing compartmentalised trays mounted on a mechanical shaker.

30 ml of one of the copper dilutions was placed into one cell in a compartmentalised tray, and this was repeated in such a way as to use 16 cells for each dilution. This procedure was also carried out for the control stream water. The 48 hour acclimated larvae were then transferred into these dilutions, and were examined and the number of deaths recorded after 3,8,16,24,48,72 and 96 hours.

All the dilutions were renewed after 48 hours to reduce loss of metal by precipitation, adsorption and uptake. The actual copper concentrations in the test dilutions were determined at the beginning and end of each 48 hour period by removing 10 ml of solution and adding Spectrosol grade nitric acid until the pH was 1.0. Subsequent analysis was carried out by flame AAS. Throughout the toxicity test the pH was kept at 5.0 ± 0.3 by adding buffer to each dilution when necessary.

8.3.2. Results

Over the first 48 hours of the toxicity test the majority of larvae (even those in the highest concentration) constructed nets for the capture of food. However in the latter half of the test period most larvae ceased net-spinning activity (including those in the control stream water).

Larvae were considered dead when they failed to respond to gentle prodding by a small glass rod. The number of larval deaths occurring over the 96 hour test period, together with the concentrations of copper measured by flame AAS, are shown in Table 8.2. None of the measured copper concentrations in the experimental dilutions varied by more than 10% of their calculated concentration. After 96 hours the total number of larval mortalities were 3,6,9,10 and 13 in the 2000, 2500, 3200, 4000 and 5000 mg l^{-1} copper dilutions respectively. There were no larval deaths in the control stream water.

The 96 hour LC_{50} value and its 95% confidence limits were calculated using the method of Litchfield and Wilcoxon (1949). A graph was constructed of the percentage larval mortality (probit scale) against copper concentration (log scale), and this is presented in Figure 8.3. A line was then drawn by eye through the points and a $(Chi)^2$ test showed this line to be a good fit ($\chi^2 = 7.68$, 3 degrees of freedom). From this graph the 96 hour LC_{50} was estimated to be 3200 mg l^{-1} copper. The upper confidence limit was calculated to be 3744 mg l^{-1} and the lower limit 2735 mg l^{-1} copper.

TABLE 8.2. Number of larval mortalities of P. conspersa occurring over a 96 hour period in 5 different copper dilutions, together with the actual concentrations of copper measured by flame AAS. Note that no mortalities occurred in the control during the 96 hour period.

CALCULATED COPPER CONCENTRATION (mg l^{-1})	2000		2500		3200		4000		5000	
	Number of Mortalities	Measured Copper Concentration (mg l^{-1})	Number of Mortalities	Measured Copper Concentration (mg l^{-1})	Number of Mortalities	Measured Copper Concentration (mg l^{-1})	Number of Mortalities	Measured Copper Concentration (mg l^{-1})	Number of Mortalities	Measured Copper Concentration (mg l^{-1})
TIME (HOURS)										
0	0	1812	0	2308	0	3016	0	3930	0	4960
3	0		0		0		0		0	
8	0		0		0		0		0	
16	0		0		0		0		0	
24	0		0		0		0		0	
48	2	1964	3	2378	1	3118	1	3888	1	5245
RENEWAL		2028		2456		3208		3900		5250
72	0		3		5		4		5	
96	1	2012	0	2546	3	3230	5	3985	7	5315
TOTAL NUMBER OF MORTALITIES	3		6		9		10		13	

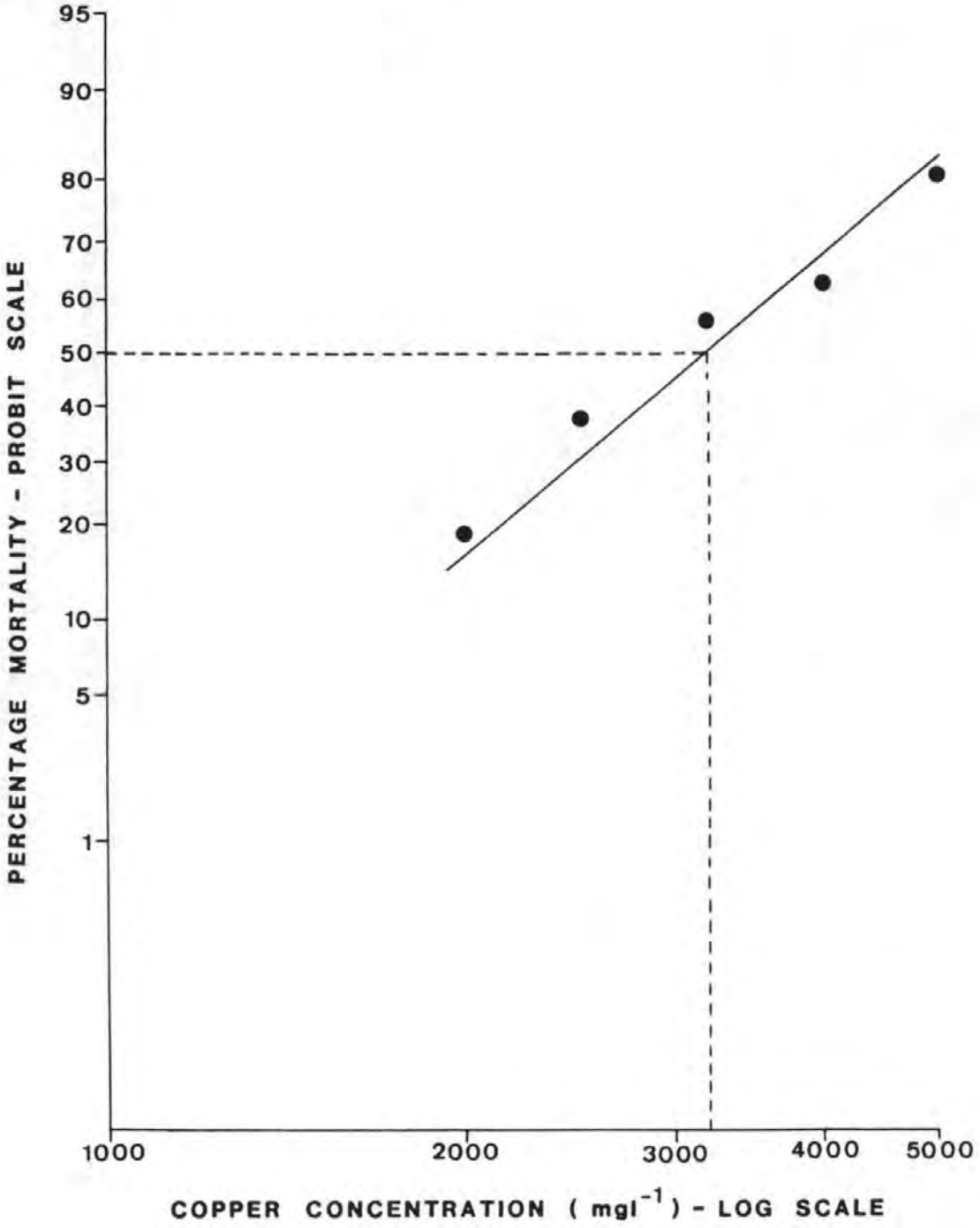


FIGURE 8.3. Relationship between the concentration (mg l^{-1}) of copper in test dilutions and the percentage mortality in instar V larvae of P. conspersa. Note that the 96 hour LC_{50} = 3200 mg l^{-1} copper.

8.3.3. Discussion

The toxicity test carried out in this investigation meets the two criteria outlined by Ward and Parrish (1982) to enable an LC_{50} to be calculated with reasonable accuracy:

(1) Except for the control, the concentration of test material in each treatment should be at least 50% of the next higher concentration.

(2) One treatment other than the control should have killed less than 35% of the organisms exposed to it (2000 mg l^{-1} in this test) and one treatment should have killed more than 65% of the organisms (5000 mg l^{-1} in this test).

Thus the calculated 96 hour LC_{50} value of 3200 mg l^{-1} should be an accurate estimate. It is recommended that the 95% confidence limits about an LC_{50} value should be less than $\pm$ 30% of the mean (American Public Health Association, 1980). The confidence limits determined in this study are 3744 and 2735 mg l^{-1}, the difference between these values and the LC_{50} concentration being only 17% and 15% respectively of the mean.

Table 8.3 summarises a number of studies that have been carried out on the toxicity of copper to freshwater macroinvertebrates. Reference to this table shows that the larvae of the trichopteran Hydropsyche betteni were able to live beyond 96 hours at a copper concentration of 64 mg l^{-1} (Warnick & Bell, 1969). After this, the highest lethal copper concentration in these studies was the 14 day LC_{50} of 13.9 mg l^{-1} recorded by Nehring (1976) for the nymphs of the plecopteran Pteronarcys californica. Clearly the 96 hour LC_{50} concentration of 3200 mg l^{-1} recorded in the present study for instar V larvae of P. conspersa is several orders of magnitude greater than any value previously reported for freshwater macroinvertebrates.

TABLE 8.3. Selected studies on the lethal concentration (mg l^{-1}) of copper to freshwater macroinvertebrates. MPS = median period of survival, TL_m = median tolerance limit, LC_{50} = median lethal concentration.

SPECIES (STAGE)	pH	HARDNESS (mg l^{-1} $CaCO_3$)	RESPONSE CRITERIA	COPPER CONCENTRATION (mg l^{-1})	REFERENCE
Nais sp.	7	320	70 min MPS	1.00	Learner & Edwards, 1963
	7	18	35 min MPS	1.00	
Ephemerella subvaria (nymph)	7.25	44	48 hr TL_m	0.32	Warnick & Bell, 1969
Acroneuria lycorias (nymph)	7.25	44	96 hr TL_m	8.30	
Hydropsyche betteni (larva)	7.25	44	Lived beyond 96 hrs at 64 mg l^{-1}		
Gammarus pseudolimnaeus (adult)	7.7	45	96 hr TL_m	0.020	Arthur & Leonard, 1970
Physa integra (adult)	7.7	45	96 hr TL_m	0.039	
Campeloma decisum (adult)	7.7	45	96 hr TL_m	1.70	
Gammarus sp.	7.6	50	24-96 hr LC_{50}	1.20 - 0.91	Rehwoldt et al., 1973
Trichoptera sp. (larva)	7.6	50	"	12.1 - 6.20	
Zygoptera sp. (larva)	7.6	50	"	10.2 - 4.60	
Chironomus sp. (larva)	7.6	50	"	0.65 - 0.03	
Nais sp.	7.6	50	"	2.30 - 0.90	
Asellus meridianus	—	25	48 hr LC_{50}	1.20 2.50	Brown, 1976
Ephemerella grandis (nymph)	7-7.2	30-70	14 day LC_{50}	0.18 - 0.20	Nehring, 1976
Pteronarcys californica (nymph)	7-7.2	30-70	14 day LC_{50}	10.1 - 13.9	
Corbicula manilensis (adult)	7.6	17	96 hr LC_{50}	>2.60	Harrison et al., 1984
Chironomus tentans (larva)	7.4	71-84	96 hr LC_{50}	0.30 - 1.69	Nebeker et al., 1984a
Clistoronia magnifica (larva)	7.2-7.4	26	60% survived to pupation at 0.098 mg l^{-1}		Nebeker et al., 1984b
Potamopyrgus jenkinsi	8.0	298	96 hr LC_{50}	0.054 - 0.079	Watton & Hawkes, 1984

An LC_{50} concentration is specific to the conditions under which the toxicity test was carried out. Copper is known to be more toxic in soft waters, and the mean hardness value of 8.04 mg l^{-1} recorded at station 1 in the Darley Brook is lower than all the hardness values shown in Table 8.3. However the water at station 1 is acidic (mean pH 5.5) and it has been shown that the toxicity of copper decreases as pH decreases due to competition between H^{+} and Cu^{2+} ions (Campbell & Stokes, 1985). Although in the present study the acute toxicity test was carried out at a relatively low pH, this could have relevance in the event of further increases in copper concentration resulting from renewed mining activity, as commented on by South West Water (1984).

Different stages in the life cycle of an animal may have differing sensitivities to copper; in both the clam Corbicula manilensis (Harrison et al., 1984) and in the chironomid Chironomus tentans (Nebeker et al., 1984a) the copper sensitivity of larvae decreased in successive developmental stages. Thus in any future toxicity work it would be informative to study not only the effects of changing the water hardness and pH, but also the larval instar exposed to copper.

CHAPTER 9

LOCATION OF COPPER IN LARVAE OF PLECTROCNEMIA CONSPERSA

9.1. Introduction

In the present study it has been shown that larvae of Plectrocnemia conspersa living in the headwaters of the Darley Brook are exposed to elevated copper concentrations both in the water column and in their potential prey. Since the amount of copper required by these larvae for normal metabolic processes is comparatively small, it is important to understand how the larvae deal with excess copper.

Although a number of metal tolerance mechanisms have been demonstrated in marine invertebrates (some of which are reviewed by Bryan, 1976), relatively little work has been carried out on metal tolerance in freshwater invertebrates. Carter and Nicholas (1978) fractionated larvae of the blackfly Simulium ornatipes and showed that zinc was bound to both the 'cuticle' and 'high molecular weight' fractions. In a study on the isopod Asellus aquaticus and the leech Erpobdella octoculata (Eyres & Pugh-Thomas, 1978) it was suggested that in both species there was either a physical or chemical barrier to the uptake of lead, together with active excretion of this metal. In another study Fish and Morel (1983) showed that the cladoceran Daphnia magna can excrete moderately strong metal-binding organic compounds.

The elevated copper concentrations recorded during the present investigation in the larvae of P. conspersa living in the uppermost reaches of the Darley Brook suggest that some copper is immobilised and stored in a non-toxic form within the larvae. It is known that metals may be stored in granules in invertebrate tissues, and there are records of metal containing granules in all the major invertebrate phyla (Brown, 1982). The function of these granules is often uncertain,

although in some species they may represent a mechanism for taking potentially toxic concentrations of a metal out of circulation.

However the occurrence of metal containing granules in freshwater invertebrates has received little attention. Brown (1977b) recorded copper containing granules in the hepatopancreas of Asellus meridianus, and copper granules were also observed in the freshwater decapod Austropotamobius pallipes (Simkiss, 1979). Concretions of mainly calcium and phosphate were found in the mantle tissue of the bivalve Anodonata cygnaea (Simkiss, 1979), and calcium spherites were recorded in the sinusoidal and connective tissue spaces of the mantle of the bivalve Amblema plicata perplicata (Petit et al., 1980).

In insects, metal containing granules have generally been more fully documented in terrestrial species. There are for example records of granules (sometimes called 'concretions') in Diptera, such as Drosophila (Filshie et al., 1971; Wessing & Eichelberg, 1975; Tapp & Hockaday, 1977) and Musca domestica (Sohal et al., 1976; 1977), and in the collembolan Tomocerus minor (Humbert, 1978; 1979). One of the few records of metal containing granules in aquatic insects is the observation by Ballan-Dufrancais et al. (1971) of mineral concretions in the midgut cells of 12 insect species, including the anisopteran Gomphus sp. and the chironomid Chironomus plumosus.

To the author's knowledge there is only one report in the literature on metal containing granules in Trichoptera. Mineral concretions containing a number of metals (calcium, magnesium, manganese, zinc and iron) have been observed in the malpighian tubules, midgut and adipose tissue of the larvae of Phryganea varia (Lhonore, 1973). It is also interesting to note that while the alimentary canal of an adult caddis (Limnephilus sp.) has been described (Mortimer, 1965), there are no detailed accounts on the gross structure and histology of the larval

digestive and excretory systems, apart from Lhonore (1973) who described the mesenteron of P. varia.

Using histochemical, electron microscopy and X-ray microanalytical techniques, the aim of the present study was to determine precisely where copper is stored in the larvae of P. conspersa collected from the headwaters of the Darley Brook. Furthermore it would be informative to determine how this copper is stored and what is its ultimate fate.

9.2. Light microscopy and histochemical analysis

9.2.1. Materials and Methods

Instar III larvae of P. conspersa were collected from station 1 in the Darley Brook and from the Aylesbeare stream, and were transported back to the laboratory in compartmentalised trays filled with water taken from the site of collection. This particular instar was selected for this work (and for transmission electron microscopy) mainly because its small size allows the penetration of fixative and other chemicals into whole larvae. In the laboratory these larvae were fixed in 70% ethanol for 2 hours at 4°C, and were then sectioned and stained by the following techniques:

Technique A Masson's Trichrome Stain

Prior to being stained the larvae were dehydrated in ethanol (60 minutes in 90% ethanol, 180 minutes in 100% ethanol), and then immersed in xylene for two periods each of 15 minutes. The larvae were subsequently embedded in wax, and 7 µm thick transverse sections were cut using a Reichart-Jung 1130 Rotary Microtome (Cambridge Instruments Limited, Cambridge). These sections were mounted on egg-albuminised slides.

The following staining technique is based on the procedure outlined by Drury and Wallington (1980), which was modified for use in this investigation:

1. Take sections to water.
2. Stain in Mayer's haematoxylin for 15 minutes.
3. Rinse in tap water for 5 seconds.
4. Place in acid alcohol for 3 seconds.
5. Place in lithium carbonate solution for 1 minute.
6. Rinse in distilled water (2 x 1 minute rinses).
7. Stain in acid fuchsin for 5 seconds.

8. Rinse in distilled water (3 x 30 seconds rinses).

9. Place in phosphomolybdic acid for 1½ minutes.

10. Rinse in distilled water (2 x 30 seconds rinses).

11. Stain in Mallory's stain for 8 seconds.

12. Rinse in distilled water (2 x 1 minute rinses).

13. Dehydrate in ethanol (1 minute in 70% ethanol, 2 x 2 minutes in 100% ethanol), clear in xylene (2 x 2 minutes) and mount in DPX (BDH Chemicals, Poole).

This staining technique was used in conjunction with dissection to help understand the internal morphology and histology of the larva.

Technique B Rubeanic acid method for copper

This technique is based on the procedure outlined by Drury and Wallington (1980), but unlike their method in which sections are stained, in this study whole larvae were placed in the staining solution prior to sectioning:

1. Make a stock solution of 0.1% rubeanic acid in absolute alcohol. 5 ml of this stock solution is added to 100 ml of 10% aqueous sodium acetate. The pH of this staining solution is adjusted to 7.4 using acetic acid.

2. Immerse larvae in the staining solution for 48 hours at 37°C.

3. Dehydrate through an alcohol series, i.e. 60 minutes in 70% ethanol, 60 minutes in 90% ethanol and 180 minutes in 100% ethanol.

4. Clear in xylene (2 x 15 minutes).

5. Embed in wax, cut 7 µm thick transverse sections which are placed on egg-albuminised slides.

6. Take sections to water.

7. Stain in eosin for 1 or 2 seconds.

8. Rinse in distilled water (2 x 15 seconds).

9. Dehydrate in ethanol (1 minute in 70% ethanol, 2 x 2 minutes in 100% ethanol), clear in xylene (2 x 2 minutes) and mount in DPX.

Rubeanic acid is a copper specific stain, which forms a greenish-black precipitate of copper rubeanate in tissues containing excess copper (normal quantities of copper cannot be stained).

9.2.2. Results

Dissection of a P. conspersa larva (see Figure 9.1) shows that the alimentary canal may be divided into fore-, mid- and hind-gut regions. The foregut consists of an oesophagus which leads into a crop, which is encircled by bands of muscle. Either side of the oesophagus are the ventral ducts of a pair of white salivary glands, which run along the length of the larva to a point approximately halfway along the midgut. At the posterior end of the crop there is a muscular proventriculus.

Next comes the larval midgut which has a thinner wall than the foregut. Six blindly ending malpighian tubules (of approximately equal length) open into the alimentary canal near the junction of the hindgut with the midgut. Two of these are relatively straight and directed anteriorly, nearly reaching the crop. The other four, more tightly coiled, are directed posteriorly and make contact with the hindgut. All the malpighian tubules are reddish brown in colour, with a darker spiral muscle strand running along their length. The hindgut is the shortest section of the digestive tract and ends in a rectum.

Plate 9.1 shows a typical transverse section through the midgut region of an instar III larva of P. conspersa from the Darley Brook. On either side of the midgut a salivary gland has been sectioned, revealing red stained contents. Lying ventrally to each salivary gland is a malpighian tubule of smaller diameter, which is stained purple and contains more darkly stained material around part of its periphery. A band of similar darkly stained material is associated with the integument.

Closer examination of a malpighian tubule of an instar III larva from the Darley Brook (see Plate 9.2) showed that a nucleus and lumen were clearly discernible. The wall of the tubule is just one cell thick.

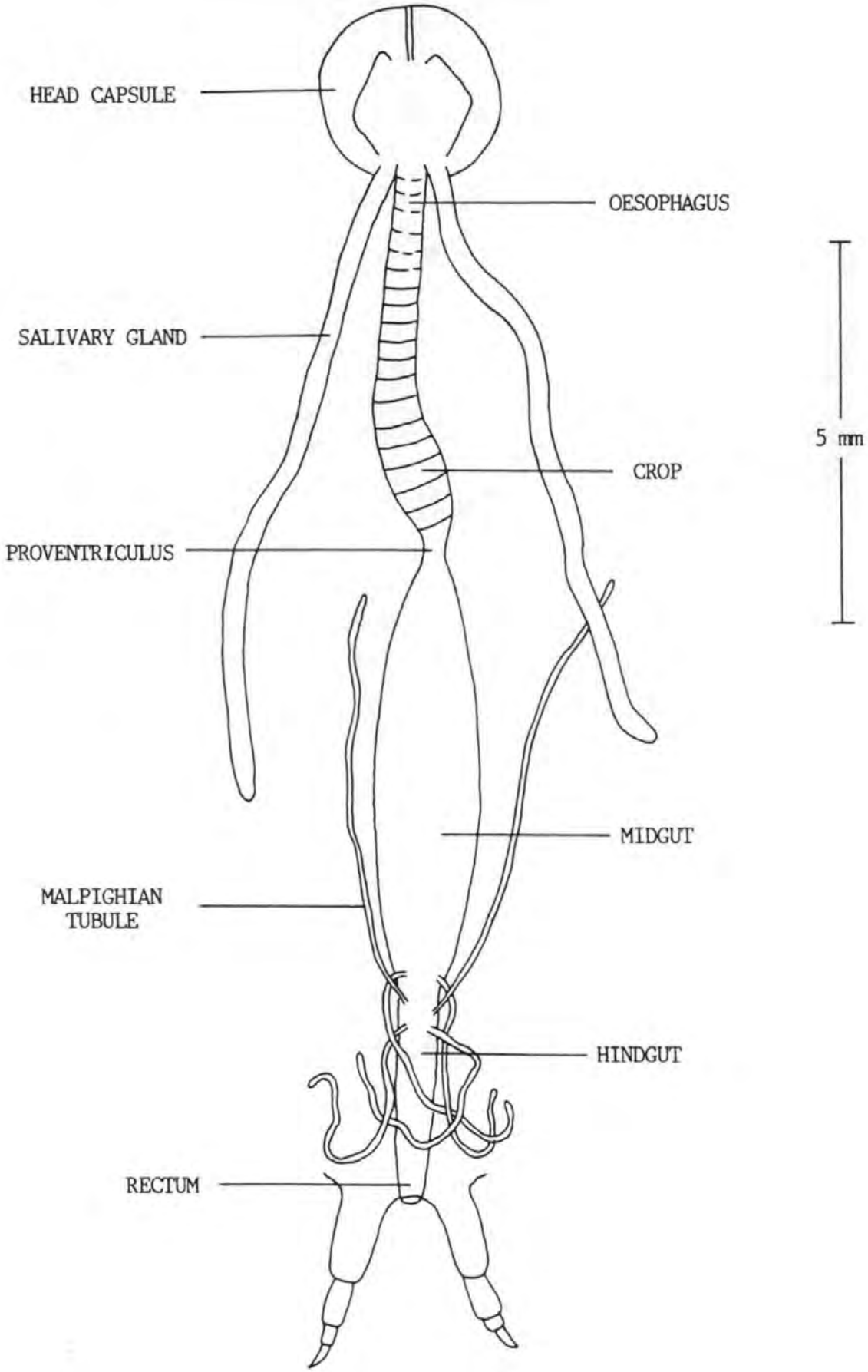


FIGURE 9.1. Dorsal view of the alimentary canal of an instar V larva of P. conspersa.

PLATE 9.1. Transverse section (x50) through the midgut region of an instar III larva of P. conspersa from the Darley Brook stained using Masson's trichrome staining technique. E enteric epithelium, FB fat body, H haemocoel, I integument, MT malpighian tubule, MW midgut wall, SG salivary gland, SL sternal longitudinal muscle, TL tergal longitudinal muscle, TSL tergosternal longitudinal muscle.

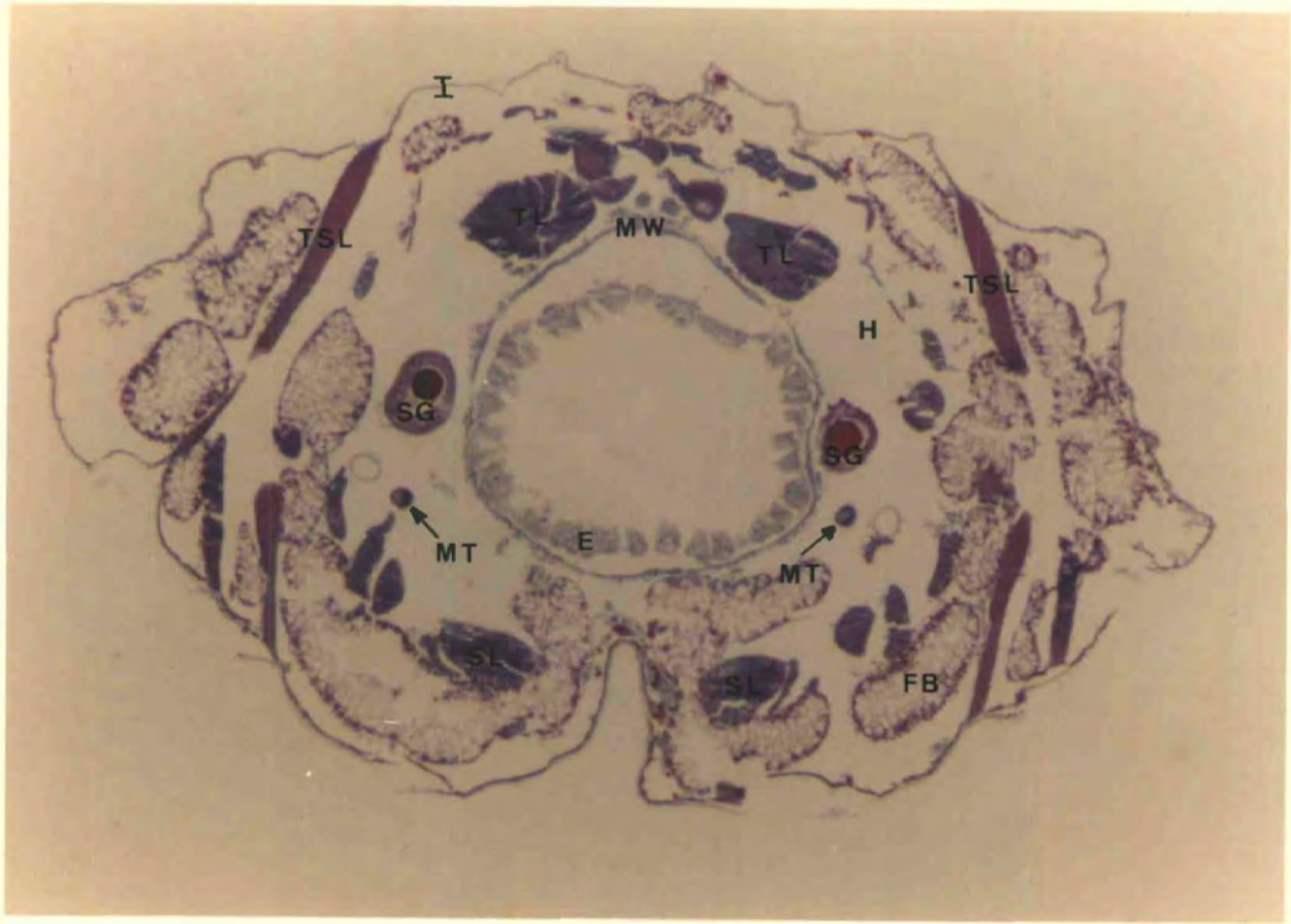
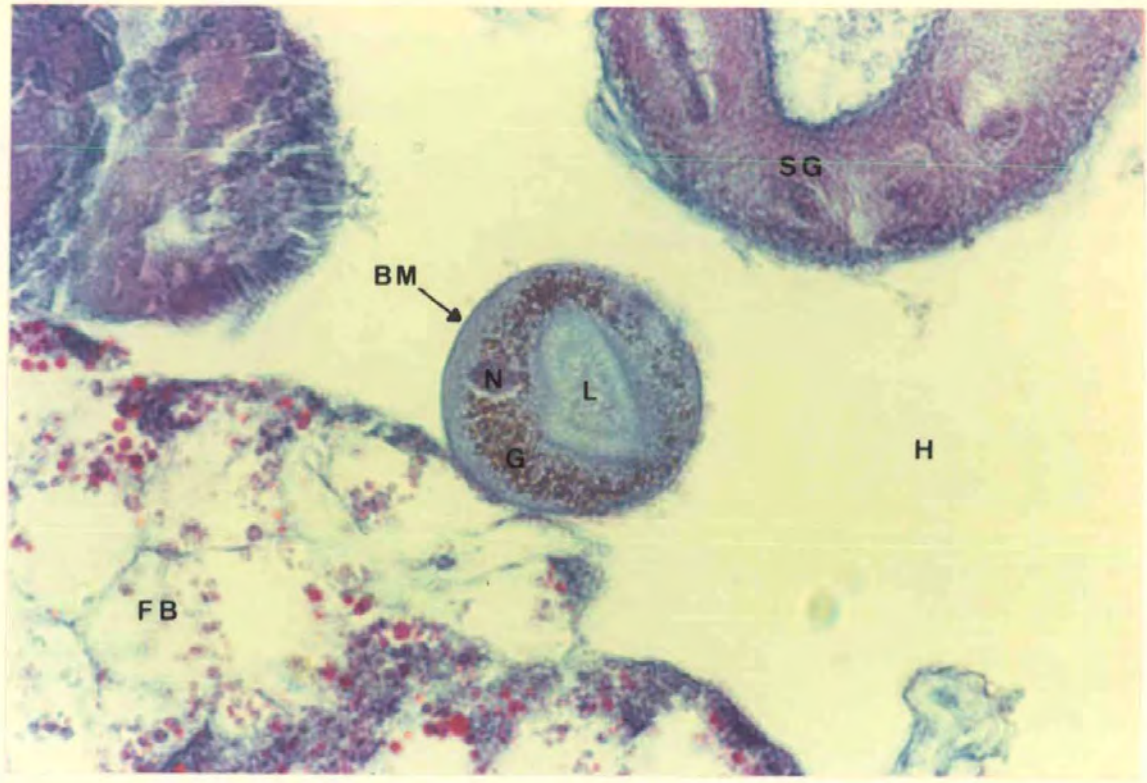
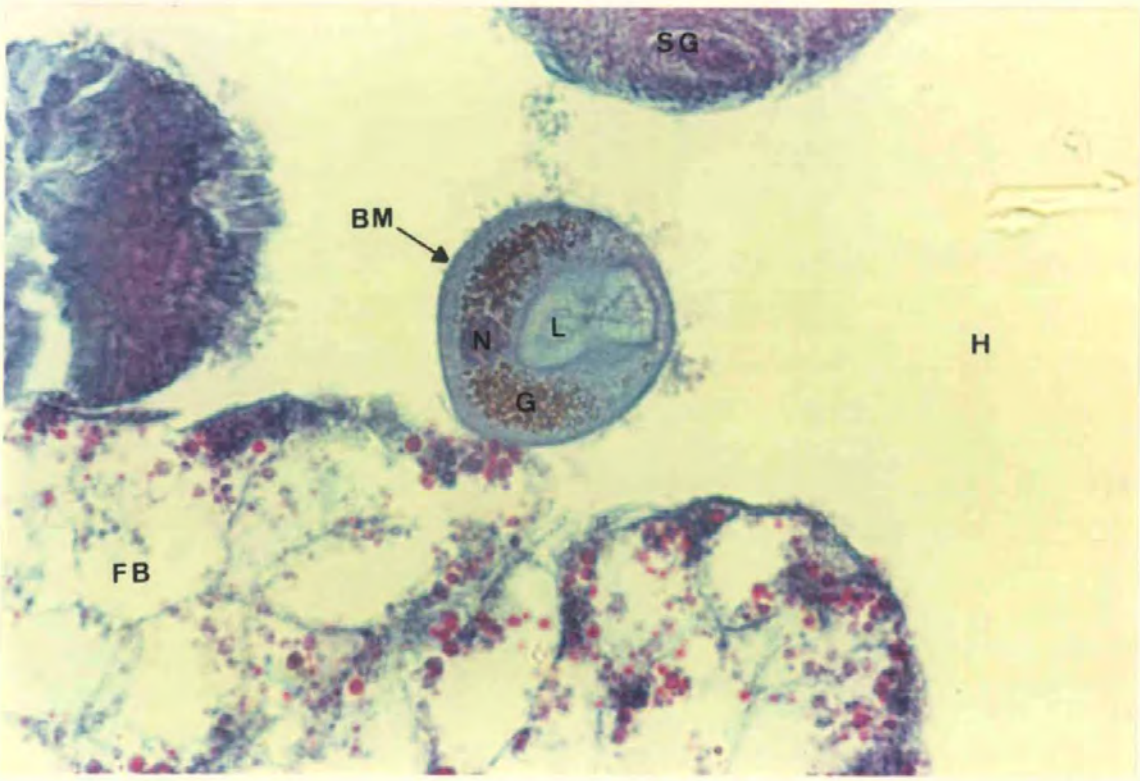

I
TSL
TL
MW
TL
TSL
H
SG
SG
MT
E
MT
SL
SL
FB

PLATE 9.2. Transverse sections (x900) through the malpighian tubule of an instar III larva of P. conspersa from the Darley Brook stained using Masson's trichrome staining technique.
BM basement membrane, FB fat body, G granules, H haemocoel, L lumen of tubule, N nucleus of excretory cell, SG salivary gland.


SG
BM
N
L
G
H
FB


SG
BM
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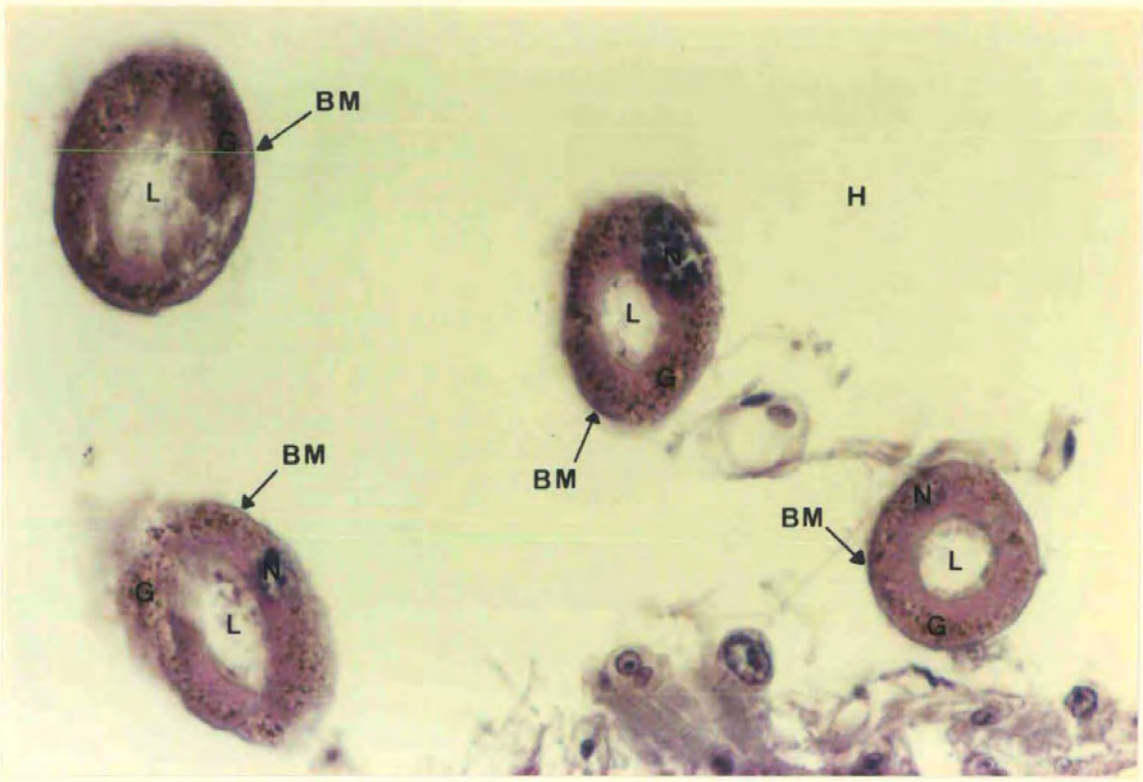
Since no more than one nucleus was seen in a section there must be no more than one cell encircling the lumen. Dense staining subspherical granules were observed towards the side of the cell containing the nucleus, and in some tubules the large numbers of these granules caused distortion of the lumen. There were no granules present in the lumen itself.

The malpighian tubules in instar III larvae from the Aylesbeare stream (see Plate 9.3) also contained granules, which were similar in appearance to those observed in the malpighian tubules of the Darley Brook larvae. However in the former there were significantly fewer granules, and in nearly all the specimens examined the lumen was circular and undistorted.

The band of darkly stained material associated with the integument was, at higher magnification (see Plate 9.4), seen to consist of a layer of subcuticular/epidermal granules. These granules were found in all the sections examined, and are similar in appearance to the granules observed in the cells of the malpighian tubules (i.e. darkly stained and subspherical). Furthermore they were found to be continuous around the entire larva. Although subcuticular granules were also evident in instar III larvae from the Aylesbeare stream (see Plate 9.5), the numbers appeared to be significantly less than in the Darley Brook larvae.

The rubeanic acid method for copper failed to provide a positive result in any of the transverse sections of instar III larvae of P. conspersa taken from either the Darley Brook or the Aylesbeare stream. Pearse (1972) has suggested that unreactive copper in tissues may be released by placing dewaxed sections over a beaker of concentrated HCl for 15 minutes. Thus larvae from the Darley Brook were fixed, embedded in wax and sectioned. These sections were dewaxed, treated

PLATE 9.3. Transverse sections (x800) through the malpighian tubules of an instar III larva of P. conspersa from the Aylesbeare stream stained using Masson's trichrome staining technique. BM basement membrane, G granules, H haemocoel, L lumen of tubule, N nucleus of excretory cell.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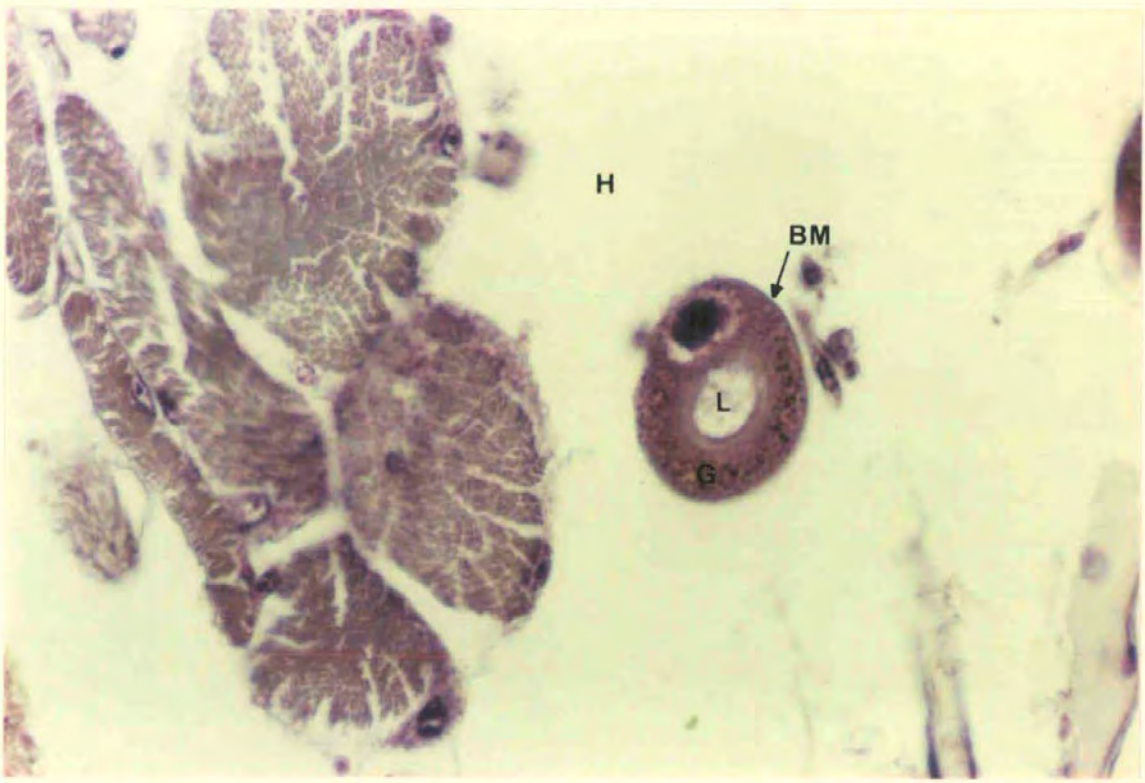
H
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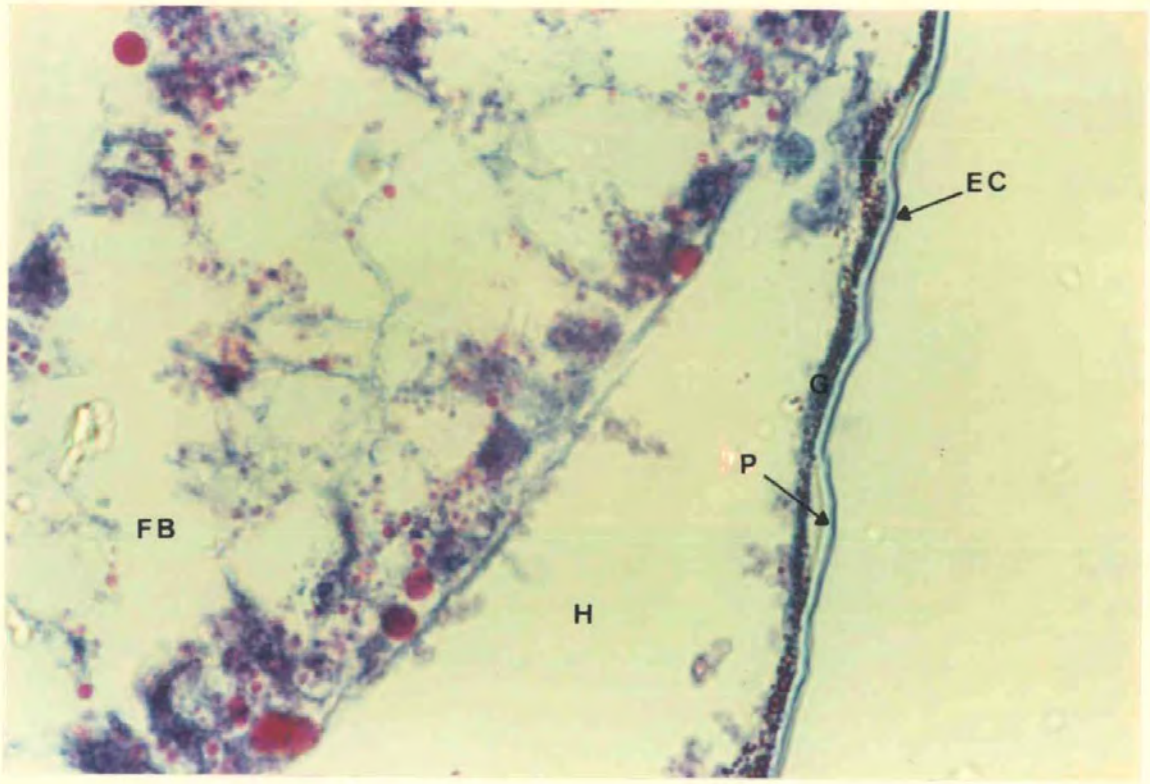
G

PLATE 9.4. Transverse sections (A x900; B x1100) through the integument of an instar III larva of P. conspersa from the Darley Brook stained using Masson's trichrome staining technique. EC epicuticle, FB fat body, G granules, H haemocoel, P procuticle.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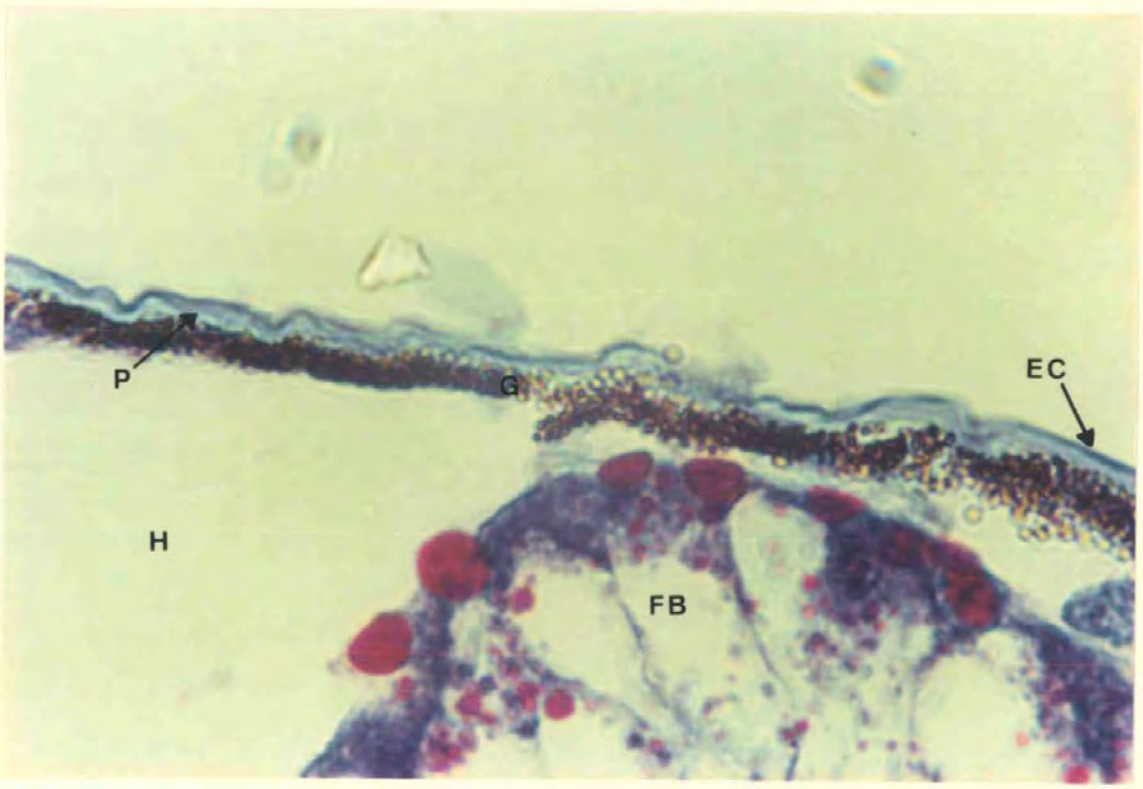
B
P
G
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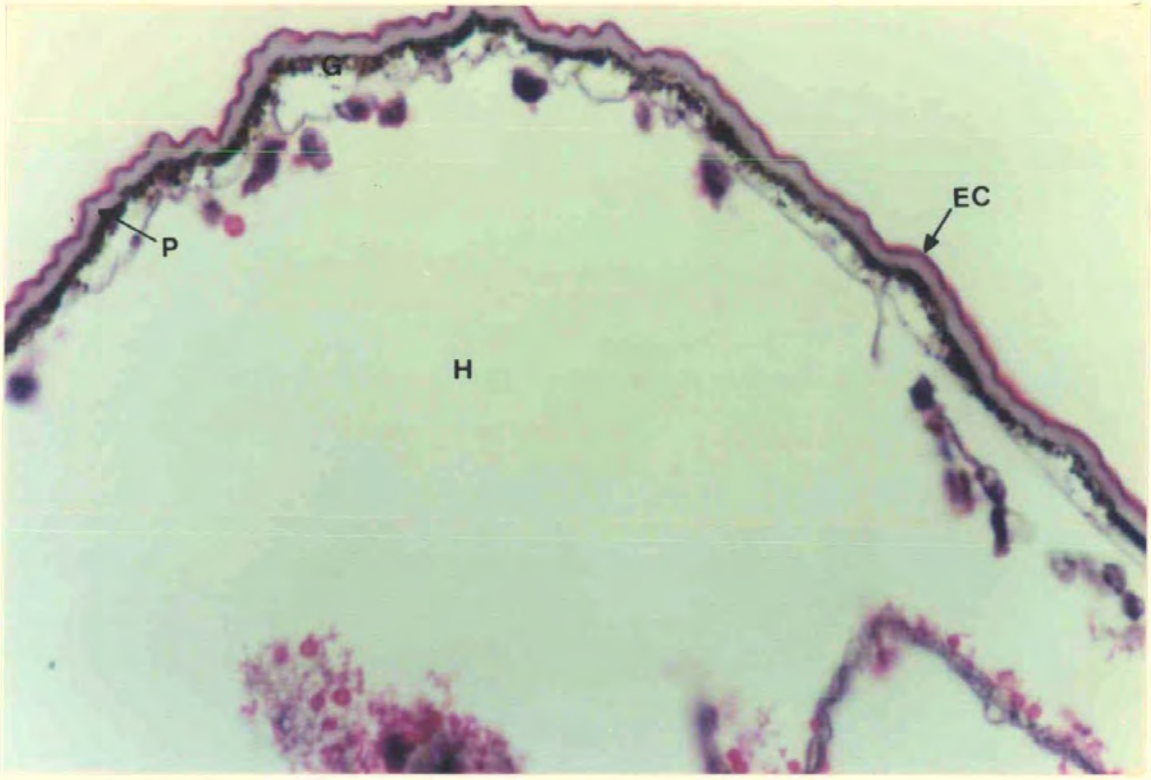
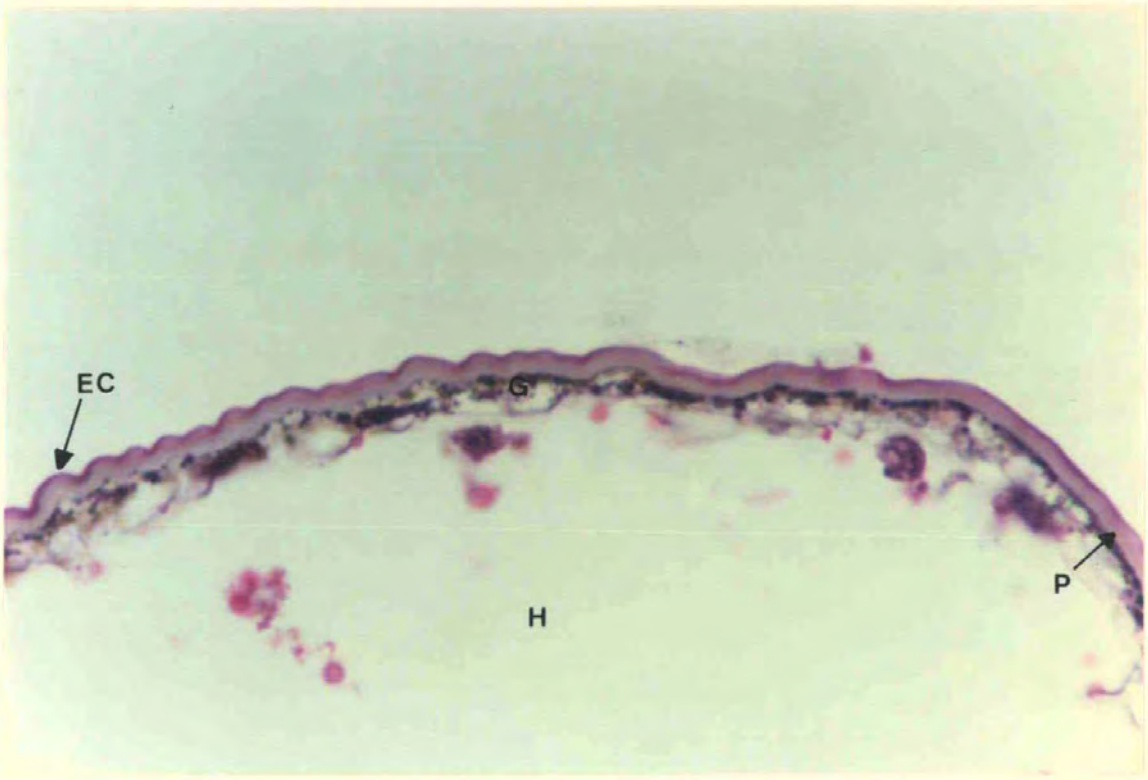
PLATE 9.5. Transverse sections (x700) through the integument of an instar III larva of P. conspersa from the Aylesbeare stream stained using Masson's trichrome staining technique. EC epicuticle, G granules, H haemocoel, P procuticle.


G
P
EC
H


EC
G
P
H

with HCl and then placed in the staining solution for periods of between 1 and 48 hours. However in all of these sections no positive reaction occurred with the rubeanic acid.

9.3. Scanning electron microscopy and X-ray microanalysis

9.3.1. Materials and Methods

When an electron beam hits a solid specimen, X-rays are emitted as a result of electron transitions between the K, L, M and N shells of an atom. Each element possesses a discrete and characteristic spectrum of X-rays. One technique for collecting and counting these X-rays is energy-dispersive analysis. In this technique a small piece of semi-conducting silicon is sandwiched between two gold electrodes. When an X-ray photon strikes the silicon crystal a pulse of current flows between the electrodes. This pulse is amplified and passed on to a multichannel analyser. In this way a complete X-ray spectrum is built up, with energy on the horizontal axis and number of photons counted on the vertical axis (Goodhew, 1975; Morgan, 1985).

Pupal cases and instar III larvae of P. conspersa were collected at station 1 in the Darley Brook. In the laboratory, fragments of ecdysed larval cuticle were removed from the pupal cases, and this material together with the instar III larvae was frozen and subsequently freeze-dried (600 Pa pressure, -55°C, 24 hours). The specimens were then mounted on 99.9% spectroscopically pure carbon stubs (Agar Aids Limited, Stansted). One advantage of using instar III larvae is that a whole larva could be mounted on a carbon stub. Each carbon stub had already been fixed to a wider diameter aluminium stub to allow it to be placed in the specimen holder. Both the aluminium stub and the specimen holder were coated in graphite paint to reduce interference during X-ray microanalysis, since the specimen holder is made of brass (i.e. copper and zinc).

Non-conducting specimens must be coated before analysis; however the coating will absorb X-rays as they are emitted from the specimen, and the coating also emits its own characteristic X-rays. Therefore the

specimens were coated in carbon (Edwards E306A vacuum coating system; Severn Science Limited, Bristol) rather than gold which is usually used in scanning electron microscopy.

Some of the electrons that hit a solid specimen will be scattered around the specimen chamber. This could result in the emission of X-rays from structures within the chamber, leading to an erroneous spectrum. To reduce this effect, a piece of graphite-coated paper was placed around the stub (after carbon-coating) to absorb electrons scattered from the specimen.

The mounted, carbon-coated specimens were placed inside a JEOL JSM35-C Scanning Electron Microscope (JEOL (UK) Limited, London), fitted with an X-ray spectrophotometer (Link Analytical Limited, High Wycombe). Typical operating conditions are shown in Table 9.1. An accelerating voltage of 24 KeV was selected because the most efficient production of X-rays generally occurs when the bombarding electrons have about 3 times the X-ray energy (for copper the K_{α} energy is 8.05 KeV). 'Dead time' refers to the period of time when the EDAX system is not recording incoming X-ray photons. In this system the recording time is automatically extended to compensate for this dead time, e.g. in Table 9.1 the total analysis time is 100 seconds (live time) + 15 seconds (dead time) = 115 seconds.

X-ray mapping is a useful means of qualitatively assessing the gross distribution of an element in a specimen. In this technique a window is set up so that only X-rays characteristic of the element of interest are collected and counted. These X-rays are used to produce bright dots on the display screen, with dots appearing only at positions corresponding to the presence of the selected element. Thus the closer the dots are, the greater the concentration and the brighter the image will appear. A photograph is taken of this image, although in this

TABLE 9.1. Typical operating conditions used in scanning electron microscopy with an energy dispersive detection system.

Accelerating voltage	24 KeV
Specimen current	2.5^{-10} A
Count rate	1800 c/s
Live time	100 secs
Average dead time	15%

study it was found to be necessary to take a series of multiple exposures (usually 4 or 5) to build up a satisfactory elemental map.

9.3.2. Results

A typical X-ray spectrum obtained from the analysis of an entire instar III larva of P. conspersa is shown in Figure 9.2. The larva was found to contain phosphorus, sulphur, chlorine, potassium and calcium. In addition, copper was the only heavy metal that was detectable in the larva. The carbon stub upon which the specimen was mounted contained none of the elements determined in the larva.

The gross distribution of copper in an entire instar III larva of P. conspersa is shown by the elemental map presented in Plate 9.6. As a second example an X-ray distribution map for copper of a leg from an instar V larva of P. conspersa is shown in Plate 9.7. It can be seen that the distribution of the dots (which corresponds to the occurrence of copper) is such that the map is almost a direct reproduction of the entire leg. The only part of the leg which is indistinct on the map is the tarsal claw. Although the reason for this is unknown, it may have been due to X-rays from this region being absorbed by the femur. In both maps (Plates 9.6B and 9.7B) copper appears to be distributed evenly throughout the entire body of the larva. However since electrons can pass through the larva, it is not possible to determine if this copper is associated with the cuticle, or whether it occurs in deeper tissues.

Pieces of ecdysed larval cuticle obtained from pupal cases collected from the Darley Brook were found to have a similar elemental composition (see Figure 9.3) to the entire instar III larvae (see Figure 9.2). Thus X-ray peaks for phosphorus, sulphur, chlorine, potassium, calcium and copper were clearly evident. Again none of these elements was detectable in the carbon stub upon which the specimen was mounted. Thus Figure 9.3 shows conclusively that copper is associated with the larval cuticle.

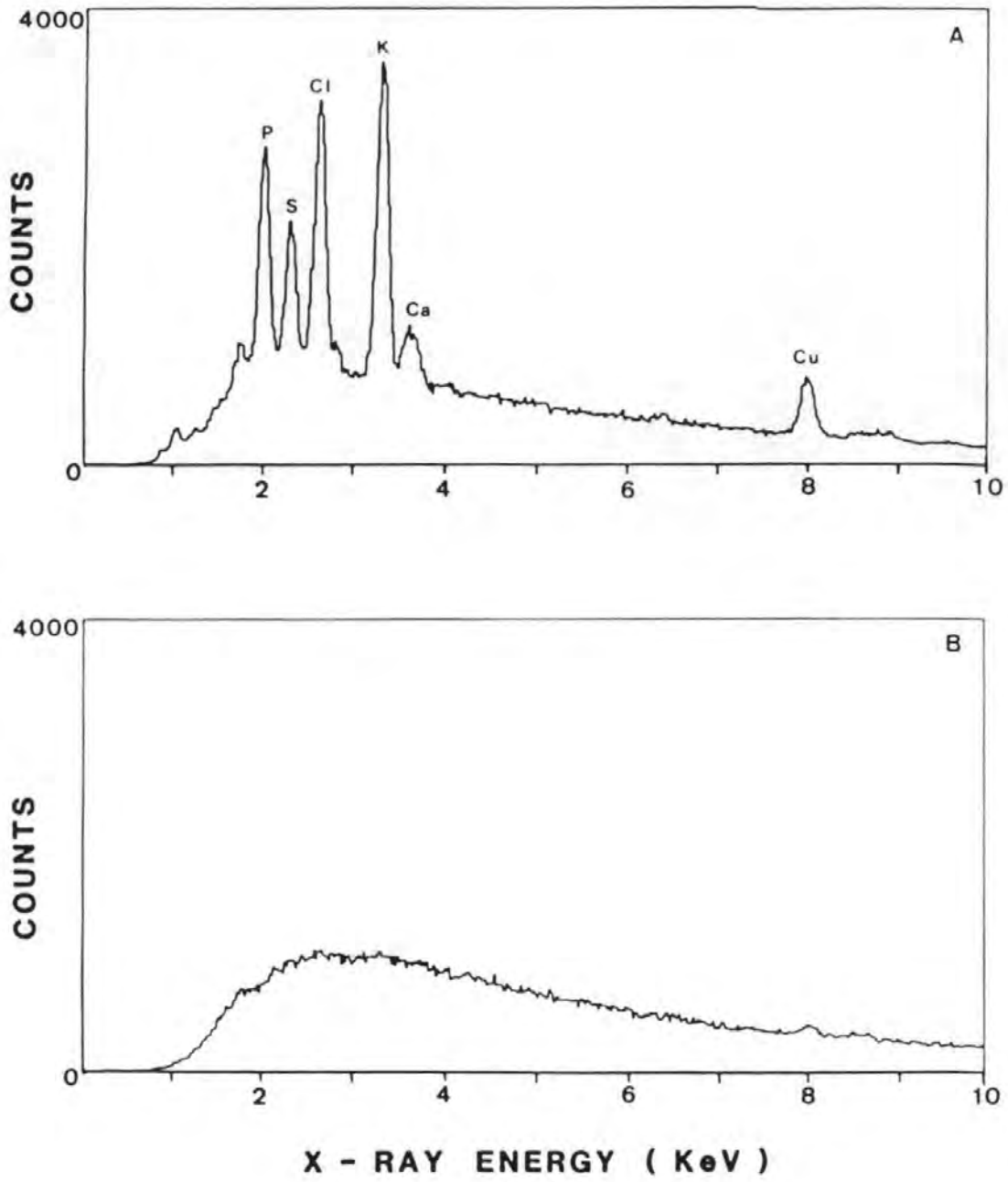
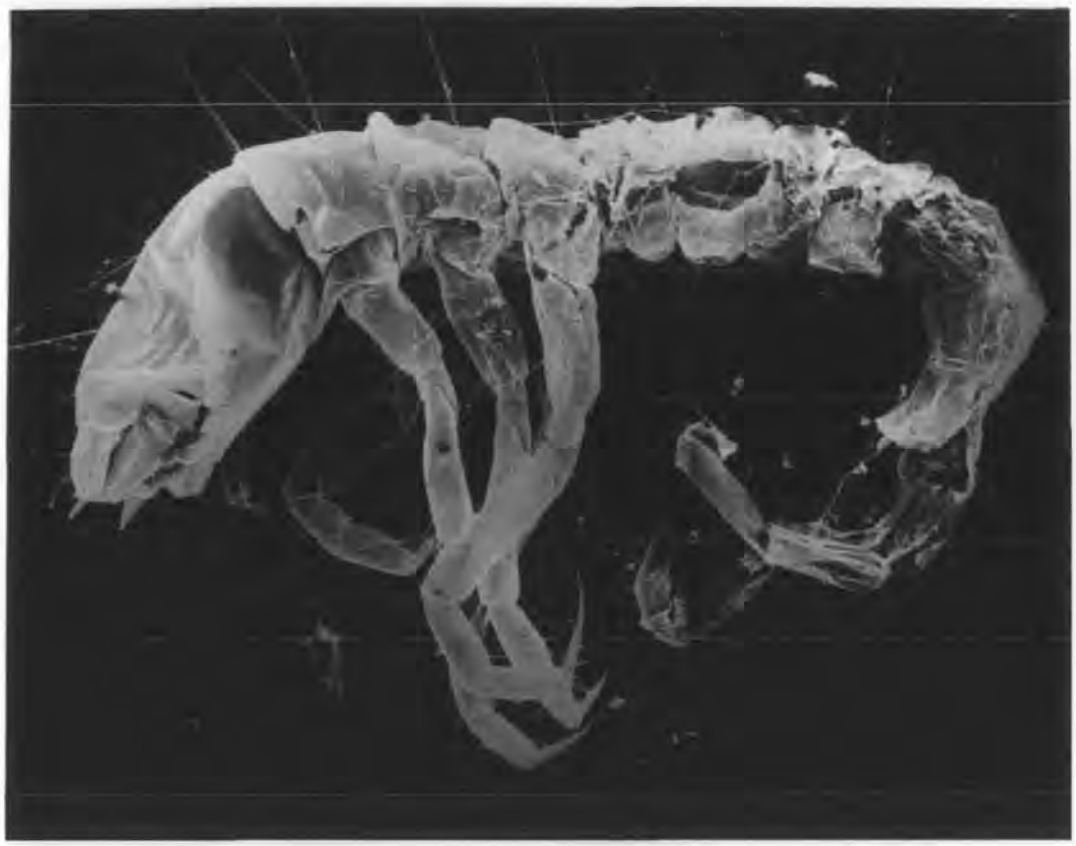
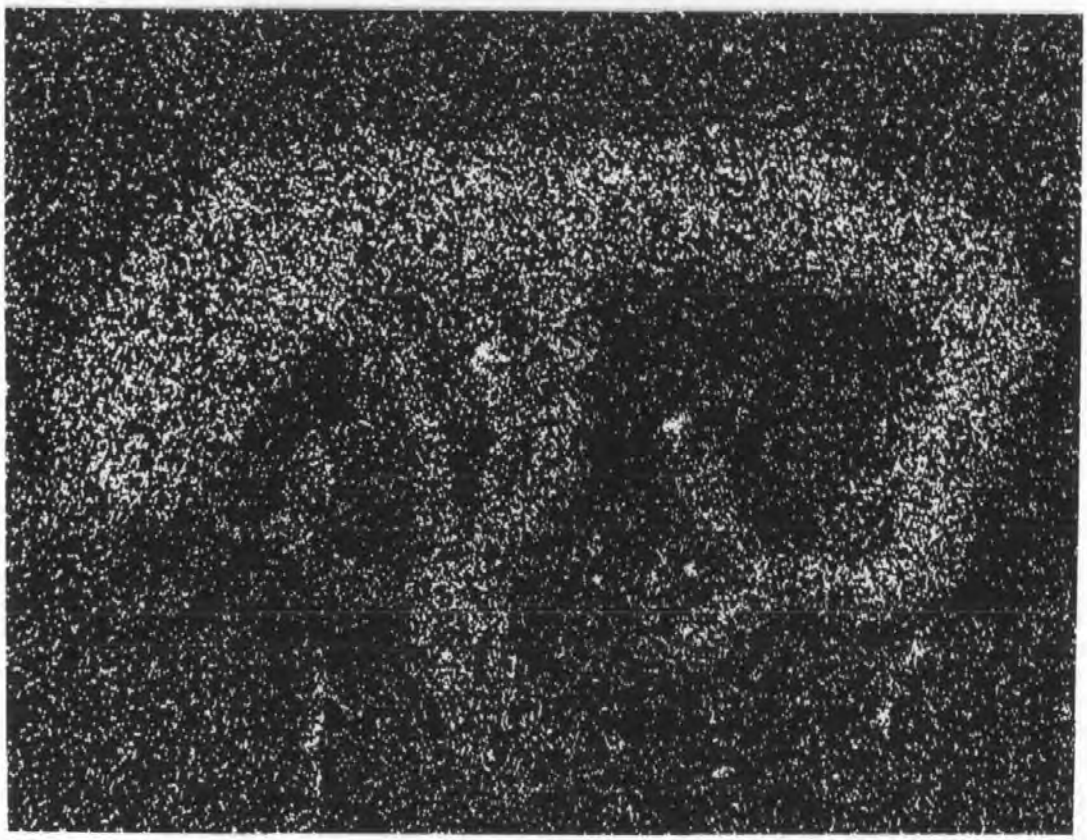


FIGURE 9.2. X-ray spectra of A). an instar III larva of P. conspersa from the Darley Brook and B). the carbon stub upon which the larva was mounted.

PLATE 9.6. A). Micrograph (x150) of an instar III larva of *P. conspersa* from the Darley Brook, together with B). an X-ray distribution map for copper of this larva.

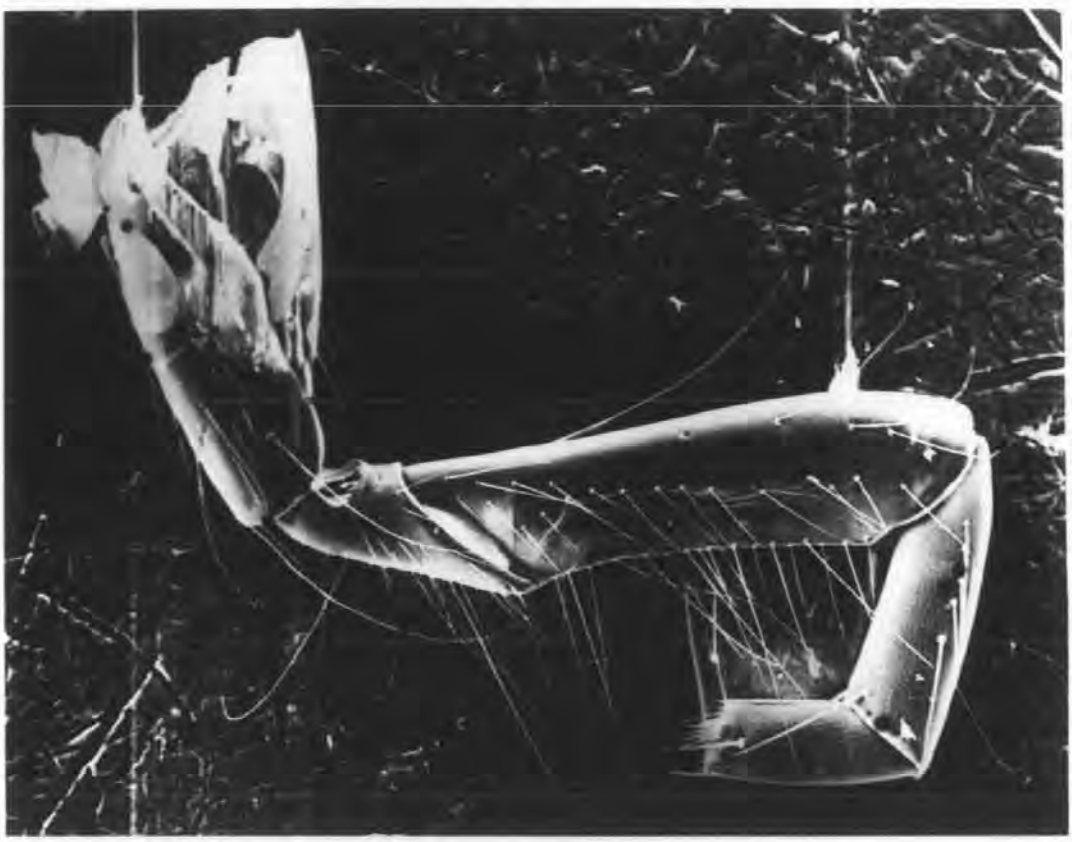


A



B

PLATE 9.7. A). Micrograph (x95) of a leg from an instar V larva of P. conspersa from the Darley Brook, together with B). an X-ray distribution map for copper of this leg.



A

B

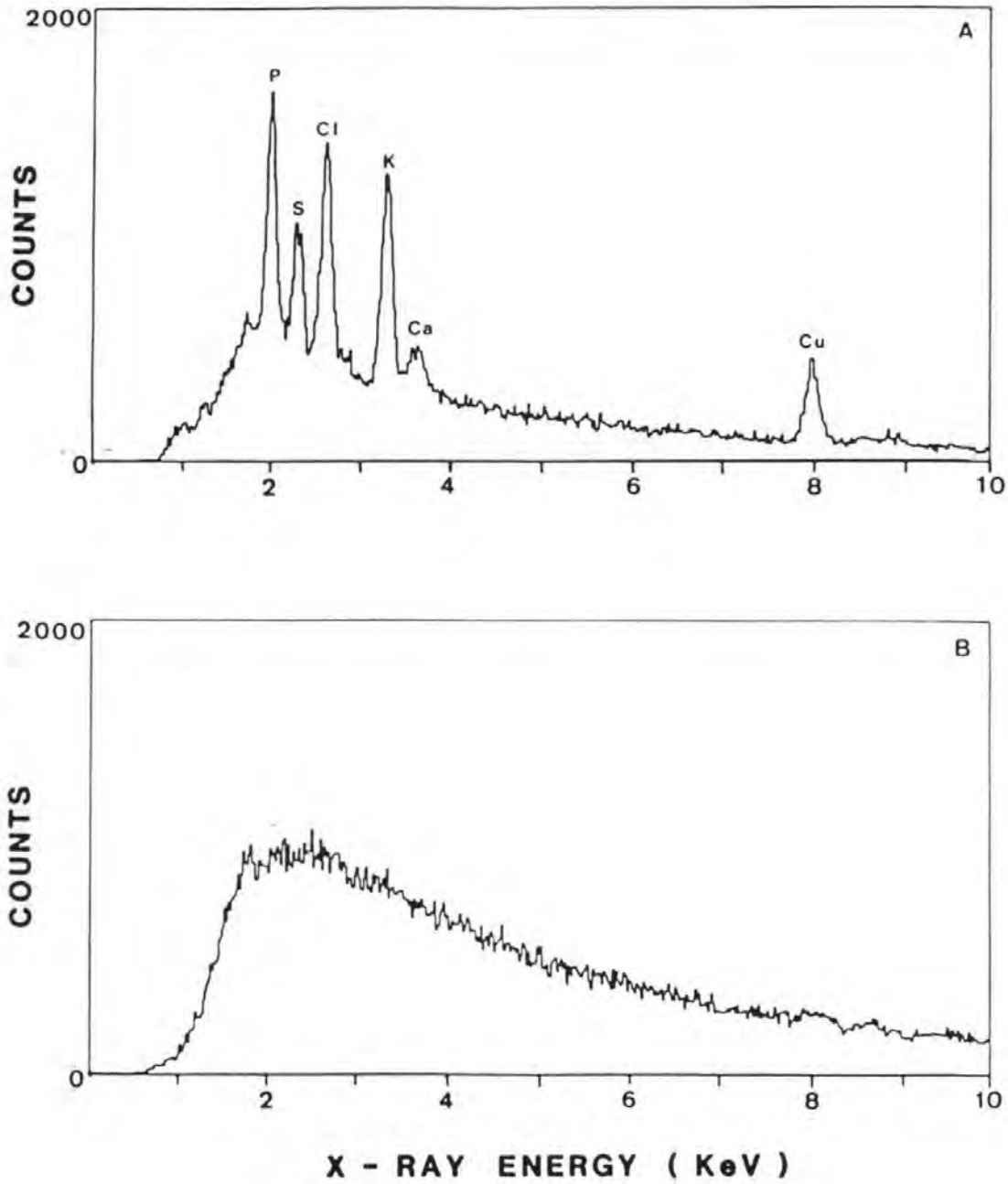


FIGURE 9.3. X-ray spectra of A). pieces of ecdysed larval cuticle obtained from pupal cases of P. conspersa from the Darley Brook and B). the carbon stub upon which the cuticle was mounted.

Although copper was detected in the malpighian tubules of an instar V larva from the Darley Brook, this element was not found in the salivary glands. A typical X-ray spectrum of a malpighian tubule is shown in Figure 9.4, and it was found to contain the same six elements that were present in both the entire larva and the ecdysed larval cuticle (see Figures 9.2 and 9.3 respectively).

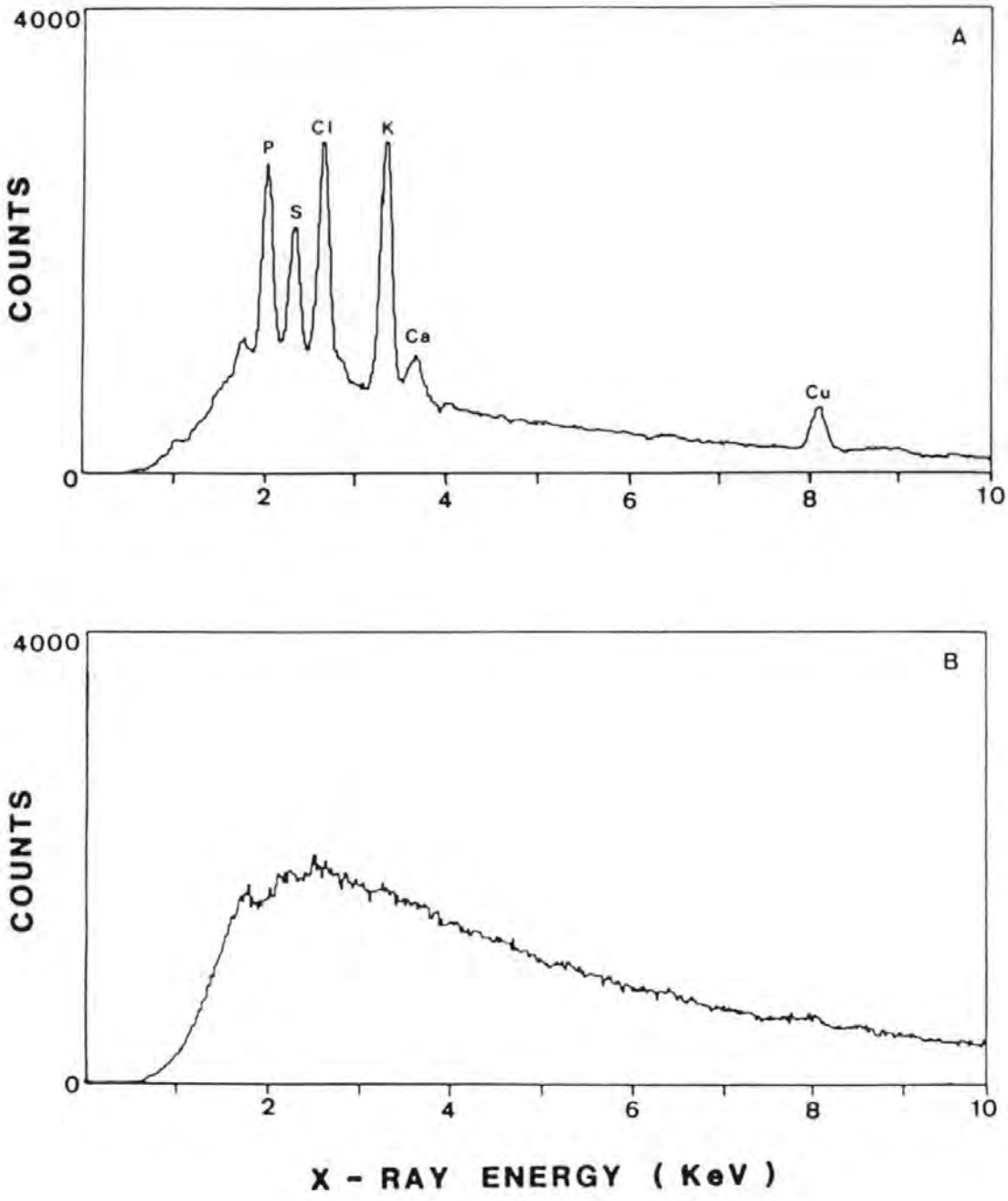


FIGURE 9.4. X-ray spectra of A). a malpighian tubule of an instar V larva of P. conspersa from the Darley Brook and B). the carbon stub upon which the tubule was mounted.

9.4. Transmission electron microscopy and X-ray microanalysis

9.4.1. Materials and Methods

Instar III larvae of P. conspersa collected from station 1 in the Darley Brook were prepared for transmission electron microscopy by the following procedure:

1. Fix in 5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.0 - 7.5) at 4°C for 24 hours.
2. Rinse in cold buffer, 3 changes of 15 minutes.
3. Post fix in 1% osmic acid in cold buffer for 60 minutes; then rinse in cold buffer (3 x 15 minutes). Note that for specimens used in elemental analysis this step is omitted.
4. Dehydrate through an alcohol series: 30% ethanol (10 minutes), 50% ethanol (10 minutes), 70% ethanol (10 minutes), 90% ethanol (15 minutes), 100% ethanol (2 x 20 minutes).
5. Place in propylene oxide, 2 changes of 10 minutes.
6. 1 : 3 Spurr's resin to propylene oxide for 60 minutes.
7. 2 : 3 Spurr's resin to propylene oxide for 3 hours.
8. Place in pure resin mixture overnight.
9. Transfer specimens to capsules filled with pure resin, and cure at 70°C for 8 hours.

The resin blocks were then trimmed using a razor blade to prepare them for ultramicrotomy. Two types of section were cut using a Sorvall Porter-Blum MT2-B Ultramicrotome (Sorvall Inc., Connecticut, USA).

(a) Sections for transmission electron microscopy. These were cut from the larvae that had been post-fixed in osmic acid. 'Gold' sections (approximate thickness 60 to 80 nm) were cut and mounted on copper grids. To increase contrast the sections were stained for 15 minutes in uranyl acetate, rinsed in distilled water, and then stained for 15 minutes in lead citrate. The grids were then rinsed again in distilled

water and dried on a piece of filter paper. These sections were examined using a Philips EM300 Transmission Electron Microscope (Philips (UK) Limited, Cambridge).

(b) Sections for elemental analysis. These were taken from larvae which had not been post-fixed. 'Purple' sections (approximately 200 nm in thickness) were cut and mounted on titanium grids. The grids were then coated in carbon. These specimens were examined using a JEOL 200CX Transmission Electron Microscope, fitted with an X-ray spectrophotometer (Link Analytical Limited, High Wycombe).

Using the above procedures it was possible to examine the ultrastructure of the integument and malpighian tubules of instar III larvae of P. conspersa. Furthermore any structures of interest that were found could be analysed for their elemental composition by X-ray microanalysis (see p.314).

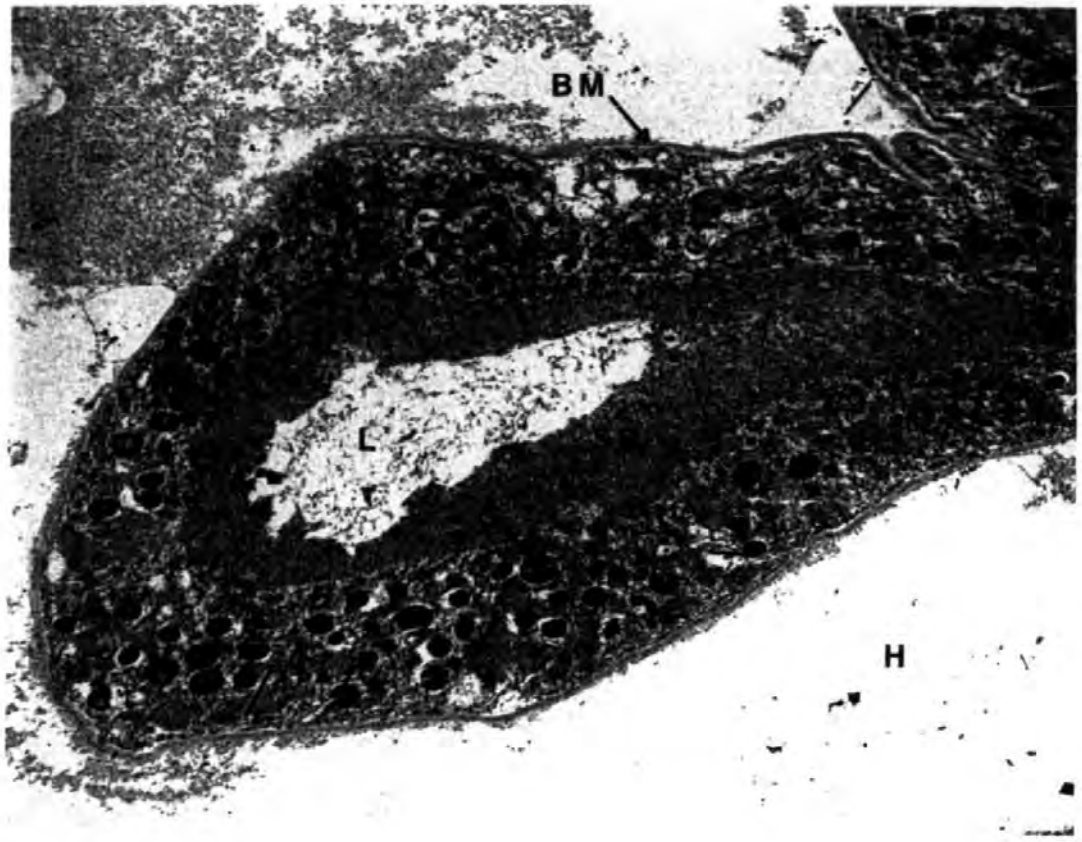
9.4.2. Results

When viewed in transverse section (see Plate 9.8) the malpighian tubule of an instar III larva of P. conspersa is seen to be just one cell thick, since no more than one cell was observed in any of the sections examined. Each epithelial cell has an inner striated margin, while in the opposite basal region, the cell is attached to a basement membrane. Within the cell cytoplasm mitochondria and granules are clearly evident. The subspherical intracellular granules are on average 0.5 μm in diameter. Furthermore the granules are uniformly electron dense (i.e. they lack internal structuring) and they are not membrane bound. Randomly scattered excretory material is found in the lumen of the tubule, but there are no granules.

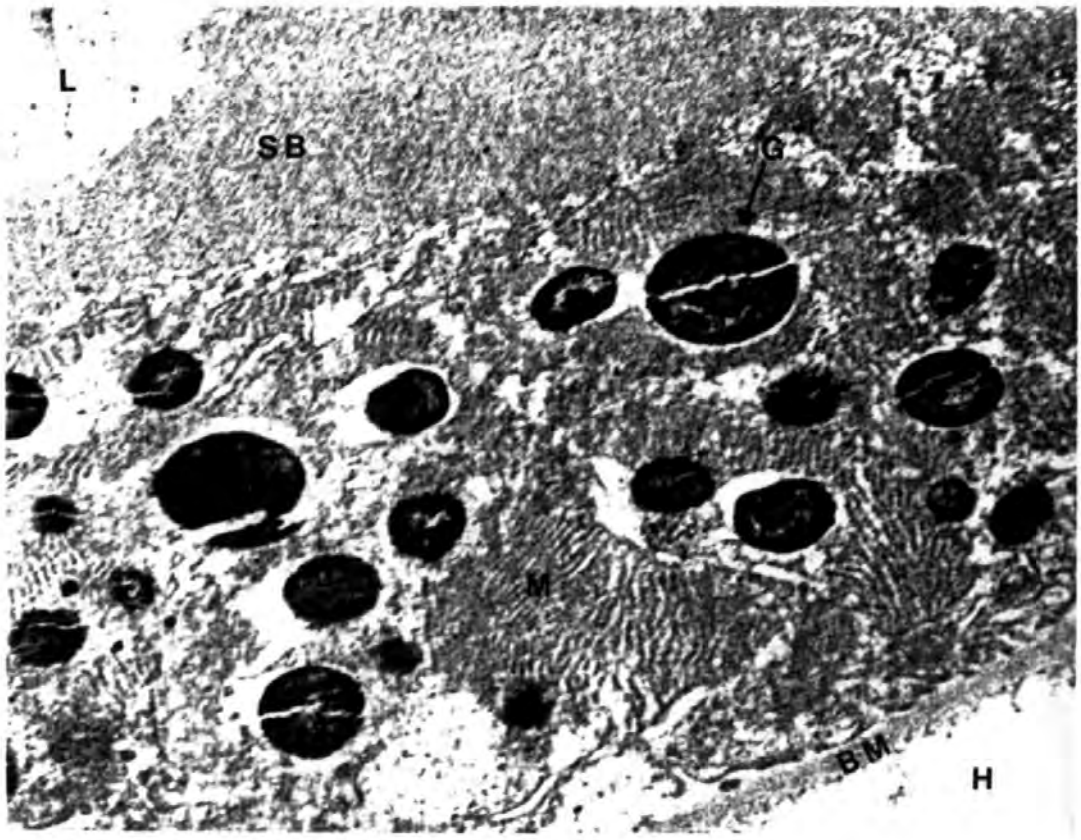
A typical X-ray spectrum of an intracellular granule in the malpighian tubule is shown in Figure 9.5, together with spectra of the adjacent cytoplasm and nearby resin. As silicon and chlorine were found in the cytoplasm and resin, these elements probably arise from contamination (silicon from the knife edge, silicon detector and vacuum oil; chlorine from Spurr's resin). The titanium peak is due to the titanium grids upon which the sections were mounted.

Since copper is used for a number of components in and around the specimen chamber, a small 'background' peak for copper was obtained. However the pronounced K_{α} peak for copper in the granule (as shown in Figure 9.5A) together with the additional K_{β} peak, is indicative of the high amount of copper in the granule. The presence of sulphur is represented by a clearly defined peak in the X-ray spectrum for the granule, but was absent from the cytoplasm and resin. Therefore the above evidence suggests that the granule contains significant amounts of copper and sulphur.

PLATE 9.8. Micrographs (A x9300; B x32000) of a section through a malpighian tubule of an instar III larva of P. conspersa from the Darley Brook. BM basement membrane, G granule, H haemocoel, L lumen of tubule, M mitochondrion, SB striated border.

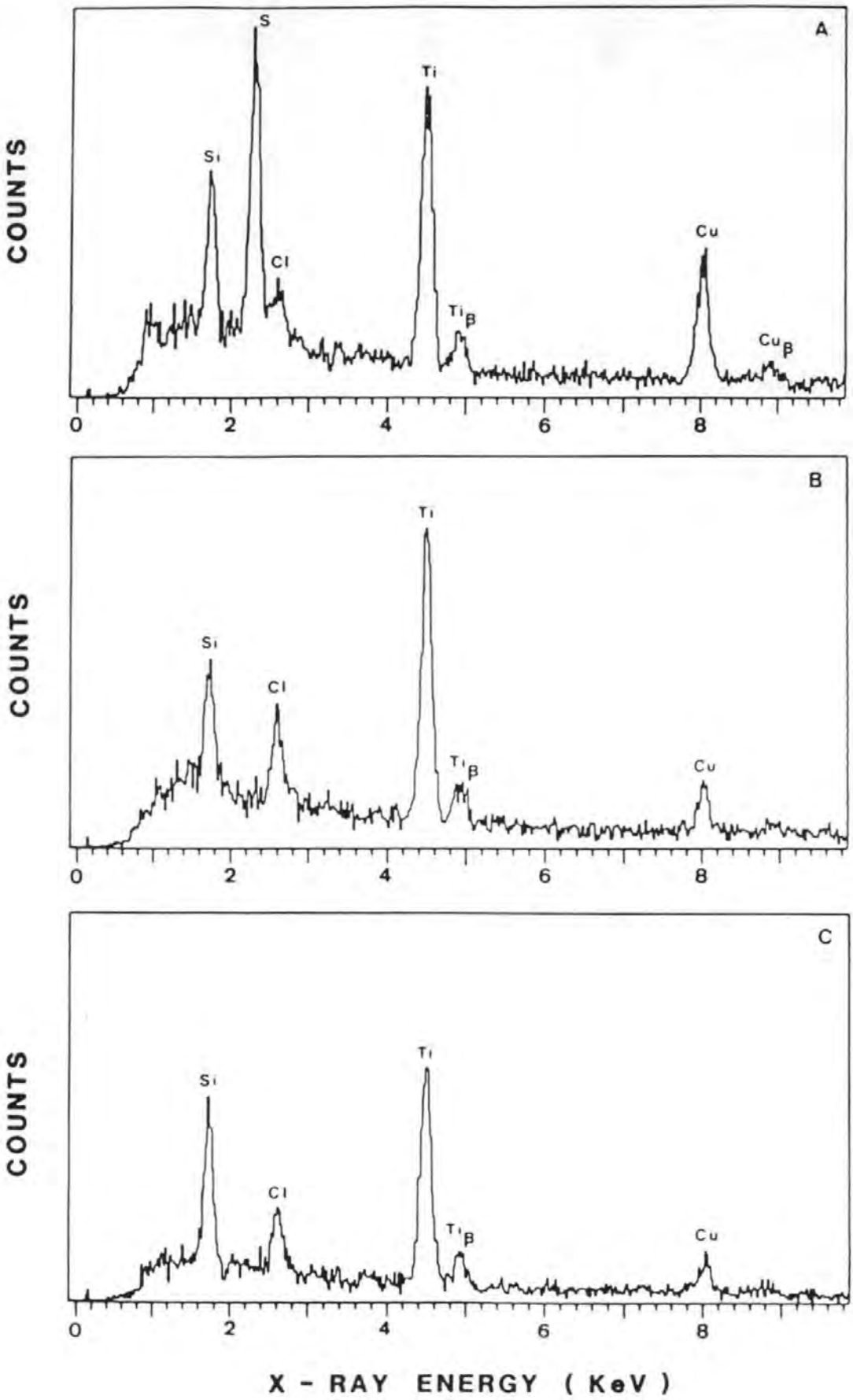


A



B

FIGURE 9.5. X-ray spectra of A). an intracellular granule, B). the adjacent cytoplasm and C). nearby resin in a section of a malpighian tubule of an instar III larva of P. conspersa from the Darley Brook.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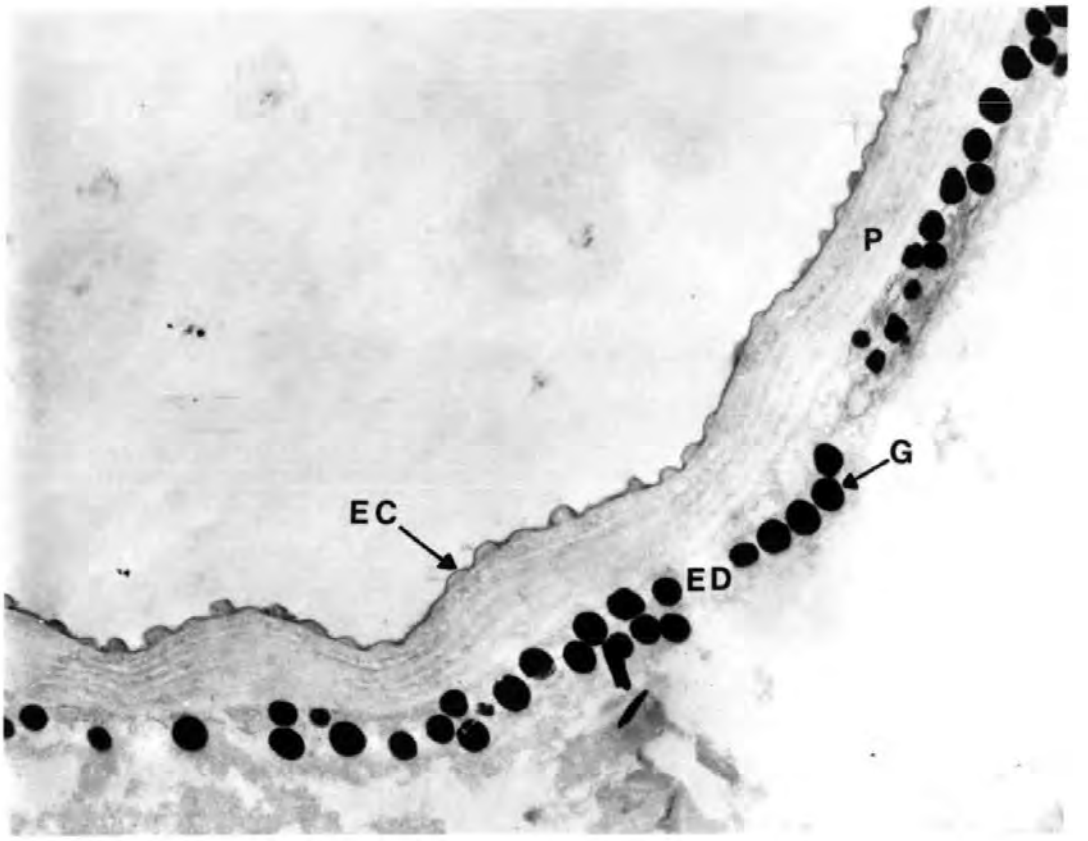
X - RAY ENERGY (KeV)

It can be seen from Plate 9.9 that in an instar III larva of P. conspersa the cuticle beneath the epicuticle appears as an undifferentiated procuticle, but there is a clear lamellar structure parallel to the surface due to the layered arrangement of microfibres. Granules can be seen in the subcuticular/epidermal region, and a number of them appear to be intracellular. The granules have a similar structure, shape and size as the granules observed in the cells of the malpighian tubule (see Plate 9.8).

The elemental composition of a subcuticular granule (and of the adjacent procuticle and nearby resin) are shown by the X-ray spectra presented in Figure 9.6. It is interesting to note that the spectrum for the subcuticular granule consisted of the same five elements that were present in the spectrum of the intracellular granule in the malpighian tubule (see Figure 9.5).

Thus significant amounts of copper (for which a K_{β} peak was also recorded) and sulphur were again present in the subcuticular granule. In contrast to the X-ray spectra shown in Figure 9.5, where the peaks for silicon and chlorine are of approximately similar magnitude in all three spectra, the peaks for these two elements in the subcuticular granule (see Figure 9.6A) differ slightly from those in the procuticle or resin spectra. Although the reason for this is uncertain, it is evident that the presence of silicon and chlorine in the subcuticular granule may in part be due to contamination (since they were also present in the procuticle and resin). The titanium grid upon which the section was mounted gave rise to the titanium peaks in the spectra.

PLATE 9.9. Micrographs (A x6600; B x18300) of a section through the integument of an instar III larva of P. conspersa from the Darley Brook. EC epicuticle, ED epidermis, G granule, P procuticle showing clear lamellar structure.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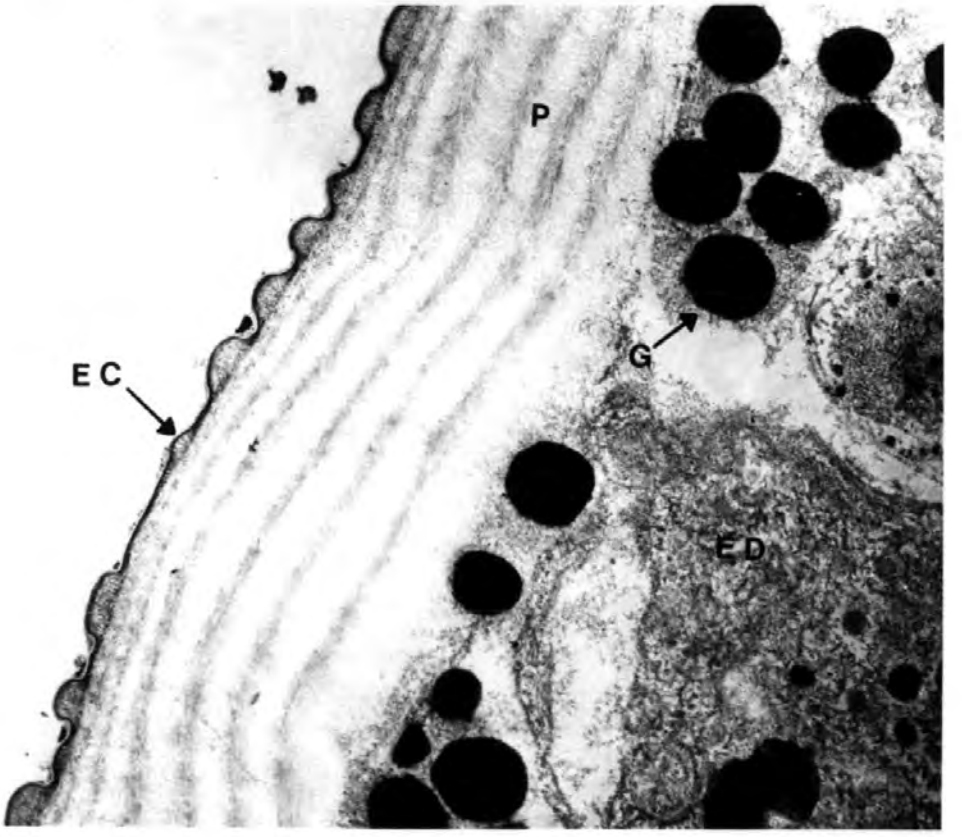
B
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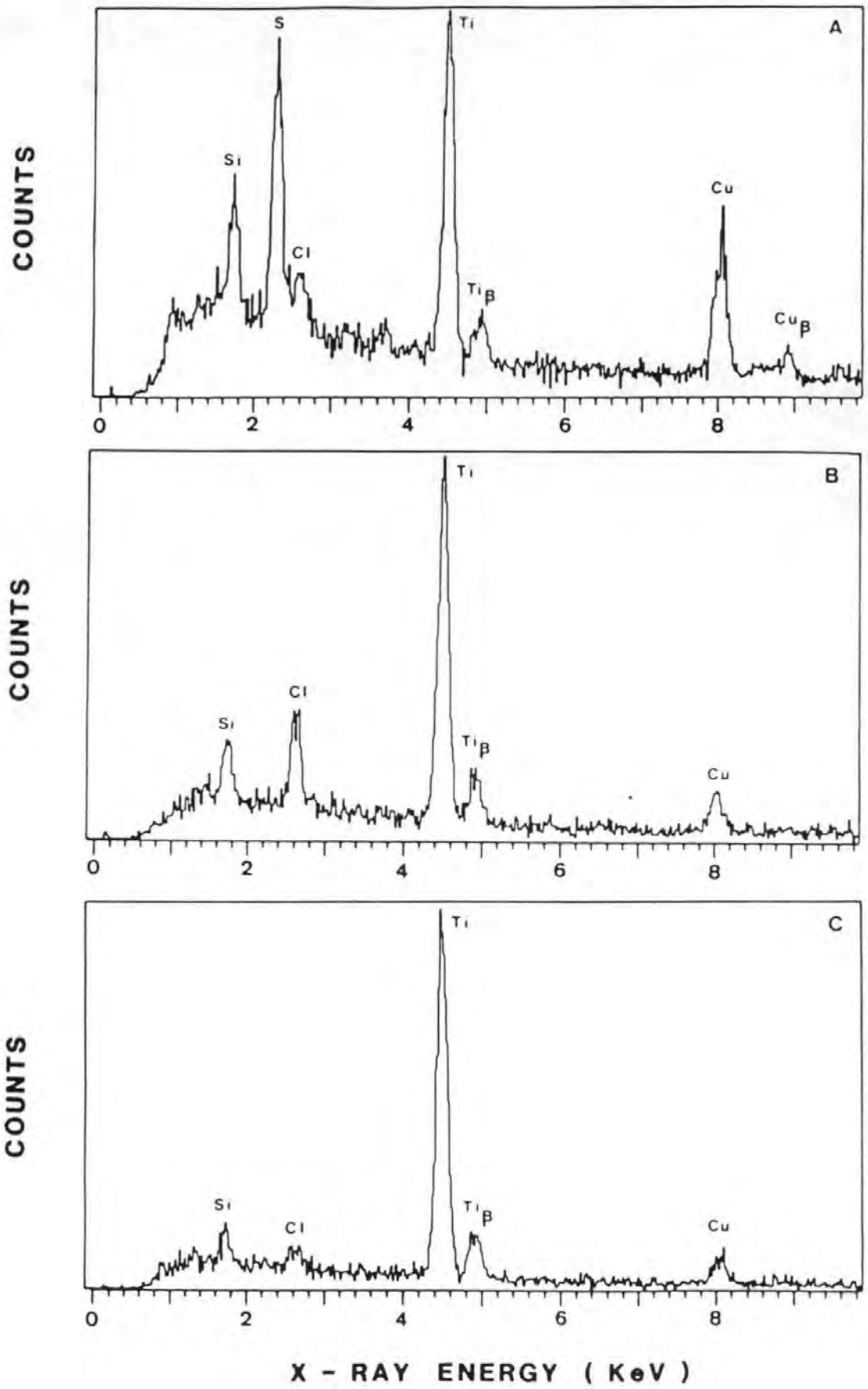
G
EC
ED

FIGURE 9.6. X-ray spectra of A). a subcuticular granule, B). the procuticle and C). nearby resin in a section of the integument of an instar III larva of P. conspersa from the Darley Brook.


A
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Cu β
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Ti β
Cu
C
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Cu
0
2
4
6
8
COUNTS
COUNTS
COUNTS
X - RAY ENERGY (KeV)

9.5. Discussion

In the present study it has been shown that significant amounts of copper and sulphur occur in granules in instar III larvae of P. conspersa collected from station 1 in the Darley Brook. These granules are only found in the cells of the malpighian tubules and in the subcuticular region. Whether granules occur in these same regions in other larval instars of P. conspersa requires further investigation, although in this study copper was found to be present in the malpighian tubules of an instar V larva from station 1 (see p.325). It is interesting to note that there do not appear to be any accounts of copper containing granules in other aquatic insect larvae.

In P. conspersa larvae the wall of the malpighian tubule is just one cell thick (see Plates 9.2 and 9.8). The cell structure is similar to that found in the malpighian tubules of most insects (Wigglesworth, 1972), i.e. the cells are associated externally with a tough basement membrane, the cytoplasm is rich in mitochondria and there is a large nucleus, and the inner surface of the cell is extended into cytoplasmic filaments which form a striated margin. The copper containing granules within these cells (see Plate 9.8) are subspherical (on average 0.5 μm diameter), homogeneous in structure and do not appear to be membrane bound.

Several workers have recorded granules (often termed 'concretions') in the malpighian tubules of terrestrial insects. Wigglesworth and Salpeter (1962) observed spherical mineral deposits which were concentrically laminated in the malpighian tubule cells of the hemipteran Rhodnius prolixus, and they suggested that these contained phosphates or carbonates of calcium and magnesium. Intracellular and extracellular concrements with concentric layers of mucopolysaccharides, in between

which were calcium, magnesium, sodium, potassium, phosphates and carbonates, have been observed in the malpighian tubules of two species of Drosophila, namely D. melanogaster and D. hydei (Wessing & Eichelberg, 1975). Sohal et al. (1976) have observed granules in the lumen and three different types of concretions in the malpighian tubules of Musca domestica, and phosphorus, sulphur, chlorine, potassium, calcium, iron, copper and zinc were detected in these structures.

When a section through the integument of an instar III larva of P. conspersa from the Darley Brook was viewed under the transmission electron microscope (see Plate 9.9), there was found to be a number of granules in the subcuticular/epidermal region, and at least some of these granules appeared to be intracellular. What is interesting about these granules is that they are of a similar shape, structure, size and composition (i.e. both contain copper and sulphur) to the granules observed in the cells of the malpighian tubule. One of the few other records of metal containing granules in the outer layer of an aquatic invertebrate is that by Bryan (1974), who showed that in the polychaete Nereis diversicolor taken from copper contaminated waters, copper was stored as fine granules in the epidermis as well as in parts of the nephridia.

One study of particular interest and relevance to the present investigation is that by Lhonore (1973), who studied the distribution and abundance of metals in tissues of the larval and pupal stages of the caddis Phryganea varia. In the larvae of this species calcium and chlorine were associated with pigments in the hypodermis, and concretions composed of calcium, magnesium and chlorine were found in the cells of the malpighian tubules; however the size, shape and structure of the pigment granules and the concretions was not commented upon. In these larvae the cells of the mesenteron were the most important metal store,

containing phosphate concretions (3 to 4 μm diameter) consisting of calcium, magnesium, manganese, zinc, iron and chlorine. By contrast no granules were observed during the present study in the midgut cells of P. conspersa. Furthermore it is interesting to note that in the work on P. varia, no copper was detected in any of the larval or pupal tissues.

In a review on the form and function of metal containing granules in invertebrate tissues, Brown (1982) distinguishes (on the basis of composition) three major granule types. These are (i) copper containing granules, which usually also contain sulphur and perhaps calcium, (ii) calcium containing granules, which are either of high purity or contain several metals and (iii) iron containing granules, where iron may be present as ferritin. In most of the studies on copper containing granules, the granules are intracellular, homogeneous and spherical, with a diameter of between 0.5 and 2.0 μm. The composition of these granules is remarkably consistent, with copper and sulphur the most important constituents. Thus the structure, shape, size and composition of the granules in the cells of the malpighian tubules and in the subcuticular region of P. conspersa larvae would appear to be comparable with copper containing granules in other invertebrates.

The occurrence of copper containing granules in Nereis diversicolor (Bryan, 1974) and in Musca domestica (Sohal et al., 1976) has already been commented upon in this discussion. Tapp and Hockaday (1977) observed granules in the cuprophilic cells of the midgut of larvae of Drosophila melanogaster; most of these subspherical granules (approximately 0.5 μm diameter) were uniformly electron dense, and X-ray microanalysis showed them to contain significant amounts of copper and sulphur. It is interesting that in this latter study carried out on the larvae of an insect, intracellular granules were observed which were very similar

in size, appearance and composition to the granules found during the present investigation in the larvae of P. conspersa.

The significant amount of sulphur in the granules in P. conspersa larvae (see Figures 9.5 and 9.6) is noteworthy since this element has been shown to be important in the metal tolerance mechanisms of three widely separate groups of organisms, namely fungi (Ashworth & Amin, 1964; Ashida, 1965), higher plants (Antonovics et al., 1971) and the macroinvertebrate Asellus meridianus (Brown, 1977b). The presence of sulphur in the copper containing granules in P. conspersa larvae leads us to speculate as to whether the copper is present in an organic or inorganic form. Whilst it is not possible using the methods employed in the current study to make this discrimination, the negative reaction with rubeanic acid in the present investigation may suggest that the copper is protein-bound (Pearse, 1972). In the amphipod Corophium volutator (Icely & Nott, 1980) and in the barnacle Balanus balanoides (Walker, 1977), copper is bound as an organic complex. This copper may be complexed with metallothioneins (proteins) or with sulphur rich amino acids (e.g. cystine and methionine).

Observations with the light microscope showed that granules similar in appearance to those observed in the Darley Brook larvae of P. conspersa were present (though in noticeably smaller amounts) in the malpighian tubules and subcuticular region of larvae from the uncontaminated stream near Aylesbeare. Both the cuticle and the malpighian tubules in P. conspersa larvae are pigmented, and it is suggested that the granules observed in the subcuticular region and in the malpighian tubules are primarily pigment granules. Although it is not known whether these granules naturally contain copper as part of their normal composition, it is interesting to note that Kikkawa et al. (1955) have shown that dark melanin pigments (and possibly other pigments)

in insect cuticles are composed of metallic complex salts containing copper, and also cobalt and iron.

If the granules observed in the Darley Brook larvae are pigment granules, then it is possible that excess copper entering the body or within the body could be stored within these granules, which if they naturally contain copper are already available to take on this role. Another observation in the present study was that there appeared to be significantly larger numbers of granules in the malpighian tubules and in the subcuticular region of larvae from the copper contaminated stream. This may suggest that elevated copper concentrations in the surrounding water, and indeed within the larval haemolymph, may stimulate further granule production.

The epicuticle in all insects is extremely thin (1 to 4 μm), and the permeability of the cuticle to water in aquatic larvae is inversely proportional to the degree of development of the epicuticle (Wigglesworth, 1972). Since the whole cuticle in P. conspersa is thin and membranous (see Plate 9.9) it is reasonable to assume that copper could pass from the surrounding water through the cuticle and be incorporated into the subcuticular granules. It is further likely that the copper in the intracellular granules in the malpighian tubules of P. conspersa larvae has been taken up from the larval haemolymph in which the malpighian tubules are freely bathed.

Earlier on in this discussion it was suggested that the role of the granules in P. conspersa was the immobilisation of excess copper within the larva, and this is supported by studies on granules in other invertebrates. For example, in Crustacea, copper containing granules are used as a store for copper from the blood and represent a mechanism for taking potentially toxic concentrations of metal out of circulation (Brown, 1982).

In Nereis diversicolor (Bryan, 1974), Balanus balanoides (Walker, 1977), Corophium volutator (Icely & Nott, 1980), Asellus meridianus (Brown, 1977b) and Ostrea edulis (George et al., 1978) copper containing granules probably result from the production of metal-binding proteins or metallothionein in invertebrates exposed to elevated copper concentrations.

In the work of Brown (1978) on Asellus meridianus, she showed that intracellular granules in the hepatopancreas were capable of storing either copper or lead. If the pigment granules in the larvae of P. conspersa are capable of storing metals other than copper, then it would in part explain the previously described (see p.208) occurrence of larvae of this species in other metal contaminated waters.

In the present investigation copper containing granules were not observed in the lumen of the malpighian tubules. This suggests that the granules in the cells of the malphigian tubule are probably not passed into the lumen, but are retained in the cells.

Before the ultimate fate of the subcuticular/epidermal granules can be discussed, it is necessary to briefly consider the events that take place during moulting of the cuticle. In an insect which is about to moult, the epidermal cells enlarge and apolysis takes place, i.e. the cuticle becomes detached through retraction from the epidermis. The epidermal cells now secrete a new epicuticle and then a procuticle, with the space between the old and new cuticles becoming filled by moulting fluid. This fluid digests and dissolves the inner layers of the old cuticle, which are absorbed by the epidermal cells. Finally ecdysis occurs, i.e. the rupture and shedding of the old cuticle.

From the above sequence of events it is likely that the subcuticular granules are associated with the old cuticle. If the

copper in these granules is not leached out by the moulting fluid (which contains a protease and a chitinase), then it can be assumed that a number of copper containing granules are shed with the old cuticle. It is interesting to note that the ecdysed larval skins of P. conspersa have been shown to contain copper (see Figure 9.3A), and furthermore it has been possible to quantify this copper (see Tables 7.11 and 7.12).

CHAPTER 10

GENERAL DISCUSSION

The present investigation has yielded information on the nature and characteristics of the different compartments in a copper contaminated mine drainage stream, and this in turn has provided some insight into possible interrelationships. Furthermore work has focused on Plectrocnemia conspersa, with a study of its life cycle and population characteristics, the measurement of copper concentrations in single specimens of different instars, and an investigation of metal tolerance mechanisms in this species. To the author's knowledge there are no other such detailed studies on an aquatic insect occurring in a metal contaminated freshwater ecosystem.

In the uppermost reaches of the Darley Brook the water quality is relatively constant over time due to the groundwater source of this stream. Indeed this constancy in water quality is typical of many mine drainage streams (Patterson & Whitton, 1981; Foster, 1982a). Thus the aquatic organisms in the headwaters of the Darley Brook are continuously exposed to an elevated copper concentration (e.g. at station 1 the mean copper concentration in the water is 0.86 mg l^{-1}; see Table 3.3), which is higher than any other copper concentration recorded in the waters of the mine drainage streams in the U.K. (Brown, 1977a; Burton & Peterson, 1979; Foster, 1982a; Jones et al., 1985); also between 95 and 97% of the copper in the headwaters of the Darley Brook is in a soluble form. Furthermore the high hydrogen ion concentration in the Darley Brook (mean pH 5.5 at station 1; see Table 3.3), together with the low amount of organic material in the water, means that a significant fraction of the copper (between 12 and 19% at station 1; see Table 3.6) is in the free cupric ion form, a species known to be

toxic to many aquatic organisms including a large number of macroinvertebrates (Andrew et al., 1977; Dodge & Theis, 1979).

The relatively constant nature of the water quality in the Darley Brook is reflected by the small amount of temporal variation observed in the metal concentrations in the sediments in this stream. It has also been demonstrated in the present study that these sediments act as an important sink for metals, e.g. at stations 1, 2 and 3 neither arsenic nor iron were detectable in the water column but they were concentrated in the sediments. Elevated copper concentrations were recorded in the sediments at these three upstream stations. Indeed the overall mean leachable copper concentration of 6985 $\mu g\ g^{-1}$ recorded at station 3 (see Table 4.3) is higher than any copper concentration reported in sediments of other mine drainage streams in South West England (Aston et al., 1974; Brown, 1976; 1977a; Aston & Thornton, 1977; Thornton & Webb, 1977). Thus a constant and elevated concentration of copper in the sediments is available to rooted macrophytes and any deposit feeders living in the headwaters of the Darley Brook.

The uppermost reaches of the Darley Brook represent a biologically hostile environment, and only those plants and animals which can tolerate the low pH and elevated copper concentrations can survive in this stream. This has resulted, as in most mine drainage streams, in a very restricted floral and faunal community.

The aquatic vegetation at station 1 is very prominent (partly because there is a lack of grazing invertebrates along this riffle section), with excessive growth at certain times of the year. However the plant community is reduced to the filamentous green alga Microspora sp. and the leafly liverwort Jungermannia atrovirens. At station 2 (pool)

the rush Juncus bulbosus grows in the muddy substratum. It is interesting to note that Microspora has been found in other copper contaminated streams (Foster, 1982a), and that Satake et al. (1983) have recorded another species of Jungermannia (namely J. vulcanicola) growing in an acidic metal enriched stream. It is evident from the present study that J. bulbosus is able to tolerate the elevated copper and hydrogen ion concentrations in the Darley Brook, and the resistance of this species to low pH is further corroborated by the work of Nilssen (1980) who noted that J. bulbosus was abundant in acidified lakes in Southern Norway.

There are however a number of plants which have been recorded by other workers in copper contaminated mine drainage waters, but which are absent from the Darley Brook. For example, Mclean and Jones (1975), Burton and Peterson (1979) and Jones et al. (1985) have all noted the occurrence of the liverwort Scapania undulata in mine drainage waters with elevated copper concentrations. Examples of other aquatic plants which have been found in copper contaminated waters include the bryophyte Fontinalis squamosa which occurred in tributaries of the River Clarach where the copper concentration in the water was 0.60 mg l^{-1} (Mclean & Jones, 1975), and the macrophyte Equisetum fluviatile which Ray & White (1976) recorded in mine drainage waters having a copper concentration of 0.16 mg l^{-1}.

In view of the existence of copper tolerant plants at station 1 and their occurrence in large quantities at certain times of the year (together with their relatively high copper concentrations), it is likely that the aquatic vegetation makes a significant contribution to the rapidly changing conditions in the uppermost reaches of the Darley Brook. This would partly account for the contrast in conditions at stations 1 and 3, e.g. the mean copper concentrations in the water

at these two riffle stations are 0.86 and 0.67 mg l^{-1} respectively (see Table 3.3). The removal of metals from mine waste waters by algal blooms has been commented upon by Sterritt and Lester (1979), who suggest that a combination of bacterial and algal systems could provide suitable treatment for mine waste waters, producing a metal-free effluent with low acidity.

There is a reduced faunal community at station 1, which is reflected by the lower species richness and significantly lower values of the Shannon-Weaver function H' recorded on both occasions when the community structure was investigated at this station, compared to the values for the invertebrate community in the clean control stream (see Table 6.7). The community at station 1 consists of chironomid larvae, coleopteran larvae and adults (Ilybius fuliginosus and three species of Agabus) and larvae of the trichopteran P. conspersa.

In general, the invertebrates most tolerant to metal pollution in freshwater appear to be tubificid worms and insect larvae, especially certain species of Chironomidae, Ephemeroptera, Plecoptera and some species of campodeiform caddis larvae (Gower, 1980). With specific reference to copper, the Chironomidae in particular appear to be of some significance. This was evident in the early study of Butcher (1946) who recorded only chironomid larvae (and one caddis species) in the River Dove (where the copper concentration in the water was 0.12 mg l^{-1}) below its confluence with the copper polluted River Churnet. Another significant observation was that of Surber (1959) who found that the chironomid Cricotopus bicinctus was extremely tolerant to copper (at concentrations as high as 2.2 mg l^{-1}) as well as chromium. In a more recent survey of copper pollution in two lotic ecosystems, one of which was an experimental stream for evaluating the impact of copper, Winner et al. (1980) reported a relationship in which the

proportion of chironomids in macroinvertebrate communities increases with the degree of pollution, and they further suggest that the percentage of chironomids in a sample could be a useful index of heavy metal pollution.

All the Chironomidae found during the present study in the Darley Brook (except Zavrelimyia sp.) have also been recorded by Armitage in hill streams in Northern England, contaminated by zinc at concentrations of 1.5 mg l^{-1} (Armitage; personal communication). Details of the Chironomidae recorded in the River Nent system in the Northern Pennines (which is also contaminated by zinc) are given in Armitage and Blackburn (1985), and by comparison it is interesting to note that far fewer chironomid species were found along the Darley Brook.

In the present study it was shown that the copper concentration in the water decreases along the length of the Darley Brook (see Figure 3.2A), and it is instructive to consider some aspects of changes in the macroinvertebrate communities which may be associated with this copper gradient.

In the uppermost reaches of the Darley Brook (i.e. station 1) chironomid larvae and larvae of P. conspersa occurred in significant numbers. By comparison, in the studies of Winner et al. (1975; 1980) and Sheehan and Winner (1984) on three copper contaminated streams (which also showed a clear gradient in the copper concentration in the water), Chironomidae dominated the community at the most polluted stations (0.12 mg l^{-1} copper in the water), and Trichoptera (along with chironomids) were numerically abundant at sites of intermediate pollution.

Several species of Coleoptera were also recorded at station 1 in the Darley Brook, and it is interesting to note that two of the Agabus species (namely A. bipustulatus and A. guttatus) have also been

found within a restricted macroinvertebrate community in a zinc contaminated stream (Armitage, 1980). In the copper polluted experimental stream studied by Winner et al. (1980), Coleoptera attained maximum densities at intermediate copper concentrations.

Stonefly nymphs were completely absent from the headwaters of the Darley Brook, and indeed a maximum of only three individuals were collected at any of the sampling stations along the main channel, i.e. station X (April 1986, see Appendix 5F) where the copper concentration was 0.14 mg l^{-1}. This may suggest that Plecoptera are less tolerant to copper than those insect orders found in the uppermost reaches of the Darley Brook, and this sensitivity to copper is further indicated by the observation of Sheehan and Winner (1984) that Plecoptera were only common at a station where the copper concentration was < 0.004 mg l^{-1}. Although Plecoptera may therefore be relatively sensitive to copper, it is worth noting that they are known to be tolerant to lead and zinc (Hawkes, 1972; Hynes, 1974).

In the present study mayfly nymphs were found to be confined to the lower reaches of the Darley Brook (i.e. stations X and F; see Figure 2.6), and Winner et al. (1980) also found that Ephemeroptera occurred only in the least polluted reaches of two copper contaminated streams. Clearly the data from both these studies suggests that mayfly nymphs (as well as stonefly nymphs) may be sensitive to copper pollution.

Although there is no information in the literature on the sensitivity of P. conspersa to metals, some work has been carried out on the net-spinning caddis Hydropsyche. Warnick and Bell (1969) found that larvae of H. betteni were able to live beyond a 96 hour test period at copper concentrations of upto 64 mg l^{-1}, and Williams et al. (1985) showed

that H. angustipennis was the most resistant species of ten freshwater macroinvertebrates to cadmium, with a 96 hour LC_{50} value of 520 mg l^{-1}. However copper (at a concentration as low as 0.125 mg l^{-1}) has been shown to cause anomalies in hydropsychid nets, either by affecting the secretion of silk or the spinning process itself (Besch et al., 1979; Petersen & Petersen, 1983).

The aquatic vegetation in the headwaters of the Darley Brook contained metal levels which were considerably higher than those in the surrounding environment, and one way of expressing the degree of accumulation is by a 'concentration factor' (or 'enrichment ratio') i.e. the ratio of the concentration of an element in biological material (expressed as $\mu g\ g^{-1}$ dry weight) to that in the environment, e.g. water, sediment or previous trophic level (expressed in ppm). For aquatic plants, concentration factors are most simply and meaningfully calculated for those species living exclusively or predominantly in the water column. Thus in the Darley Brook, since water is believed to be the main source of copper for Microspora (a filamentous green alga), concentration factors in this plant have been calculated and these are shown in Table 10.1. This table shows the mean copper concentrations in Microspora, which was collected every three months at station 1 from January 1984 to July 1985, together with the concentration of copper in the water on these same occasions.

Reference to Table 10.1 shows that in both years the highest concentration factors were recorded when the concentration of copper in the water was lowest and vice-versa. Thus in 1984 concentration factors ranged from 2430 (January) to 3232 (July), and similarly in 1985 the values were between 2981 (January) and 3816 (July). Although there appears to be a straightforward relationship between copper concentrations

TABLE 10.1. Concentrations of copper in the water (mg l^{-1}) and in Microspora sp. (µg g^{-1}) at station 1 in the Darley Brook every three months from January 1984 to July 1985, together with the calculated concentration factors.

	DATE	COPPER CONCENTRATION		CONCENTRATION FACTOR
		WATER (mg l^{-1})	Microspora sp. (µg g^{-1})	
1984	23/1	0.99	2406	2430
	25/4	0.88	2617	2974
	18/7	0.82	2650	3232
	19/10	0.86	2467	2869
1985	17/1	0.89	2653	2981
	17/4	0.80	2541	3176
	18/7	0.74	2824	3816

in the water and concentration factors, it is complicated by the increased plant growth and metabolism in summer, which would probably result in a higher concentration in plant tissue even if the concentration in the water remained exactly the same throughout the year.

Thus in the present investigation it has been shown that concentration factors in Microspora vary over time, and one criticism that can be made of other workers who have calculated concentration factors in freshwater algae is that they have usually only done this for one sampling occasion. For example, Trollope and Evans (1976) calculated a concentration factor for copper of 16000 for Microspora collected from water near zinc smelting wastes, where the concentration of copper in the water was 0.06 mg l^{-1}. It is interesting to note that in this study the copper concentration in the water was less than that recorded at station 1 in the Darley Brook, but the alga accumulates copper to a greater extent than Microspora at station 1. Stokes (1979) has selected or calculated concentration factors from a number of studies on algae in copper contaminated waters and, for example, in the case of Cladophora values were found to range between 1000 and 3500.

It should be noted that for aquatic plants which are more closely associated with the sediment it is difficult to calculate concentration factors, since it is unclear whether the sediment or the water represents the major metal source. For this reason concentration factors were not calculated in J. bulbosus (which has a root system) or in J. atrovirens (which may possibly take up metals from the sediment via the rhizoidal fraction of the plant).

A number of workers have calculated or made reference to concentration factors for metals in aquatic invertebrates (Brown, 1977b; Namminga & Wilhm, 1977; Willis, 1983). Namminga and Wilhm (1977) recorded a concentration factor of 540 for copper in samples of chironomid

larvae taken from a stream where the copper concentration in the water was 0.0035 mg l^{-1}. By comparison, concentration factors for copper of approximately 470 (January 1986) and 1225 (July 1986) have been recorded in the present investigation in samples of chironomid larvae collected from station 1 (mean copper concentration in water, 0.86 mg l^{-1}). Furthermore concentration factors (in relation to the water) calculated for instar V larvae of P. conspersa also taken from station 1 were always in excess of 64 (this lowest concentration factor being recorded in January 1985). However the concentration of copper in P. conspersa larvae has been shown in the present study to be influenced by a number of factors (which are considered later on in this discussion) including size, the time of year when the larvae are sampled, and the concentration of copper in the surrounding water and in the prey. Clearly these must all be taken into account when determining concentration factors, and yet none of the workers who have calculated concentration factors in aquatic invertebrates have considered the importance of all of these influences.

In the present study the technique developed with graphite furnace AAS has yielded detailed information on the variation of copper concentrations between individuals, between different stages of the life cycle and between different populations of P. conspersa exposed to different water qualities.

On a number of occasions when different larval instars of P. conspersa were collected from either station 1 or 4, there was found to be an exponential decrease in copper concentration with increasing body weight (see Figure 7.1), which is indicative of a surface to volume phenomenon. This suggests that copper is associated with the larval cuticle and this was confirmed by the analysis of shed larval skins

by graphite furnace AAS (see Tables 7.11 and 7.12) and X-ray microanalysis (see Figure 9.3). Smock (1983b) has also found an exponential decrease in the concentration of several metals with increasing body weight in aquatic insects, and he demonstrated that in the mayfly Stenacron interpunctatum chromium is associated with the shed exuviae of the nymphs.

The concentrations of copper in larval instars II, III, IV and V of P. conspersa collected from stations 1 and 4 were found to vary with time (see Table 7.3), and a more detailed investigation of instar V larvae from these two stations showed that the copper concentrations exhibited a clear seasonality, with a summer maximum and a winter minimum (see Figure 7.3). Results from the detailed quantitative sampling and the subsequent copper analysis of the major prey items of P. conspersa larvae undertaken at station 1 (i.e. chironomid and coleopteran larvae) on two occasions support the hypothesis that the summer maximum in the copper concentration of P. conspersa larvae was due partly to more prey being consumed and partly to an increase in the copper concentration of the prey itself.

It is interesting to speculate on the possible factors which caused the copper concentrations in the chironomid larvae at station 1 to be significantly higher in July 1986 than in January 1986 (mean concentrations were 1054 and 407 $\mu g\ g^{-1}$ respectively; see Table 7.13). It has previously been mentioned (see pages 63 and 103) that the small amount of temporal variation in the copper concentrations in the water and sediment at station 1 is random and shows no discernible pattern over time. However the concentrations of copper in Microspora and in J. atrovirens were higher in the summer than in the preceding winter (see Figure 5.1). Thus temporal variation in metal concentrations

in the plants and invertebrates is taking place against a background of relatively constant metal concentrations in the water and sediments. No doubt the biotic variation is a reflection of seasonal changes in the metabolic activities of the plants and animals, including the feeding patterns of the invertebrates.

It may also be noted that there was some increase in water temperature at station 1 during the summer (see Figure 3.3) and this could have led to an increase in the uptake of copper from the water by the aquatic macroinvertebrates. Indeed, Harding et al. (1981) found that concentrations of zinc, cadmium and lead in a number of aquatic invertebrates were highest in August and they suggested that the higher water temperature caused an increase in metal uptake.

A density distribution map for P. conspersa is shown in Figure 10.1. This map, previously unpublished, is reproduced with the permission of Dr L.R. Taylor, Rothamsted Insect Survey. The map is based on the mean annual catch of this species at 64 light trap sites over a six year period (1966-1971). However it should be noted that large catches from a single trap may have an exaggerated effect on the pattern shown in Figure 10.1, whilst a blank area does not necessarily indicate the absence of P. conspersa, but its lower density in that part.

It can be seen from Figure 10.1 that large catches of P. conspersa were made in upland areas (most of Scotland and North West Wales) since this species is characteristically found in the small acid and cool streams in these areas. However it is also clear from the map and observations made during the current investigation that P. conspersa is more widely distributed, occurring at lower altitudes and under a wider range of conditions.

In South West England, P. conspersa and P. geniculata may be found as part of a diverse invertebrate community in clean streams.

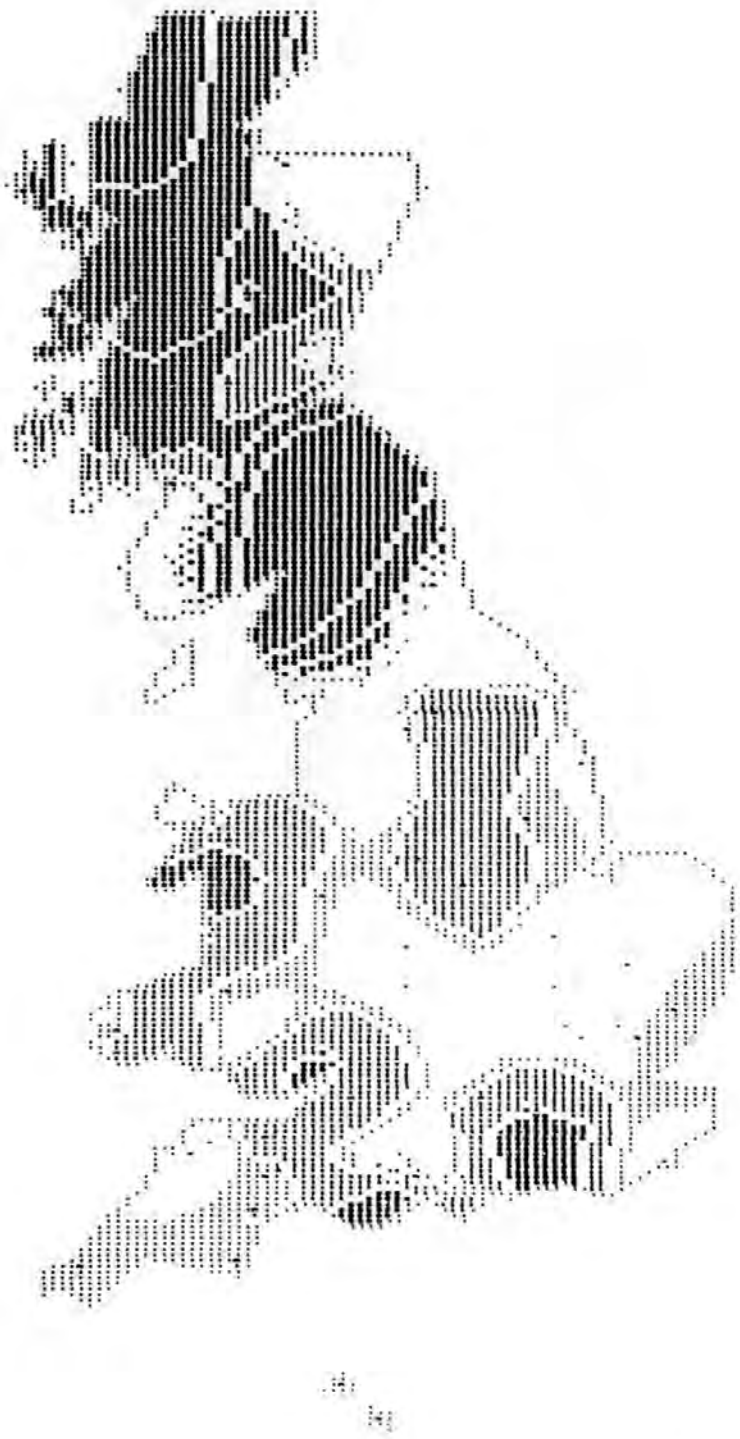

FIGURE 10.1. Density distribution map for P. conspersa, produced by computer using the Symap V Program of the Laboratory for Computer Graphics, Harvard. Information is based on the mean annual catch of P. conspersa at 64 Rothamsted light trap sites over the period 1966-1971. Layering intervals are approximately logarithmic : 0, 1-2, 3-9, 10-31, 32-99, 100-315, 316-999, 1000-3161 (Unpublished map from the Rothamsted Insect Survey).

However a striking feature of copper contaminated streams in this region, particularly in Cornwall, is the prominence of P. conspersa. In several of the streams examined during this work having copper concentrations ranging from 0.1 to 0.4 mg l^{-1} P. conspersa was found to be relatively abundant but within a more diverse community than in the Darley Brook, and in the headwaters of the Darley Brook with very high copper concentrations (as much as 0.99 mg l^{-1} at the adit) P. conspersa is at its most abundant. Indeed final instar larvae attained densities of over 100 m^{-2} at station 1 in June 1984 (see Figure 6.3).

It has previously been mentioned (see p.208) that a number of workers have recorded the presence of P. conspersa in streams subject to elevated concentrations of iron, copper, zinc and aluminium (i.e. Hildrew & Townsend, 1976; Brown, 1977a; Armitage, 1980; Stoner et al., 1984 respectively). These studies, together with the present investigation, suggest that P. conspersa is a metal tolerant species. This suggestion is corroborated by the survival of the Aylesbeare stream larvae in the headwaters of the Darley Brook during the transfer experiment, since larvae from this population have no selective pressure upon them to be metal tolerant as they occur in a metal uncontaminated stream.

One aim of the present study was to investigate how metal tolerance is achieved in P. conspersa larvae. Histological and ultrastructural techniques showed the occurrence of granules in the cells of the malpighian tubules and in the subcuticular region of P. conspersa larvae (see Plates 9.8 and 9.9 respectively). The granules in both these regions were subspherical (on average 0.5 μm diameter) and showed no internal structuring. Furthermore X-ray microanalysis

of the granules showed them to contain significant amounts of copper and sulphur (see Figures 9.5 and 9.6).

It is suggested that these copper containing granules are primarily pigment granules, but they also provide a mechanism for taking potentially toxic concentrations of copper (and possibly other metals) out of circulation. It is further proposed that some of the subcuticular granules may be lost with the old cuticle during moulting, and as mentioned earlier on in this discussion, copper has been shown to be associated with the shed larval skin.

In addition to the immobilisation of copper in the granules, there is also the possibility that some copper is excreted by P. conspersa. Indeed larvae in highly copper contaminated waters may excrete copper at a greater rate than larvae living in waters with a lower copper concentration, and this could give rise to the relationship between the concentration of copper in the larvae and in the water that is shown in Figures 7.5 and 7.6. Furthermore the excretion of copper may partly account for the significant decrease between the copper concentration in instar V larvae and in prepupae (see Table 7.10), and possibly between the amount of copper in prepupae and subsequent instars.

There are only a few studies on metal tolerance mechanisms in freshwater invertebrates, and one study of particular relevance to the present investigation is the work by Brown (1976; 1977b; 1978) who studied tolerance in Asellus meridianus, an isopod which occurs in the metal contaminated rivers Hayle and Gannel. In this species intracellular granules were observed in the hepatopancreas, and it was demonstrated that there is competition between copper and lead for inclusion into these granules. Furthermore adaptation to metals in A. meridianus is probably reinforced by a genetic influence, as tolerance to lead continued in the F2 generation cultured from individuals

collected from the lead contaminated River Gannel. However as P. conspersa as a species is metal tolerant, it is unlikely that copper tolerance in the population in the headwaters of the Darley Brook is a form of tolerance requiring genetic reinforcement within that population.

Future work

It is felt that future work on the Darley Brook should take the form of mainly laboratory-based studies on the aquatic plants and animals. For example, there are relatively few accounts in the literature on the toxicity of copper to freshwater macroinvertebrates, and although a 96 hour LC_{50} value was determined for instar V larvae of P. conspersa from station 1 (i.e. 3200 mg l^{-1} copper; see Figure 8.3), this is an area which warrants further investigation. It has been demonstrated that different larval instars of freshwater invertebrates have different sensitivities to copper (Anderson et al., 1980; Harrison et al., 1984; Nebeker et al., 1984a) and this could be investigated in P. conspersa. Furthermore the results of toxicity experiments on copper using larvae of P. conspersa from different populations could be compared. An examination is also required of the effect of modifying factors such as hardness and pH, which have been shown to affect the toxicity of copper to freshwater invertebrates (e.g. Learner & Edwards, 1963; Stephenson, 1983; Campbell & Stokes, 1985).

It would also be interesting to study the uptake of different chemical species of copper by P. conspersa larvae. Dodge and Theis (1979) demonstrated that copper uptake by larvae of Chironomus tentans was significantly inhibited by copper complexation with glycine and other organic ligands. By contrast, both free cadmium and cadmium bound to nitrilotriacetic acid was accumulated by larvae of Hydropsyche sp. (Dressing et al., 1982).

In further studies, larvae of P. conspersa might be exposed to elevated concentrations of metals other than copper, to determine the toxicity of these metals to this species. Such information would be useful in view of the reported occurrence of P. conspersa in other metal contaminated waters (see p.208). Furthermore if the larvae from these toxicity experiments were examined by ultrastructural techniques (such as X-ray microanalysis) then it could be determined whether the granules in the malpighian tubules and subcuticular region serve to store other metals in addition to copper. It has previously been mentioned in this discussion that Brown (1978) showed that granules in the cells of the hepatopancreas of A. meridianus are capable of storing either copper or lead.

The radioactive isotope ^{64}Cu may be used to study the rate of uptake of copper from the water by P. conspersa, and to determine if this rate varies in different larval instars and in different populations of P. conspersa. Using this isotope, Bryan (1974) demonstrated that copper-tolerant individuals of the polychaete Nereis diversicolor absorbed copper more quickly than non-tolerant individuals, suggesting that the copper-tolerant worms may be better able to chelate and detoxify copper as it enters the body. However it should be noted that the half-life of ^{64}Cu is relatively short (12.8 hours), limiting its use to short-term uptake experiments.

In the present investigation work has focused on P. conspersa and only a small amount of information has been collected on the other macroinvertebrates in the headwaters of the Darley Brook. This has included density estimates of the coleopteran larvae and adults at station 1 over a two year period (see Figure 6.11), and the measurement of copper concentrations in coleopteran and chironomid larvae at this station on two occasions (see Table 7.13). Clearly there is a need for

further investigation of these invertebrates, and the Coleoptera in particular are relatively easy to collect, identify and handle. Such an investigation could entail similar work to that undertaken on P. conspersa, i.e. the measurement of copper in individual larvae and adults, toxicological studies and examination by histological and ultrastructural techniques. In the latter case, it is interesting to note that Bayon and Martoja (1974) have recorded the presence of mineral concretions composed of several elements in the cells of the malpighian tubules and in the wall of the mesenteron in larvae of Agabus bipustulatus.

The metal resistances of green algae collected from metal contaminated rivers has been studied by Foster (1982b), who showed that Microspora stagnorum was very resistant to both copper and zinc. In view of this, it would be interesting to investigate the sensitivity of the aquatic vegetation at station 1 (which includes Microspora), where there is a particularly high level of copper. In addition, an ultrastructural study of these plants is required, especially as electron dense bodies (possibly containing copper and chromium) have been recorded in the aquatic moss Fontinalis antipyretica (Mouvet, 1984).

Finally, one compartment of the Darley Brook that was not investigated in the present study was that associated with the micro-organisms. Patrick and Loutit (1976) demonstrated that a number of metals (including copper) were concentrated by bacteria and subsequently passed on to the tubificid worms consuming these bacteria. Thus it would be instructive to investigate the response of the microbial community (including bacteria) to conditions in the Darley Brook, as well as any contribution they make to the processing and transfer of copper.

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APPENDIX 1

1A. Flame AAS operating conditions for water analysis

OPERATING CONDITIONS	METAL			
	COPPER	IRON	CALCIUM	MAGNESIUM
WAVELENGTH (nm)	324.7	248.2	422.7	285.2
LAMP CURRENT (mA)	4.0	5.0	4.0	4.0
SLIT WIDTH (nm)	0.5	0.2	0.5	0.5
INTEGRATION TIME (sec)	8.0	8.0	8.0	8.0
AIR/C_2H_2 FLOW (l min^{-1})	11.0/1.50	13.0/2.75	13.0/2.00	12.0/1.70
LINEAR WORKING RANGE (mg l^{-1})	0.05-1.00	0.05-0.50	1.00-5.00	0.10-0.50

Background correction found unnecessary

1B. Flame AAS operating conditions for sediment analysis

OPERATING CONDITIONS	METAL				
	COPPER	IRON	CALCIUM	MAGNESIUM	ALUMINIUM
WAVELENGTH (nm)	324.7	248.2	422.7	285.2	309.3
LAMP CURRENT (mA)	4.0	5.0	4.0	4.0	10.0
SLIT WIDTH (nm)	0.5	0.2	0.5	0.5	0.5
INTEGRATION TIME (sec)	8.0	8.0	8.0	8.0	8.0
N_2O/C_2H_2 FLOW (l min^{-1})	5.0/11.0	5.0/11.0	5.7/11.0	5.5/11.0	6.0/11.0
LINEAR WORKING RANGE (mg l^{-1})	0.05-2.00	0.05-0.50	0.10-2.00	0.10-0.50	1.00-10.00

Background correction found unnecessary

1C. Flame AAS operating conditions for plant analysis

OPERATING CONDITIONS	METAL				
	COPPER	IRON	CALCIUM	MAGNESIUM	ALUMINIUM
WAVELENGTH (nm)	324.7	248.2	422.7	285.2	309.3
LAMP CURRENT (mA)	4.0	5.0	4.0	4.0	10.0
SLIT WIDTH (nm)	0.5	0.2	0.5	0.5	0.5
INTEGRATION TIME (sec)	8.0	8.0	8.0	8.0	8.0
N_2O/C_2H_2 FLOW (l min^{-1})	5.0/11.0	5.0/11.0	5.7/11.0	5.5/11.0	6.0/11.0
LINEAR WORKING RANGE (mg l^{-1})	2.00-6.00	1.50-5.00	1.00-4.00	0.10-0.50	1.00-10.00

Background correction found unnecessary

1D. Graphite furnace AAS operating conditions for arsenic determination

WAVELENGTH	: 193.7 nm	LAMP CURRENT	: 9.0 mA
SLIT WIDTH	: 1.0 nm	INJECTION SIZE	: 25 µl
PURGE GAS	: Nitrogen	WORKING RANGE	: 1 to 10 ng ml^{-1}

DEUTERIUM BACKGROUND CORRECTION

TUBES COATED WITH PYROLYTIC GRAPHITE

PHASE	TEMPERATURE (°C)	TIME (sec)	RAMP
1	200	5.0	8
2	750	5.0	6
3	1400	5.0	6
4	2400	5.0	0

1E. Graphite furnace AAS operating conditions for copper determination

WAVELENGTH	: 327.4 nm	LAMP CURRENT	: 3.5 mA
SLIT WIDTH	: 1.0 nm	INJECTION SIZE	: 25 µl
PURGE GAS	: Nitrogen	WORKING RANGE	: 1 to 50 ng ml^{-1}

DEUTERIUM BACKGROUND CORRECTION

NON-COATED GRAPHITE TUBES

PHASE	TEMPERATURE (°C)	RAMP (x5 sec)
1	100	7
2	200	8
3	750	5
4	900	6
5	1800	0
6	1800	1

APPENDIX 2

2A. Concentrations of copper (mg l^{-1}) in unfiltered (UF) and filtered (F) water samples collected each month from January 1984 to July 1985 at stations 1 to 3.

		STATION 1		STATION 2		STATION 3	
		UF	F	UF	F	UF	F
1984	23/1	0.99	0.93	0.94	0.92	0.77	0.74
	20/2	0.79	0.77	0.69	0.69	0.57	0.55
	20/3	0.86	0.83	0.85	0.82	0.74	0.71
	25/4	0.88	0.85	0.68	0.66	0.64	0.63
	21/5	0.93	0.92	0.89	0.89	0.76	0.75
	18/6	0.86	0.83	0.71	0.69	0.61	0.58
	18/7	0.82	0.80	0.71	0.71	0.58	0.56
	20/8	0.78	0.77	0.69	0.67	0.59	0.52
	24/9	0.86	0.84	0.82	0.81	0.68	0.67
	19/10	0.86	0.85	0.82	0.81	0.70	0.68
	21/11	0.91	0.90	0.85	0.84	0.71	0.69
	13/12	0.95		0.86		0.74	
1985	17/1	0.89		0.84		0.73	
	20/2	0.82		0.77		0.64	
	15/3	0.91		0.87		0.73	
	17/4	0.80		0.77		0.65	
	17/5	0.87		0.83		0.69	
	21/6	0.79		0.74		0.59	
	18/7	0.74		0.70		0.57	

2A. Concentrations of copper (mg l^{-1}) in unfiltered water samples collected each month from August 1984 to July 1985 at stations A to E.

		STATIONS				
		A	B	C	D	E
1984	20/8	0.84		0.81		
	24/9	0.88		0.87		
	19/10	0.90		0.89		
	21/11	0.94	0.82	0.92		
	13/12	0.99	0.87	0.96		
1985	17/1	0.94	0.73	0.91		
	20/2	0.88	0.80	0.83		
	15/3	0.95		0.92		
	17/4	0.90		0.86	0.27	0.11
	17/5	0.87		0.87	0.32	0.11
	21/6	0.80		0.80	0.24	0.15
	18/7	0.76		0.75	0.32	0.13

2B. Concentrations of calcium (mg l^{-1}) in unfiltered (UF) and filtered (F) water samples collected each month from January 1984 to July 1985 at stations 1 to 4.

		STATION 1		STATION 2		STATION 3		STATION 4	
		UF	F	UF	F	UF	F	UF	F
1984	23/1	1.08	1.04	1.11	1.09	1.21	1.20	2.28	2.22
	20/2	1.23	1.23	1.26	1.24	1.40	1.36	2.93	2.89
	20/3	1.44	1.38	1.46	1.45	1.47	1.45	3.31	3.29
	25/4	1.93	1.92	3.09	3.06	1.72	1.68	3.79	3.78
	21/5	2.37	2.33	2.84	2.73	2.54	2.51	4.02	4.02
	18/6	1.47	1.43	1.38	1.37	1.71	1.65	2.42	2.40
	18/7	1.04	1.02	1.02	1.01	1.05	1.04	2.10	2.07
	20/8	1.32	1.29	1.29	1.28	1.38	1.36	3.08	3.00
	24/9	1.23	1.20	1.24	1.24	1.30	1.28	3.63	3.58
	19/10	1.49	1.49	1.48	1.47	1.55	1.53	2.82	2.78
	21/11	1.67	1.64	1.80	1.74	1.99	1.96	2.97	2.81
	13/12	1.53		1.70		1.51		2.89	
1985	17/1	1.41		1.49		1.45		2.76	
	20/2	1.34		1.35		1.36		2.33	
	15/3	1.11		1.15		1.11		2.07	
	17/4	1.36		1.45		1.40		2.53	
	17/5	1.21		1.29		1.27		2.19	
	21/6	1.21		1.31		1.31		2.57	
	18/7	1.35		1.41		1.39		2.94	

2B. Concentrations of calcium (mg l^{-1}) in unfiltered water samples collected each month from August 1984 to July 1985 at stations A to F.

		STATIONS					
		A	B	C	D	E	F
1984	20/8	1.34		1.33			
	24/9	1.24		1.24			
	19/10	1.50		1.49			
	21/11	1.70	1.55	1.68			
	13/12	1.54	1.45	1.53			
1985	17/1	1.43	1.35	1.41			
	20/2	1.37	1.27	1.36			
	15/3	1.12		1.11			
	17/4	1.40		1.38	1.80	2.72	2.84
	17/5	1.27		1.24	1.76	2.23	2.31
	21/6	1.30		1.24	1.70	2.40	2.42
	18/7	1.40		1.36	1.78	2.57	2.54

2C. Concentrations of magnesium (mg l^{-1}) in unfiltered (UF) and filtered (F) water samples collected each month from January 1984 to July 1985 at stations 1 to 4.

		STATION 1		STATION 2		STATION 3		STATION 4	
		UF	F	UF	F	UF	F	UF	F
1984	23/1	1.16	1.15	1.13	1.11	1.07	1.04	1.01	0.97
	20/2	1.36	1.20	1.27	1.22	1.25	1.17	1.05	1.02
	20/3	1.00	0.96	0.99	0.97	0.99	0.95	0.83	0.79
	25/4	1.00	0.95	1.00	0.98	0.93	0.90	0.80	0.75
	21/5	1.16	1.16	1.11	1.09	1.04	1.03	1.01	0.97
	18/6	1.05	1.05	1.00	1.00	1.00	1.00	0.90	0.90
	18/7	0.88	0.86	0.86	0.85	0.85	0.85	0.75	0.74
	20/8	1.04	0.99	1.01	1.01	1.01	1.01	0.96	0.92
	24/9	1.18	1.12	1.10	1.10	1.10	1.10	0.90	0.90
	19/10	1.02	0.99	0.99	0.99	0.99	0.99	0.89	0.89
	21/11	1.30	1.20	1.27	1.20	1.25	1.20	1.07	1.00
	13/12	1.24		1.22		1.19		1.05	
1985	17/1	1.12		1.10		1.10		1.00	
	20/2	1.10		1.00		1.00		0.95	
	15/3	1.00		0.97		0.90		0.80	
	17/4	1.05		1.00		0.97		0.85	
	17/5	1.10		1.10		1.10		0.90	
	21/6	0.90		0.83		0.80		0.75	
	18/7	1.20		1.20		1.20		0.90	

2C. Concentrations of magnesium (mg l^{-1}) in unfiltered water samples collected each month from August 1984 to July 1985 at stations A to F.

		STATIONS					
		A	B	C	D	E	F
1984	20/8	1.10		1.05			
	24/9	1.20		1.18			
	19/10	1.08		1.02			
	21/11	1.34	1.12	1.30			
	13/12	1.26	1.24	1.25			
1985	17/1	1.20	1.09	1.12			
	20/2	1.15	1.00	1.10			
	15/3	1.05		1.00			
	17/4	1.05		1.05	1.05	1.65	1.72
	17/5	1.15		1.10	1.20	1.75	1.75
	21/6	1.10		1.00	1.00	1.55	1.55
	18/7	1.30		1.20	1.30	1.73	1.80

2D. Conductivity ($\mu S\ cm^{-1}$) expressed as the K_{25} value measured each month from January 1984 to July 1985 at stations 1 to 4 and A to C.

		STATIONS						
		1	2	3	4	A	B	C
1984	23/1	85	84	78	84			
	20/2	87	88	82	88			
	20/3	74	75	65	76			
	25/4	77	67	51	54			
	21/5	66	65	58	73			
	18/6	54	52	52	67			
	18/7	62	60	57	69			
	20/8	52	56	52	70	53		52
	24/9	70	68	62	78	70		70
	19/10	86	88	76	88	86		86
	21/11	78	80	72	78	80	77	79
	13/12	70	65	75	86	78	75	78
1985	17/1	78	77	69	85	76	71	76
	20/2	82	81	72	88	83	78	82
	15/3	76	76	68	86	76		75
	17/4	92	92	79	88	93		92
	17/5	101	90	86	102	100		100
	21/6	82	82	75	85	80		80
	18/7	72	72	64	74	71		72

2E. pH measured in the water each month from January 1984 to July 1985 at stations 1 to 4 and A to C.

		STATIONS						
		1	2	3	4	A	B	C
1984	23/1	5.1	5.1	5.1	5.7			
	20/2	5.0	5.0	5.0	5.9			
	20/3	5.7	5.7	6.1	7.4			
	25/4	6.0	6.0	6.1	7.0			
	21/5	5.4	5.4	5.6	6.4			
	18/6	6.1	6.4	6.4	6.9			
	18/7	5.4	5.4	5.7	6.8			
	20/8	5.7	5.7	6.0	6.0	5.3		5.5
	24/9	6.1	6.3	6.4	6.9	5.6		5.7
	19/10	5.4	5.5	5.7	6.1	5.0		5.3
	21/11	5.4	5.4	5.8	6.0	5.0	5.4	5.2
	13/12	5.3	5.5	5.5	6.1	5.2	5.5	5.3
1985	17/1	4.9	5.0	5.1	6.1	4.9	5.2	4.9
	20/2	5.4	5.4	5.4	5.9	5.1	5.7	5.1
	15/3	5.4	5.7	5.7	5.8	5.2		5.3
	17/4	5.6	5.6	5.7	6.1	5.1		5.5
	17/5	5.8	5.8	5.9	6.4	5.3		5.4
	21/6	6.0	6.0	6.1	7.1	5.6		5.8
	18/7	5.7	5.7	5.8	6.6	5.4		5.6

2F. Dissolved oxygen (mg l^{-1}) measured each month from January 1984 to July 1985 at stations 1 to 4 and A to C.

		STATIONS						
		1	2	3	4	A	B	C
1984	23/1	9.57	10.73	10.30	10.81			
	20/2	9.20	10.90	11.04	10.90			
	20/3	9.89	12.20	11.67	12.87			
	25/4	9.35	11.10	10.49	11.24			
	21/5	9.09	9.98	7.78	10.52			
	18/6	10.01	10.04	7.85	9.91			
	18/7	9.37	10.07	9.32	10.01			
	20/8	11.02	10.45	7.19	8.60	6.42		7.92
	24/9	9.77	10.51	8.41	9.13	5.57		7.37
	19/10	8.62	9.30	10.08	9.84	4.63		6.43
	21/11	10.25	11.42	11.48	11.48	5.85	11.05	7.55
	13/12	9.02	10.02	10.61	10.55	4.95	10.08	7.55
1985	17/1	7.67	9.83	10.05	10.72	4.35	8.87	5.90
	20/2	9.04	9.92	10.31	10.85	4.82	10.55	7.06
	15/3	8.51	10.21	10.15	10.75	4.48		6.25
	17/4	10.04	10.90	10.85	11.27	6.29		8.72
	17/5	9.66	10.90	10.54	11.25	4.80		6.25
	21/6	8.20	9.90	10.39	9.70	5.62		6.35
	18/7	8.53	10.50	10.59	10.08	4.48		6.03

2G. Percentage oxygen saturation measured each month from January 1984 to July 1985 at stations 1 to 4 and A to C.

		STATIONS						
		1	2	3	4	A	B	C
1984	23/1	82.7	92.4	89.2	91.8			
	20/2	81.7	96.3	97.1	95.4			
	20/3	89.1	108.3	103.4	110.4			
	25/4	86.6	106.4	98.3	106.5			
	21/5	84.2	97.5	72.0	97.8			
	18/6	96.1	100.3	78.7	96.4			
	18/7	87.6	100.3	91.8	95.3			
	20/8	105.8	108.2	72.3	84.8	47.8		62.7
	24/9	90.2	94.1	74.3	84.6	45.2		62.1
	19/10	79.2	85.7	92.3	91.1	42.2		60.2
	21/11	91.8	101.7	103.4	103.0	51.3	100.1	67.2
	13/12	81.4	90.0	95.5	94.8	44.2	91.0	67.1
1985	17/1	69.5	87.3	88.1	93.6	39.8	77.8	53.3
	20/2	81.4	89.4	91.7	94.6	43.0	94.1	63.5
	15/3	76.9	93.7	89.9	96.2	40.1		56.0
	17/4	91.3	102.5	100.2	100.5	55.8		78.8
	17/5	89.4	103.4	101.7	104.3	43.3		56.6
	21/6	74.6	92.6	97.3	90.9	50.5		56.9
	18/7	78.7	98.2	98.5	94.4	40.0		54.7

2H. Water temperatures (°C) measured each month from January 1984 to July 1985 at stations 1 to 4 and A to C.

		STATIONS						
		1	2	3	4	A	B	C
1984	23/1	9.9	9.7	9.7	9.1			
	20/2	10.3	9.9	9.9	9.4			
	20/3	10.9	10.5	10.1	9.8			
	25/4	12.2	12.2	12.2	11.3			
	21/5	12.5	12.9	12.7	12.3			
	18/6	13.3	15.0	15.4	12.8			
	18/7	13.5	15.3	15.9	13.4			
	20/8	13.7	16.3	16.9	14.5	10.9		10.9
	24/9	11.7	12.8	13.6	12.2	10.9		10.8
	19/10	11.4	11.6	11.5	11.9	10.7		10.7
	21/11	10.7	10.6	10.5	10.7	10.8	10.5	10.6
	13/12	10.7	10.4	9.8	10.5	10.8	10.7	10.7
1985	17/1	10.2	9.3	9.0	8.9	10.7	10.5	10.5
	20/2	10.6	10.4	10.1	9.3	10.7	10.2	10.6
	15/3	10.8	11.1	10.4	9.7	10.9		10.8
	17/4	11.3	12.0	11.6	10.1	10.7		11.0
	17/5	12.1	12.8	13.6	11.8	11.0		11.3
	21/6	12.5	13.6	14.4	12.4	10.9		11.5
	18/7	13.0	14.8	15.8	13.0	11.1		11.6

2I. Total inorganic phosphate (mg l^{-1}) and total oxidised nitrogen (mg l^{-1}) measured in unfiltered non-acidified water samples collected each month from November 1984 to August 1985, and in October 1985, at stations 1 to 4.

		TOTAL INORGANIC PHOSPHATE (mg l^{-1})				TOTAL OXIDISED NITROGEN (mg l^{-1})			
		STATIONS				STATIONS			
		1	2	3	4	1	2	3	4
1984	21/11	0.03	0.03	0.025	0.05	1.20	1.40	1.40	2.00
	13/12	0.03	0.03	0.03	0.04	0.90	1.00	1.10	1.65
1985	17/1	0.05	0.03	0.02	0.04	1.10	2.50	1.30	1.50
	6/2	0.035	0.03	0.02	0.05	1.00	1.50	1.10	1.70
	19/2	0.03	0.03	0.03	0.06	0.90	0.90	1.00	1.45
	15/3	0.02	0.02	0.02	0.06	1.65	1.00	1.35	1.75
	17/4					1.10	1.65	1.25	1.55
	17/5	0.01	0.02	0.01	0.04	0.95	0.95	1.10	1.80
	21/6	0.04	0.05	0.04	0.09	1.10	1.05	1.55	3.20
	18/7	0.02	0.02	0.02	0.02	1.70	1.70	2.30	3.30
	16/8	0.01	0.01	0.01	0.03	1.20	1.50	2.20	2.10
	14/10	0.06	0.04	0.04	0.08	1.50	1.50	1.50	1.75

2I. Total inorganic phosphate (mg l^{-1}) and total oxidised nitrogen (mg l^{-1}) measured in unfiltered non-acidified water samples collected each month from November 1984 to August 1985, and in October 1985, at stations A and C to F.

		TOTAL INORGANIC PHOSPHATE (mg l^{-1})					TOTAL OXIDISED NITROGEN (mg l^{-1})				
		STATIONS					STATIONS				
		A	C	D	E	F	A	C	D	E	F
1984	21/11	0.04	0.03	0.025	0.03		1.20	1.30	2.10	3.25	
	13/12	0.04	0.03	0.025	0.035		1.00	1.10	1.90	3.35	
1985	17/1	0.05	0.04				1.20	1.15			
	6/2	0.02	0.02	0.025	0.03	0.025	1.10	1.25	1.75	3.20	3.40
	19/2	0.04		0.03	0.04	0.04	1.00		1.50	3.50	4.15
	15/3	0.03	0.02	0.02	0.03	0.03	1.30	1.20	1.30	3.00	3.35
	17/4						1.20	2.05	1.75	3.00	3.30
	17/5	0.03	0.02	0.01	0.01	0.01	1.30	1.15	2.25	2.95	3.00
	21/6	0.05	0.05	0.04	0.03	0.03	1.00	0.95	2.00	2.90	2.95
	18/7	0.06	0.03	0.02	0.045	0.04	1.50	1.60	3.10	3.80	4.10
	16/8	0.04	0.02	0.02	0.01	0.03	1.50	1.50	2.70	3.20	3.30
	14/10	0.04	0.04	0.03	0.06	0.05	1.35	1.40	3.00	3.75	3.65

APPENDIX 3

3A. Leachable and exchangeable copper concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 1 and 2, together with a mean concentration and standard error (S.E.).

STATION 1	LEACHABLE							MEAN	S.E.
1984	23/1	1894	2131	2566	2070	2380	1904	2158	109
	25/4	1948	2377	2465	1840	1658	1935	2037	129
	18/7	2107	2248	2263	2434	2082	2177	2218	52
	19/10	2370	2277	2097	2311	1926	2086	2177	69
1985	17/1	2235	2325	2480	2368	2243	2384	2339	38
	17/4	1825	1888	1889	2076	2221	2161	2010	67
	18/7	2351	2106	1705	2156	1877	2005	2033	92
STATION 1	EXCHANGEABLE								
1984	23/1	669	790	620	698	737	634	691	26
	25/4	730	782	698	677	669	597	692	25
	18/7	787	744	808	850	750	851	798	19
	19/10	818	749	783	743	700	776	762	16
1985	17/1	743	799	757	799	759	773	771	9
	17/4	711	660	670	708	740	842	721	27
	18/7	753	572	602	587	587	722	637	32
STATION 2	LEACHABLE								
1984	23/1	2168	2050	2079	2201	2145	2198	2140	26
	25/4	2303	1822	2581	2534	2049	2372	2276	119
	18/7	2062	2649	2224	2222	2561	2457	2362	93
	19/10	2347	2455	2237	2222	2457	2198	2319	48
1985	17/1	2387	2595	2312	2435	2672	2635	2506	60
	17/4	2061	2102	2287	2170	2226	2021	2144	41
	18/7	2236	2544	2522	2489	2333	2087	2368	75
STATION 2	EXCHANGEABLE								
1984	23/1	949	1035	935	954	971	901	958	18
	25/4	966	1054	994	1201	956	1154	1054	42
	18/7	1051	1159	1257	1047	1193	1170	1146	34
	19/10	1148	1028	1010	930	1178	951	1040	41
1985	17/1	1172	1148	1283	1185	1220	1205	1202	19
	17/4	1029	898	970	1116	833	1073	986	44
	18/7	1051	1247	1236	1170	987	981	1112	49

3A. Leachable and exchangeable copper concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 3 and 4, together with a mean concentration and standard error (S.E.).

STATION 3	LEACHABLE							MEAN	S.E.
1984	23/1	6524	6726	7004	7124	7431	7081	6981	130
	25/4	6826	6264	6172	7082	6886	7156	6731	170
	18/7	7179	7619	7133	6824	7519	6994	7211	125
	19/10	6587	7422	7031	7279	7055	6602	6996	140
1985	17/1	6978	7181	6718	7063	7219	7026	7030	73
	17/4	6751	7068	6854	7295	7097	7252	7002	88
	18/7	7163	7226	7268	6170	7080	6742	6941	172
STATION 3	EXCHANGEABLE								
1984	23/1	2045	2264	1923	2046	2255	2033	2094	56
	25/4	2046	1719	1986	1808	2035	1713	1885	64
	18/7	2203	2052	2080	2036	2124	2049	2090	26
	19/10	1867	1815	1908	1918	1802	1924	1872	22
1985	17/1	2195	2153	2190	2160	2115	2263	2179	20
	17/4	2030	1862	1920	2077	2083	2284	2042	60
	18/7	2449	2139	2069	2151	2020	2013	2139	66
STATION 4	LEACHABLE								
1984	23/1	108	100	98	122	114	109	109	4
	25/4	107	113	92	93	103	97	101	3
	18/7	109	84	90	120	88	96	98	6
	19/10	86	90	84	82	76	93	85	2
1985	17/1	100	94	102	107	105	98	101	2
	17/4	86	102	100	97	100	107	99	3
	18/7	100	124	105	109	119	120	113	4
STATION 4	EXCHANGEABLE								
1984	23/1	9	17	13	14	16	14	14	1
	25/4	11	11	10	10	9	10	10	1
	18/7	18	14	11	12	15	15	14	1
	19/10	9	12	12	9	10	15	11	1
1985	17/1	14	10	15	13	13	15	13	1
	17/4	8	11	12	9	11	15	11	1
	18/7	12	13	15	18	19	18	16	1

3B. Leachable and exchangeable iron concentrations($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 1 and 2, together with a mean concentration and standard error (S.E.).

STATION 1	LEACHABLE							MEAN	S.E.
1984	23/1	19817	20574	22051	19226	17954	19986	19934	557
	25/4	17272	15690	20258	20474	19493	17915	18517	768
	18/7	20433	21323	24649	22565	22573	23664	22534	623
	19/10	20113	19712	18988	20997	19189	18497	19582	364
1985	17/1	20988	21175	20449	19971	22149	20995	20954	300
	17/4	20002	21925	20403	22828	19871	19442	20745	563
	18/7	18666	18600	17580	18409	23333	17124	18952	911

STATION 1	EXCHANGEABLE								
1984	23/1	63	58	60	60	78	50	62	4
	25/4	65	85	60	67	87	89	76	5
	18/7	55	66	61	77	65	70	66	3
	19/10	84	79	58	98	72	80	79	5
1985	17/1	82	66	82	85	100	83	83	4
	17/4	54	61	88	82	66	61	69	6
	18/7	56	93	70	82	76	68	74	5

STATION 2	LEACHABLE								
1984	23/1	17720	15438	16227	17472	19333	16952	17190	548
	25/4	18784	17028	18553	17558	15382	16966	17378	505
	18/7	18642	18025	19208	19725	18525	17965	18681	279
	19/10	16166	15419	16504	17800	16587	15972	16408	327
1985	17/1	15768	15372	15674	17725	18082	18016	16772	527
	17/4	17156	17470	17428	18220	19244	16919	17739	350
	18/7	16450	20652	15189	16798	19132	17326	17591	806

STATION 2	EXCHANGEABLE								
1984	23/1	593	435	570	491	556	499	524	24
	25/4	735	646	606	700	678	744	684	22
	18/7	505	622	657	536	516	556	565	25
	19/10	791	967	844	745	875	781	834	33
1985	17/1	636	604	709	730	676	665	670	19
	17/4	699	601	537	630	573	628	611	23
	18/7	549	733	513	634	742	681	642	39

3B. Leachable and exchangeable iron concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 3 and 4, together with a mean concentration and standard error (S.E.).

STATION 3	LEACHABLE							MEAN	S.E.
1984	23/1	15314	15938	16000	18058	17397	16313	16503	418
	25/4	17998	15881	14670	13528	16222	14181	15413	663
	18/7	17233	17399	16057	16619	17703	18154	17194	308
	19/10	13933	14083	13866	15484	15039	13770	14362	293
1985	17/1	16846	13239	15478	14624	16237	15573	15332	519
	17/4	16337	14439	14669	14013	13961	14992	14735	358
	18/7	12987	12641	15898	14574	12560	17088	14291	772
STATION 3	EXCHANGEABLE								
1984	23/1	46	51	61	61	43	53	53	3
	25/4	82	62	84	77	61	57	71	5
	18/7	94	68	92	70	84	72	80	5
	19/10	49	81	69	71	56	52	63	5
1985	17/1	56	35	36	44	57	50	46	4
	17/4	71	66	53	62	56	53	60	3
	18/7	72	70	54	66	46	84	65	6
STATION 4	LEACHABLE								
1984	23/1	14233	13372	15250	14789	15308	14519	14578	295
	25/4	15552	13823	13508	14856	13898	12738	14062	408
	18/7	16782	14983	15668	15996	16068	14700	15699	311
	19/10	13722	12400	14604	14804	14070	13868	13911	347
1985	17/1	15244	15707	15615	15903	16722	16078	15878	204
	17/4	13341	14569	13601	14743	13313	14033	13933	253
	18/7	11966	15441	14128	13559	12634	13408	13522	492
STATION 4	EXCHANGEABLE								
1984	23/1	84	82	90	104	99	80	90	4
	25/4	94	110	83	82	77	93	90	5
	18/7	88	82	77	78	84	91	83	2
	19/10	62	54	64	55	53	61	58	2
1985	17/1	78	82	94	87	90	79	85	3
	17/4	61	68	59	50	65	65	61	3
	18/7	48	72	61	67	42	46	56	5

3C. Leachable and exchangeable arsenic concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 1 and 2, together with a mean concentration and standard error (S.E.).

STATION 1	LEACHABLE							MEAN	S.E.
1984	23/1	368	292	342	362	367	303	339	14
	25/4	305	302	311	282	265	269	289	8
	18/7	322	305	314	300	332	364	322	9
	19/10	256	278	243	301	271	250	266	9
1985	17/1	313	313	323	400	345	324	336	13
	17/4	287	276	333	300	331	340	311	11
	18/7	230	270	286	262	245	274	261	8
STATION 1	EXCHANGEABLE								
1984	23/1	4.4	4.6	4.1	4.3	4.0	4.8	4.4	0.1
	25/4	3.1	3.4	4.4	3.9	3.4	3.5	3.6	0.2
	18/7	7.5	6.2	5.3	6.5	6.1	6.5	6.4	0.3
	19/10	5.5	6.1	5.8	5.0	4.7	4.8	5.3	0.2
1985	17/1	4.5	3.8	4.6	4.6	4.0	4.1	4.3	0.1
	17/4	3.3	2.8	4.8	3.5	4.9	4.0	3.9	0.3
	18/7	2.3	2.7	2.9	2.6	2.5	2.9	2.7	0.1
STATION 2	LEACHABLE								
1984	23/1	90	106	114	87	99	93	98	4
	25/4	120	91	103	98	85	99	99	5
	18/7	150	141	134	159	125	127	139	5
	19/10	104	87	74	98	121	116	100	7
1985	17/1	178	152	134	118	170	141	148	9
	17/4	101	130	97	129	120	111	114	6
	18/7	143	132	138	158	121	104	132	8
STATION 2	EXCHANGEABLE								
1984	23/1	36	39	43	42	51	54	44	3
	25/4	54	38	35	53	50	57	47	4
	18/7	68	73	65	62	52	70	65	3
	19/10	39	44	52	55	44	52	47	2
1985	17/1	65	72	78	72	76	65	71	2
	17/4	56	54	51	65	50	58	55	2
	18/7	65	60	69	79	55	41	61	5

3C. Leachable and exchangeable arsenic concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 3 and 4, together with a mean concentration and standard error (S.E.).

								MEAN	S.E.
STATION 3	LEACHABLE								
1984	23/1	154	143	135	124	137	140	138	4
	25/4	180	135	148	138	137	161	149	7
	18/7	171	201	202	217	176	189	192	7
	19/10	188	160	152	143	129	147	153	8
1985	17/1	221	173	221	159	186	178	189	10
	17/4	164	141	161	187	179	179	168	7
	18/7	199	148	198	209	176	205	189	9
STATION 3	EXCHANGEABLE								
1984	23/1	1.5	1.6	1.8	1.7	2.0	2.1	1.8	0.1
	25/4	5.6	4.5	6.5	5.4	4.5	4.3	5.1	0.3
	18/7	2.0	2.0	2.1	1.7	1.6	1.9	1.9	0.1
	19/10	3.3	2.5	3.5	2.7	2.6	4.1	3.1	0.3
1985	17/1	4.0	4.5	3.2	3.9	3.9	4.0	3.9	0.2
	17/4	2.6	3.5	2.7	3.8	3.8	2.7	3.2	0.2
	18/7	2.1	2.6	3.0	3.4	2.0	3.6	2.8	0.3
STATION 4	LEACHABLE								
1984	23/1	20	17	16	21	23	18	19	1
	25/4	16	12	10	13	19	11	14	1
	18/7	19	19	16	22	20	13	18	1
	19/10	20	17	16	26	19	24	20	1
1985	17/1	20	24	20	22	27	27	23	1
	17/4	14	19	22	27	20	24	21	2
	18/7	20	16	23	26	23	24	22	1
STATION 4	EXCHANGEABLE								
1984	23/1	2.1	1.7	1.3	1.7	1.5	1.9	1.7	0.1
	25/4	1.5	1.7	0.9	1.0	1.2	1.5	1.3	0.1
	18/7	1.6	1.8	2.2	1.6	1.4	1.8	1.7	0.1
	19/10	3.0	1.8	2.0	1.8	2.4	1.8	2.1	0.2
1985	17/1	2.7	2.4	2.9	1.9	2.2	2.7	2.5	0.2
	17/4	1.8	2.7	2.5	2.3	1.6	2.0	2.2	0.2
	18/7	2.3	1.8	1.8	2.0	1.8	2.7	2.1	0.1

3D. Leachable and exchangeable calcium concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 1 and 2, together with a mean concentration and standard error (S.E.).

								MEAN	S.E.
STATION 1	LEACHABLE								
1984	23/1	311	358	298	293	330	351	323	11
	25/4	278	301	322	294	305	275	295	7
	18/7	333	293	291	300	304	300	304	6
	19/10	268	239	246	293	270	304	270	10
1985	17/1	296	328	318	346	310	312	318	7
	17/4	362	313	340	358	334	331	339	7
	18/7	288	300	310	271	352	304	304	11
STATION 1	EXCHANGEABLE								
1984	23/1	43	51	60	63	57	41	53	4
	25/4	64	40	52	52	43	51	50	3
	18/7	61	55	52	41	47	76	55	5
	19/10	56	43	48	40	58	42	48	3
1985	17/1	68	69	64	46	61	57	61	3
	17/4	40	46	64	70	45	55	53	5
	18/7	58	60	57	53	50	74	59	3
STATION 2	LEACHABLE								
1984	23/1	350	329	371	344	383	396	362	10
	25/4	342	356	365	282	309	330	330	12
	18/7	351	379	305	346	355	387	353	12
	19/10	339	293	335	353	312	303	322	9
1985	17/1	354	401	341	337	362	339	355	10
	17/4	371	339	343	351	359	346	351	5
	18/7	366	332	324	348	318	280	328	12
STATION 2	EXCHANGEABLE								
1984	23/1	94	79	107	85	110	71	91	6
	25/4	103	80	109	91	81	84	91	5
	18/7	89	103	78	80	91	84	88	4
	19/10	88	105	72	83	91	84	87	4
1985	17/1	87	91	76	116	88	104	94	6
	17/4	80	97	86	100	85	96	90	3
	18/7	62	83	106	81	74	70	79	6

3D. Leachable and exchangeable calcium concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 3 and 4, together with a mean concentration and standard error (S.E.).

								MEAN	S.E.
STATION 3	LEACHABLE								
1984	23/1	234	293	269	282	270	264	268	8
	25/4	224	257	223	237	293	267	250	11
	18/7	251	273	262	288	242	248	260	7
	19/10	256	287	277	230	246	241	256	9
1985	17/1	223	293	217	202	237	228	233	13
	17/4	265	290	272	259	213	256	259	10
	18/7	234	271	275	286	249	196	251	13
STATION 3	EXCHANGEABLE								
1984	23/1	36	47	36	57	37	61	46	4
	25/4	52	50	51	39	40	42	46	2
	18/7	41	37	66	58	62	50	52	5
	19/10	53	50	48	36	41	38	44	3
1985	17/1	55	44	45	39	49	52	47	2
	17/4	42	50	50	49	45	34	45	2
	18/7	41	41	45	54	61	57	50	3
STATION 4	LEACHABLE								
1984	23/1	749	776	795	697	745	700	743	16
	25/4	650	682	736	711	640	651	678	16
	18/7	766	739	664	657	681	764	711	20
	19/10	655	717	694	697	619	628	668	16
1985	17/1	688	755	771	670	679	671	705	18
	17/4	710	723	608	676	710	660	681	17
	18/7	767	794	728	664	794	717	744	21
STATION 4	EXCHANGEABLE								
1984	23/1	229	181	192	206	214	162	197	10
	25/4	161	163	181	205	177	213	183	9
	18/7	165	157	168	157	179	202	171	7
	19/10	197	183	215	171	185	193	190	6
1985	17/1	176	183	208	159	172	159	176	7
	17/4	170	178	173	170	194	171	176	4
	18/7	215	211	184	179	202	209	200	6

3E. Leachable and exchangeable magnesium concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 1 and 2, together with a mean concentration and standard error (S.E.).

STATION 1	LEACHABLE							MEAN	S.E.
1984	23/1	385	414	378	392	410	370	391	7
	25/4	369	351	371	417	411	358	379	11
	18/7	412	370	407	433	400	414	406	8
	19/10	314	333	395	387	410	342	363	16
1985	17/1	338	354	323	363	307	322	334	9
	17/4	332	339	346	393	416	392	369	14
	18/7	320	327	376	347	359	406	355	13
STATION 1	EXCHANGEABLE								
1984	23/1	15	12	17	18	15	19	16	1
	25/4	15	13	9	11	8	12	11	1
	18/7	16	15	22	24	21	19	20	1
	19/10	20	17	16	15	16	19	17	1
1985	17/1	12	14	12	14	21	10	14	1
	17/4	8	9	14	14	10	13	11	1
	18/7	11	10	12	12	14	16	13	1
STATION 2	LEACHABLE								
1984	23/1	326	388	345	358	388	350	359	10
	25/4	324	342	366	391	331	339	349	10
	18/7	322	418	381	393	340	380	372	14
	19/10	402	334	374	324	332	367	355	12
1985	17/1	294	262	276	295	326	307	293	9
	17/4	321	352	351	407	371	356	359	11
	18/7	278	314	342	306	311	322	312	8
STATION 2	EXCHANGEABLE								
1984	23/1	16	17	18	25	14	17	18	1
	25/4	18	17	13	9	11	14	14	1
	18/7	15	22	12	11	10	12	14	2
	19/10	20	29	17	15	18	18	20	2
1985	17/1	20	17	15	19	20	18	18	1
	17/4	13	17	14	20	14	16	16	1
	18/7	9	16	16	13	14	13	14	1

3E. Leachable and exchangeable magnesium concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January 1984 to July 1985 at stations 3 and 4, together with a mean concentration and standard error (S.E.).

STATION 3	LEACHABLE							MEAN	S.E.
1984	23/1	320	344	366	287	338	314	328	11
	25/4	321	343	280	361	297	304	317	12
	18/7	310	360	349	390	274	349	338	17
	19/10	270	307	330	316	269	320	302	11
1985	17/1	278	218	257	249	309	320	271	16
	17/4	360	304	356	292	305	358	329	13
	18/7	259	286	253	328	319	350	299	16

STATION 3	EXCHANGEABLE								
1984	23/1	17	13	10	12	11	15	13	1
	25/4	14	17	14	18	20	19	17	1
	18/7	12	19	19	13	13	18	16	1
	19/10	20	19	25	20	21	22	21	1
1985	17/1	15	12	10	15	10	17	13	1
	17/4	14	14	18	15	10	8	13	1
	18/7	7	20	11	14	13	15	13	2

STATION 4	LEACHABLE								
1984	23/1	1504	1642	1566	1585	1692	1671	1610	29
	25/4	1606	1471	1513	1643	1520	1558	1551	26
	18/7	1702	1719	1627	1671	1551	1583	1642	27
	19/10	1366	1325	1343	1465	1428	1328	1375	23
1985	17/1	1428	1596	1432	1476	1478	1548	1493	27
	17/4	1487	1564	1487	1447	1514	1531	1505	17
	18/7	1435	1457	1381	1355	1528	1469	1437	26

STATION 4	EXCHANGEABLE								
1984	23/1	24	26	33	25	26	37	29	2
	25/4	37	40	35	46	43	40	40	1
	18/7	32	29	32	31	41	37	33	2
	19/10	37	32	45	33	30	40	36	2
1985	17/1	39	44	43	41	54	40	43	2
	17/4	22	23	22	20	26	24	23	1
	18/7	25	31	18	21	28	27	25	2

3F. Leachable and exchangeable aluminium concentrations (μg g^{-1}) in six sediment sample units taken quarterly from January to July 1985 at stations 1 and 2, together with a mean concentration and standard error (S.E.).

								MEAN	S.E.
STATION 1		LEACHABLE							
1985	17/1	9287	8964	9386	9579	9221	9538	9329	92
	17/4	8766	9413	8922	9601	9442	9331	9245	133
	18/7	10020	9060	9131	10044	10315	9922	9748	213
STATION 1		EXCHANGEABLE							
1985	17/1	1685	1562	1842	1703	1544	1679	1669	44
	17/4	1830	1656	1679	1695	1586	1835	1713	40
	18/7	2042	1814	1825	2008	1983	2040	1952	43
STATION 2		LEACHABLE							
1985	17/1	8078	7897	8140	8373	8441	8328	8209	84
	17/4	8164	8885	8366	8521	8502	8651	8514	100
	18/7	8778	9541	8654	8298	8288	9019	8763	194
STATION 2		EXCHANGEABLE							
1985	17/1	1740	1494	1715	1602	1819	1479	1641	57
	17/4	1427	1652	1416	1730	1545	1512	1547	51
	18/7	1630	1785	1759	1733	1649	1792	1724	28

3F. Leachable and exchangeable aluminium concentrations ($\mu g\ g^{-1}$) in six sediment sample units taken quarterly from January to July 1985 at stations 3 and 4, together with a mean concentration and standard error (S.E.).

								MEAN	S.E.
STATION 3		LEACHABLE							
1985	17/1	41318	38823	40315	39480	40688	41045	40278	391
	17/4	38407	37358	37947	38639	39033	39917	38550	361
	18/7	41978	39756	40735	40319	40461	39536	40464	353
STATION 3		EXCHANGEABLE							
1985	17/1	4016	4079	3557	3947	4138	3923	3943	84
	17/4	4105	3663	3525	3944	4040	3605	3813	100
	18/7	4408	3975	3862	4149	4034	4055	4080	76
STATION 4		LEACHABLE							
1985	17/1	8430	8696	8907	8854	8451	8559	8649	83
	17/4	7801	8264	7878	8088	7756	8251	8006	92
	18/7	8908	7742	8759	7892	9064	8677	8507	225
STATION 4		EXCHANGEABLE							
1985	17/1	775	841	1099	939	811	765	871	52
	17/4	909	1054	974	919	995	1140	998	36
	18/7	1001	1180	1070	870	1183	1000	1050	49

APPENDIX 4

4A. Copper concentrations ($\mu g\ g^{-1}$) in four replicate samples of Jungermannia atrovirens taken each month from January 1984 to July 1985 at station 1, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
1984	23/1	2085	2042	2033	2126	2072	22
	20/2	2146	2031	2154	2104	2109	28
	20/3	2223	2207	2252	2206	2222	11
	25/4	2165	2188	2136	2084	2143	23
	21/5	2192	2155	2211	2164	2181	13
	18/6	2536	2342	2445	2375	2425	43
	18/7	2288	2283	2367	2304	2311	20
	20/8	2396	2315	2358	2405	2369	21
	24/9	2195	2166	2191	2179	2183	7
	19/10	2269	2277	2216	2220	2246	16
	21/11	2112	2129	2110	2147	2125	9
	13/12	2129	2211	2231	2266	2209	29
1985	17/1	2388	2148	2158	2314	2252	59
	20/2	2134	2112	2149	2192	2147	17
	15/3	2120	2203	2342	2140	2201	50
	17/4	2432	2394	2430	2417	2418	9
	17/5	2247	2192	2379	2266	2271	40
	21/6	2262	2346	2403	2443	2364	40
	18/7	2435	2416	2592	2596	2510	49

4B. Copper concentrations ($\mu g\ g^{-1}$) in four replicate samples of Microspora sp. (collected at station 1) and in the root and shoot fractions of Juncus bulbosus (collected at station 2) taken quarterly from January 1984 to July 1985, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
Microspora sp.							
1984	23/1	2438	2558	2299	2328	2406	59
	25/4	2664	2566	2583	2656	2617	25
	18/7	2689	2781	2566	2562	2650	53
	19/10	2548	2448	2452	2421	2467	28
1985	17/1	2579	2775	2643	2616	2653	43
	17/4	2469	2490	2680	2523	2541	48
	18/7	2719	2866	2930	2779	2824	47
Juncus bulbosus root							
1984	23/1	2971	3259	3293	2990	3128	86
	25/4	3149	3405	3236	3348	3285	57
	18/7	3664	3539	3481	3548	3558	39
	19/10	2802	2894	3094	2930	2930	61
1985	17/1	3486	3291	3306	3348	3358	45
	17/4	3413	3232	3253	3305	3301	41
	18/7	3424	3348	3615	3504	3473	57
Juncus bulbosus shoot							
1984	23/1	974	984	1047	1017	1006	17
	25/4	959	1079	1008	1053	1025	27
	18/7	1064	1056	1199	1138	1114	34
	19/10	1043	1129	1044	1118	1084	23
1985	17/1	1068	979	1024	1042	1028	19
	17/4	1136	1026	1042	1111	1079	27
	18/7	1186	1255	1206	1067	1179	40

4C. Iron concentrations ($\mu g\ g^{-1}$) in four replicate samples of Jungermannia atrovirens taken each month from January 1984 to July 1985 at station 1, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
1984	23/1	4076	3865	3863	4098	3976	65
	20/2	4112	3985	3795	3862	3939	70
	20/3	4022	3984	4384	3910	4075	106
	25/4	4120	4166	4077	4282	4161	44
	21/5	3625	4294	4238	3925	4021	155
	18/6	4185	4258	4165	4302	4228	32
	18/7	4604	4226	4395	4514	4435	82
	20/8	4080	4508	4255	4144	4247	95
	24/9	3939	3862	3892	4001	3924	31
	19/10	4204	3840	3953	4024	4005	77
	21/11	4041	3915	3982	4131	4017	46
	13/12	3535	4126	4038	3915	3904	130
1985	17/1	4462	3916	4292	4063	4183	121
	20/2	3972	4198	4070	3729	3992	99
	15/3	3789	3771	4209	3685	3864	118
	17/4	4516	4298	4316	4192	4331	68
	17/5	4107	3904	3953	4644	4152	170
	21/6	4012	4209	4552	4525	4325	130
	18/7	3859	4191	4389	4376	4204	124

4D. Iron concentrations ($\mu g\ g^{-1}$) in four replicate samples of Microspora sp. (collected at station 1) and in the root and shoot fractions of Juncus bulbosus (collected at station 2) taken quarterly from January 1984 to July 1985, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
Microspora sp.							
1984	23/1	8948	8940	8841	9019	8937	37
	25/4	9018	9246	9111	9262	9159	58
	18/7	9266	9351	9282	9155	9264	41
	19/10	8633	8724	8700	8872	8732	51
1985	17/1	8823	9067	9017	9195	9026	77
	17/4	9091	9080	8989	9180	9085	39
	18/7	9236	9197	9069	9050	9138	46
Juncus bulbosus root							
1984	23/1	1067	1130	1115	1045	1089	20
	25/4	1197	1210	1178	1307	1223	29
	18/7	1298	1279	1222	1205	1251	23
	19/10	1142	1087	1067	1092	1097	16
1985	17/1	1139	1153	1222	1101	1154	26
	17/4	1026	1073	1141	1050	1073	25
	18/7	1144	1159	1308	1219	1208	37
Juncus bulbosus shoot							
1984	23/1	200	239	256	217	228	13
	25/4	264	225	233	248	243	9
	18/7	232	281	254	259	257	10
	19/10	234	252	266	228	245	9
1985	17/1	247	187	254	200	222	17
	17/4	295	299	257	268	280	10
	18/7	306	260	273	318	289	14

4E. Calcium concentrations ($\mu g\ g^{-1}$) in four replicate samples of Jungermannia atrovirens taken each month from January 1984 to July 1985 at station 1, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
1984	23/1	1483	1414	1506	1462	1466	20
	20/2	1392	1476	1408	1299	1394	37
	20/3	1551	1550	1523	1516	1535	9
	25/4	1740	1632	1677	1693	1686	23
	21/5	1590	1543	1562	1673	1592	29
	18/6	1375	1393	1412	1514	1424	31
	18/7	1542	1462	1509	1511	1506	17
	20/8	1432	1516	1426	1541	1479	29
	24/9	1634	1545	1543	1574	1574	21
	19/10	1620	1621	1649	1608	1625	9
	21/11	1409	1590	1505	1547	1513	·39
	13/12	1569	1615	1608	1667	1615	20
1985	17/1	1666	1595	1705	1610	1644	26
	20/2	1577	1534	1470	1462	1511	28
	15/3	1512	1482	1545	1399	1485	32
	17/4	1742	1706	1742	1669	1715	18
	17/5	1648	1752	1822	1809	1758	40
	21/6	1524	1485	1608	1535	1538	26
	18/7	1493	1389	1578	1405	1466	44

4F. Calcium concentrations ($\mu g\ g^{-1}$) in four replicate samples of Microspora sp. (collected at station 1) and in the root and shoot fractions of Juncus bulbosus (collected at station 2) taken quarterly from January 1984 to July 1985, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
Microspora sp.							
1984	23/1	1879	1868	1894	2046	1922	42
	25/4	2097	2059	2182	2133	2118	26
	18/7	2021	2016	2009	2114	2040	25
	19/10	2037	1917	2006	1930	1973	29
1985	17/1	1951	1853	1839	1867	1878	25
	17/4	2044	2160	2013	2113	2083	33
	18/7	1843	2059	1979	1918	1950	46
Juncus bulbosus root							
1984	23/1	1358	1313	1391	1338	1350	17
	25/4	1513	1468	1359	1449	1447	33
	18/7	1419	1410	1350	1398	1394	16
	19/10	1344	1299	1385	1324	1338	18
1985	17/1	1303	1358	1287	1342	1323	17
	17/4	1391	1347	1382	1402	1381	12
	18/7	1267	1303	1398	1246	1304	34
Juncus bulbosus shoot							
1984	23/1	1733	1810	1767	1844	1789	25
	25/4	1984	2012	1958	1914	1967	21
	18/7	1891	1940	1822	1907	1890	25
	19/10	1880	1830	1901	1872	1871	15
1985	17/1	1743	1735	1799	1832	1777	23
	17/4	1834	1882	1941	1790	1862	33
	18/7	1862	1794	1738	1805	1800	26

4G. Magnesium concentrations ($\mu g\ g^{-1}$) in four replicate samples of Jungermannia atrovirens taken each month from January 1984 to July 1985 at station 1, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
1984	23/1	638	633	616	658	636	9
	20/2	692	650	704	664	678	13
	20/3	617	595	668	680	640	21
	25/4	653	594	625	612	621	13
	21/5	672	673	648	691	671	9
	18/6	613	628	681	617	635	16
	18/7	639	664	698	644	661	14
	20/8	656	617	629	674	644	13
	24/9	682	698	667	672	680	7
	19/10	638	685	665	614	651	16
	21/11	638	653	571	615	619	18
	13/12	706	686	688	701	695	5
1985	17/1	672	703	713	619	677	21
	20/2	639	673	628	686	657	14
	15/3	711	691	668	636	677	16
	17/4	673	683	679	704	685	7
	17/5	639	623	615	704	645	20
	21/6	739	673	703	688	701	14
	18/7	716	656	674	728	694	17

4H. Magnesium concentrations ($\mu g\ g^{-1}$) in four replicate samples of Microspora sp. (collected at station 1) and in the root and shoot fractions of Juncus bulbosus (collected at station 2) taken quarterly from January 1984 to July 1985, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
Microspora sp.							
1984	23/1	894	860	921	816	873	23
	25/4	780	816	863	818	819	17
	18/7	896	830	816	819	840	19
	19/10	776	856	813	790	809	18
1985	17/1	908	927	903	973	928	16
	17/4	838	835	793	764	808	18
	18/7	954	999	981	1047	995	20
Juncus bulbosus root							
1984	23/1	917	987	1024	974	976	22
	25/4	922	985	926	931	941	15
	18/7	901	875	843	911	883	15
	19/10	1019	899	957	975	963	25
1985	17/1	925	971	934	888	930	17
	17/4	1036	1092	1071	1010	1052	18
	18/7	1042	983	993	970	997	16
Juncus bulbosus shoot							
1984	23/1	2134	2220	2099	2157	2153	26
	25/4	2062	2168	2150	2056	2109	29
	18/7	2220	2346	2266	2201	2258	33
	19/10	2171	2084	2131	2092	2120	20
1985	17/1	2167	2225	2102	2214	2177	28
	17/4	1976	2073	2052	2058	2040	22
	18/7	1935	2026	2038	1966	1991	25

4I. Arsenic concentrations ($\mu g\ g^{-1}$) in four replicate samples of Jungermannia atrovirens taken each month from January 1984 to July 1985 at station 1, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
1984	23/1	53	58	54	55	55	1
	20/2	57	48	59	69	58	5
	20/3	73	78	65	60	69	4
	25/4	66	61	69	63	65	2
	21/5	63	74	64	75	69	3
	18/6	69	81	89	80	80	4
	18/7	74	67	82	76	75	3
	20/8	65	64	63	68	65	1
	24/9	61	63	75	72	68	4
	19/10	61	62	65	59	62	2
	21/11	69	76	76	82	76	3
	13/12	76	72	63	69	70	3
1985	17/1	66	54	60	69	62	4
	20/2	67	56	67	72	66	4
	15/3	46	44	51	45	47	2
	17/4	71	77	66	77	73	3
	17/5	62	54	64	71	63	4
	21/6	65	74	81	62	71	5
	18/7	75	73	63	78	72	4

4J. Arsenic concentrations ($\mu g\ g^{-1}$) in four replicate samples of Microspora sp. (collected at station 1) and in the root and shoot fractions of Juncus bulbosus (collected at station 2) taken quarterly from January 1984 to July 1985, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
Microspora sp.							
1984	23/1	67	84	80	93	81	6
	25/4	90	89	103	95	94	3
	18/7	97	82	78	84	85	4
	19/10	98	99	106	105	102	2
1985	17/1	88	87	99	92	92	3
	17/4	83	87	79	86	84	2
	18/7	105	88	93	85	93	5
Juncus bulbosus root							
1984	23/1	11	12	9	12	11	0.7
	25/4	14	12	13	17	14	1.1
	18/7	15	17	14	15	15	0.7
	19/10	11	10	12	11	11	0.4
1985	17/1	10	13	14	13	13	0.9
	17/4	17	15	15	12	15	1.1
	18/7	15	21	24	20	20	1.9
Juncus bulbosus shoot							
1984	23/1	1.5	1.3	1.4	2.3	1.6	0.3
	25/4	1.7	2.1	2.0	1.7	1.9	0.1
	18/7	1.4	2.2	1.6	2.0	1.8	0.2
	19/10	1.7	1.6	1.6	2.0	1.7	0.1
1985	17/1	2.0	1.8	2.2	2.2	2.1	0.1
	17/4	2.4	2.0	2.4	2.5	2.3	0.1
	18/7	1.4	2.8	1.8	1.8	2.0	0.3

4K. Aluminium concentrations ($\mu g\ g^{-1}$) in four replicate samples of Jungermannia atrovirens and Microspora sp. (both collected at station 1) and in the root and shoot fractions of Juncus bulbosus (collected at station 2) taken either monthly or quarterly from January to July 1985, together with a mean concentration and standard error (S.E.).

						MEAN	S.E.
Jungermannia atrovirens							
1985	17/1	5584	4938	5009	5573	5276	176
	20/2	5305	5223	5375	5439	5336	47
	15/3	5051	5025	5508	4985	5142	123
	17/4	5394	5272	5591	5396	5413	66
	17/5	5264	5312	5229	5615	5355	89
	21/6	4958	5154	5383	5389	5221	104
	18/7	5122	5401	5577	5162	5316	107
Microspora sp.							
1985	17/1	7259	7106	6870	7065	7075	80
	17/4	7342	7125	7062	7305	7209	68
	18/7	7045	7158	7180	6969	7088	50
Juncus bulbosus root							
1985	17/1	2113	2107	2236	2089	2136	34
	17/4	2492	2674	2508	2525	2550	42
	18/7	2104	2322	2297	2189	2228	51
Juncus bulbosus shoot							
1985	17/1	1600	1554	1541	1682	1594	32
	17/4	1668	1716	1735	1600	1680	30
	18/7	1679	1549	1597	1686	1628	33

APPENDIX 5

5A. Number of larvae of Plectrocnemia conspersa in each instar in ten sample units taken either montly or every two months from December 1983 to November 1985 at station 1.

	INSTAR					INSTAR			
	II	III	IV	V		II	III	IV	V
1983									
6/12	0	1	0	1	20/3	0	0	0	5
	0	4	2	1		0	3	2	6
	1	5	1	1		0	1	7	8
	1	1	0	1		2	0	2	1
	0	3	2	1		0	0	1	0
	2	5	5	0		0	2	3	3
	2	5	1	1		0	0	6	2
	0	3	2	1		1	2	2	1
						0	0	1	3
1984									
23/1	0	3	6	6	25/4	0	0	6	4
	0	4	0	1		0	3	3	2
	3	2	6	0		0	0	5	5
	0	5	2	0		0	1	0	2
	0	0	3	4		0	0	3	14
	0	3	0	0		1	2	3	1
	1	1	1	1		0	0	0	3
	0	0	5	0		0	0	7	5
						0	2	3	2
						0	1	5	2
20/2	0	0	0	1	21/5	0	0	4	5
	0	2	4	2		0	3	4	1
	0	0	6	0		0	0	2	14
	0	4	2	0		0	0	8	8
	0	0	5	4		0	0	1	1
	0	1	1	1		0	0	0	9
	0	3	2	7		0	2	1	1
	3	0	0	3		0	0	0	15
						0	0	2	12
						0	1	2	1

	INSTAR					INSTAR			
	II	III	IV	V		II	III	IV	V
1984									
18/6	0	0	1	3	24/9	0	0	1	5
	0	2	1	14		0	2	0	5
	0	0	0	5		1	0	0	0
	0	2	4	2		0	0	0	3
	0	2	1	4		1	0	0	1
	0	2	2	12		0	0	0	3
	0	0	3	11		0	1	0	3
	0	0	0	3		0	0	0	4
	0	0	3	14		0	0	0	0
	0	0	2	10		1	0	0	4
18/7	0	0	4	7	19/10	0	1	0	0
	0	3	0	7		0	3	0	0
	0	1	1	4		1	4	0	2
	0	0	1	1		1	2	0	2
	0	1	0	4		0	3	2	0
	0	1	3	7		1	1	1	0
	0	0	1	5		0	2	0	5
	0	0	0	4		2	1	1	4
	0	1	0	5		0	0	1	0
	0	0	2	3		0	2	0	4
20/8	0	1	1	3	21/11	0	2	2	0
	0	0	1	4		0	0	1	1
	0	0	1	5		1	2	1	0
	0	1	1	5		0	2	2	2
	0	1	0	0		0	0	0	2
	1	0	0	0		0	2	0	3
	0	0	3	5		1	6	2	0
	0	0	0	7		2	2	1	1
	0	1	0	3		2	0	2	1
	0	0	1	2		0	0	0	4

	INSTAR					INSTAR			
	II	III	IV	V		II	III	IV	V
1984									
13/12	0	3	0	4	17/5	0	0	3	2
	0	1	2	0		0	2	0	5
	3	6	4	4		0	1	2	5
	1	1	2	3		0	0	2	5
	2	0	2	2		0	2	1	2
	0	1	0	0		0	1	6	5
	1	0	1	1		0	0	0	1
	0	1	2	1		0	0	1	1
	2	0	1	1		1	3	1	7
	2	1	5	0		0	3	1	6
1985									
17/1	2	1	2	0	18/7	0	0	4	6
	0	2	2	1		0	1	1	3
	0	1	0	1		0	0	0	1
	1	2	3	2		0	0	0	6
	1	2	1	2		0	0	0	3
	1	0	4	0		0	0	0	1
	0	2	0	0		0	0	2	4
	1	0	2	0		0	1	1	10
	1	1	2	1		0	0	0	5
	0	1	8	4		0	0	3	2
15/3	0	3	1	5	18/9	0	0	1	0
	0	0	2	2		1	0	0	0
	0	0	1	3		0	0	0	1
	0	1	7	5		0	1	1	1
	3	2	3	1		2	0	0	3
	0	0	0	2		0	0	0	0
	0	0	1	3		1	0	0	0
	0	2	1	2		0	0	1	2
	0	2	0	3		0	2	0	0
	0	1	0	4		0	0	2	0

	INSTAR			
	II	III	IV	V
1985				
20/11	1	3	1	1
	0	2	2	3
	0	1	1	2
	0	2	2	1
	1	1	4	4
	0	2	1	1
	1	0	0	0
	2	1	0	1
	0	1	1	0
	0	2	0	1

5B. Number of larvae of Plectrocnemia conspersa in each instar in ten sample units taken every two months from December 1983 to July 1985 at station 3.

	INSTAR					INSTAR			
	II	III	IV	V		II	III	IV	V
1983									
6/12	0	2	0	0	18/6	0	0	0	1
	1	0	0	0		0	0	0	0
	0	0	0	0		0	0	0	3
	0	0	0	0		0	1	2	2
	0	1	0	2		0	0	0	2
	0	0	2	0		0	0	0	0
	1	0	0	0		0	0	0	1
	0	1	0	0		0	0	2	2
						0	0	1	2
						0	0	0	1
1984									
20/2	0	0	0	0	20/8	0	0	1	0
	0	2	0	0		0	0	0	0
	0	0	1	0		0	1	0	0
	1	0	1	0		0	0	0	3
	0	0	0	0		0	0	0	0
	0	1	0	0		0	0	0	0
	0	0	1	3		0	0	3	1
	0	0	1	0		0	0	0	1
						1	0	0	0
						0	0	0	0
25/4	0	0	1	3	19/10	0	0	0	0
	0	0	1	1		0	0	0	1
	0	0	0	0		0	0	0	1
	0	0	2	0		1	0	0	0
	0	0	2	3		0	0	0	0
	1	0	0	0		0	1	1	0
	0	0	1	2		0	0	0	0
	0	0	1	5		0	0	0	0
	0	2	0	1		0	2	0	0
	0	0	1	1		0	0	0	0

	INSTAR					INSTAR			
	II	III	IV	V		II	III	IV	V
1984									
13/12	1	1	1	0	17/5	0	0	0	2
	0	0	1	0		0	0	2	4
	0	0	0	0		0	1	1	1
	0	0	0	2		0	0	0	0
	1	0	0	0		0	0	1	3
	0	0	0	0		0	0	0	1
	0	0	0	0		0	0	0	4
	0	1	0	0		0	0	0	0
	0	0	1	0		0	0	1	0
	0	0	0	0		0	0	0	0
1985									
17/1	0	0	0	0	18/7	0	0	0	3
	0	1	2	0		0	0	1	0
	0	1	0	0		0	0	0	0
	0	1	0	0		0	1	1	3
	0	0	2	0		0	0	0	0
	1	0	0	0		0	0	2	1
	0	0	0	0		0	0	0	1
	0	0	0	0		0	0	0	1
	0	0	0	1		0	0	0	0
	0	0	0	0		0	0	1	0
15/3	0	0	0	0					
	0	1	0	0					
	0	0	0	2					
	0	0	0	0					
	0	0	0	0					
	0	0	2	2					
	0	1	0	0					
	0	0	0	1					
	1	0	1	0					
	0	0	0	0					

5C. Number of larvae of Plectrocnemia conspersa in each instar in ten sample units taken monthly or every two months from December 1983 to November 1985 at station 4.

	INSTAR					INSTAR			
	II	III	IV	V		II	III	IV	V
1983									
6/12	0	1	0	0	20/3	0	0	4	0
	0	3	0	1		0	0	1	0
	0	0	0	0		0	0	1	0
	3	0	1	0		0	0	1	2
	0	0	0	0		0	0	0	0
	0	0	0	0		1	0	0	0
	1	0	0	1		0	0	0	0
	0	1	0	0		0	3	1	1
	0	0	1	2		0	1	0	0
	0	2	2	0					
1984									
23/1	1	0	0	0	25/4	0	0	3	3
	0	1	3	0		0	4	1	0
	1	0	1	0		0	1	0	1
	0	0	1	3		0	0	1	1
	0	0	0	0		0	1	3	1
	0	0	0	0		0	0	0	0
	0	0	0	1		0	1	0	0
	0	1	0	0		0	0	0	0
						0	0	0	2
						0	1	1	2
20/2	0	1	1	0	21/5	0	0	2	0
	1	0	0	0		0	1	2	2
	0	1	0	0		0	0	0	1
	0	1	0	1		0	1	0	0
	0	1	4	1		0	0	0	3
	0	0	0	0		0	0	1	2
	0	0	1	0		0	0	0	0
	0	1	0	1		0	0	2	5
						0	1	1	0
						0	0	1	2

	INSTAR					INSTAR			
	II	III	IV	V		II	III	IV	V
1984									
18/6	0	0	0	0	24/9	0	0	0	0
	0	0	0	4		0	2	1	1
	0	0	1	2		0	0	0	1
	0	0	0	0		0	0	0	0
	0	0	0	0		0	0	0	1
	0	0	0	1		0	0	1	0
	0	0	1	1		0	0	0	0
	0	0	0	5		0	0	1	1
	0	0	1	1		0	0	0	0
	0	0	0	2		1	0	0	0
18/7	0	0	0	1	19/10	0	0	0	0
	0	0	0	1		0	2	0	0
	0	0	0	2		0	0	0	0
	0	0	0	0		0	0	0	1
	0	0	1	4		0	0	0	0
	0	0	0	2		2	0	1	0
	0	0	0	3		0	0	1	0
	0	0	0	0		0	0	0	1
	0	0	0	2		0	0	0	0
	0	0	0	0		0	1	0	0
20/8	0	0	0	0	21/11	0	2	0	0
	0	1	0	0		0	0	0	0
	0	0	0	0		0	0	0	0
	0	0	0	1		0	0	0	0
	0	0	0	0		0	0	0	0
	0	0	1	1		1	0	0	1
	1	0	0	1		0	0	0	0
	0	0	0	0		0	1	0	0
	0	0	0	0		0	0	0	0
	0	1	0	0		0	0	1	1

	INSTAR					INSTAR			
	II	III	IV	V		II	III	IV	V
1984									
13/12	0	0	0	0	17/5	0	0	1	1
	0	1	0	0		0	0	0	0
	0	1	0	2		0	0	2	2
	0	0	0	0		0	0	0	0
	0	0	1	0		0	0	0	1
	0	0	0	0		0	2	1	0
	1	0	0	0		0	0	1	0
	0	0	0	0		0	0	1	2
	0	0	0	0		0	1	3	2
	1	1	0	0		0	0	0	0
1985									
17/1	0	2	1	0	18/7	0	0	0	6
	0	0	0	0		0	0	0	5
	0	1	0	0		0	0	0	5
	0	2	1	0		0	0	0	1
	0	0	0	0		0	0	0	1
	0	0	1	1		0	0	2	2
	0	0	0	0		0	0	1	3
	1	0	0	0		0	0	0	0
	0	1	0	0		0	0	0	0
	0	0	0	0		0	0	1	5
15/3	0	0	1	0	18/9	0	2	0	0
	0	2	0	0		0	0	0	0
	0	0	1	0		1	1	0	0
	0	0	0	0		0	0	0	0
	0	2	3	0		0	0	0	1
	0	0	1	0		1	0	1	1
	0	0	0	0		0	0	0	0
	0	1	0	0		0	0	0	0
	0	0	1	1		0	0	1	0
	1	0	0	0		0	0	0	0

	INSTAR			
	II	III	IV	V
1985				
20/11	0	0	1	0
	0	0	0	0
	0	3	1	0
	0	0	0	0
	0	1	0	0
	0	4	0	0
	1	0	0	0
	1	0	1	2
	0	0	1	0
	0	0	0	1

5D. Number of larvae of Plectrocnemia conspersa in each instar in four sample units taken every two months from January 1984 to January 1985 at station 2.

	INSTAR					INSTAR			
	II	III	IV	V		II	III	IV	V
1984									
23/1	1	2	8	3	21/11	2	3	5	4
	0	6	6	5		0	4	1	2
	1	0	4	3		0	2	3	3
	2	5	2	1		1	7	1	4
					1985				
20/3	0	2	7	5	17/1	1	1	2	2
	1	2	1	3		0	1	8	6
	0	1	2	3		4	4	4	2
	0	2	3	4		2	4	4	1
21/5	0	1	2	4					
	0	0	0	2					
	0	2	0	6					
	0	0	3	1					
18/7	0	1	3	1					
	0	2	1	3					
	0	0	0	2					
	0	0	0	2					
24/9	0	1	1	4					
	1	3	1	3					
	1	0	0	0					
	0	0	0	2					

5E. Composition of the macroinvertebrate communities in ten sample units taken in July 1984 at stations 1, D, X and F in the Darley Brook and at station 4, together with the total number of invertebrates in each sample unit.

		STATION 1									
PLECOPTERA	Diura bicaudata										
	Chloroperla torrentium										
	Nemoura cambrica										
EPHEMEROPTERA	Baetis rhodani										
TRICHOPTERA	Plectrocnemia conspersa	11	10	6	2	5	11	6	4	6	5
	Rhyacophila dorsalis										
	Hydropsyche instabilis										
	Sericostoma personatum										
	Drusus annulatus										
	Potamophylax sp.										
COLEOPTERA	Agabus guttatus (larvae)	3	1	2	2	6	4	1	7	4	0
	Agabus guttatus (adults)	1	1	0	0	1	2	0	1	1	0
	Agabus biguttatus (larvae)	0	3	0	0	3	4	3	4	1	1
	Agabus biguttatus (adults)	0	0	0	0	1	2	1	1	1	0
	Ilybius fuliginosus (larvae)	0	1	2	0	1	1	1	0	3	1
	Ilybius fuliginosus (adults)	0	0	1	0	0	0	1	0	1	1
DIPTERA	Simulium naturale										
	Pedicia sp.	1	0	0	0	0	0	0	0	0	0
	Dicranota sp.										
	Limnophora sp.										
	Atherix sp.										
	Eukiefferiella sp.	8	1	0	3	0	1	5	0	0	2
	Cricotopus sp.	4	0	1	0	2	6	0	2	0	2
	Orthocladius sp.	0	8	0	0	2	0	0	0	7	0
	Macropelopia sp.	0	0	4	0	7	1	0	3	0	3
	Diamesa sp.	2	3	1	0	0	1	0	3	1	0
	Polypedilum sp.	0	1	0	1	0	1	0	5	0	0
ARACHNIDA	Hydracarina (1 species)	0	0	0	1	0	0	0	0	0	0
TRICLADIDA	Polycelis nigra										
	Phagocata vitta										
Total Number of Invertebrates		30	29	17	9	28	34	18	30	25	15

		STATION D									
PLECOPTERA	Diura bicaudata										
	Chloroperla torrentium	0	0	0	0	0	0	0	0	0	1
	Nemoura cambrica										
EPHEMEROPTERA	Baetis rhodani										
TRICHOPTERA	Plectrocnemia conspersa	1	1	4	2	4	3	1	2	1	1
	Rhyacophila dorsalis										
	Hydropsyche instabilis										
	Sericostoma personatum										
	Drusus annulatus										
	Potamophylax sp.										
COLEOPTERA	Agabus guttatus (larvae)	1	0	0	0	0	2	0	0	0	0
	Agabus guttatus (adults)	0	0	0	0	0	0	1	1	0	0
	Agabus biguttatus (larvae)	0	0	0	0	2	0	0	0	0	0
	Agabus biguttatus (adults)	0	1	0	0	0	0	0	0	0	0
	Ilybius fuliginosus (larvae)	0	0	1	0	0	0	0	1	0	0
	Ilybius fuliginosus (adults)	0	0	1	0	0	0	0	0	0	0
DIPTERA	Simulium naturale										
	Pedicia sp.										
	Dicranota sp.										
	Limnophora sp.										
	Atherix sp.										
	Eukiefferiella sp.	0	0	0	2	3	0	0	0	0	1
	Cricotopus sp.	0	0	1	0	2	0	1	0	0	0
	Orthocladius sp.	0	0	2	0	0	1	0	0	3	0
	Macropelopia sp.	0	0	0	2	4	0	0	0	0	0
	Diamesa sp.	0	3	0	0	0	1	0	0	0	0
	Polypedilum sp.	1	0	3	0	0	0	1	2	0	0
ARACHNIDA	Hydracarina (1 species)										
TRICLADIDA	Polycelis nigra										
	Phagocata vitta	0	0	1	0	0	0	0	0	0	0
Total Number of Invertebrates		3	5	13	6	15	7	4	6	4	3

		STATION X									
PLECOPTERA	Diura bicaudata										
	Chloroperla torrentium										
	Nemoura cambrica										
EPHEMEROPTERA	Baetis rhodani	0	0	7	3	4	3	0	4	4	1
TRICHOPTERA	Plectrocnemia conspersa	1	0	1	0	2	0	0	1	0	1
	Rhyacophila dorsalis	0	0	0	0	0	0	0	1	0	0
	Hydropsyche instabilis										
	Sericostoma personatum										
	Drusus annulatus										
	Potamophylax sp.										
COLEOPTERA	Agabus guttatus (larvae)										
	Agabus guttatus (adults)										
	Agabus biguttatus (larvae)										
	Agabus biguttatus (adults)										
	Ilybius fuliginosus (larvae)										
	Ilybius fuliginosus (adults)										
DIPTERA	Simulium naturale										
	Pedicia sp.										
	Dicranota sp.										
	Limnophora sp.										
	Atherix sp.										
	Eukiefferiella sp.	3	0	6	11	0	9	7	0	7	12
	Cricotopus sp.	4	2	1	0	6	0	3	0	6	0
	Orthocladius sp.	0	2	8	3	0	4	1	5	0	6
	Macropelopia sp.	0	0	1	0	6	0	2	3	0	0
	Diamesa sp.	3	0	0	5	3	4	3	0	1	0
	Polypedilum sp.	0	3	1	7	1	4	6	0	1	8
ARACHNIDA	Hydracarina (1 species)	0	0	0	0	0	0	0	1	0	0
TRICLADIDA	Polycelis nigra	0	1	0	0	0	0	0	0	0	0
	Phagocata vitta										
Total Number of Invertebrates		11	8	25	29	22	24	22	15	19	28

		STATION F									
PLECOPTERA	Diura bicaudata										
	Chloroperla torrentium										
	Nemoura cambrica										
EPHEMEROPTERA	Baetis rhodani	2	3	2	0	3	0	2	0	3	5
TRICHOPTERA	Plectrocnemia conspersa	1	0	1	2	1	2	1	4	1	0
	Rhyacophila dorsalis										
	Hydropsyche instabilis										
	Sericostoma personatum										
	Drusus annulatus										
	Potamophylax sp.										
COLEOPTERA	Agabus guttatus (larvae)										
	Agabus guttatus (adults)										
	Agabus biguttatus (larvae)										
	Agabus biguttatus (adults)										
	Ilybius fuliginosus (larvae)										
	Ilybius fuliginosus (adults)										
DIPTERA	Simulium naturale										
	Pedicia sp.										
	Dicranota sp.										
	Limnophora sp.										
	Atherix sp.										
	Eukiefferiella sp.	0	3	6	0	0	1	0	0	3	5
	Cricotopus sp.	4	0	0	2	9	1	4	1	0	0
	Orthocladius sp.	0	2	0	2	0	1	0	1	2	0
	Macropelopia sp.	3	1	0	0	1	3	0	1	0	0
	Diamesa sp.	3	0	1	3	0	0	4	0	3	0
	Polypedilum sp.	1	0	0	1	0	0	2	5	0	0
ARACHNIDA	Hydracarina (1 species)	4	3	3	0	6	3	3	7	2	5
TRICLADIDA	Polycelis nigra										
	Phagocata vitta										
Total Number of Invertebrates		18	12	13	10	20	11	16	19	14	15

		STATION 4									
PLECOPTERA	Diura bicaudata	3	1	2	0	3	2	2	0	0	1
	Chloroperla torrentium	11	14	12	0	2	6	3	6	6	1
	Nemoura cambrica	0	0	2	0	0	0	0	0	3	0
EPHEMEROPTERA	Baetis rhodani	1	0	0	1	0	2	0	0	4	0
TRICHOPTERA	Plectrocnemia conspersa	1	1	2	0	5	2	3	0	2	0
	Rhyacophila dorsalis	0	0	0	0	1	0	0	0	0	0
	Hydropsyche instabilis	1	0	1	0	0	0	0	0	0	0
	Sericostoma personatum	0	7	0	0	0	1	2	0	0	2
	Drusus annulatus	0	1	0	0	0	2	1	0	0	1
	Potamophylax sp.	0	0	0	0	1	0	0	1	0	2
COLEOPTERA	Agabus guttatus (larvae)										
	Agabus guttatus (adults)										
	Agabus biguttatus (larvae)										
	Agabus biguttatus (adults)										
	Ilybius fuliginosus (larvae)										
	Ilybius fuliginosus (adults)										
DIPTERA	Simulium naturale	0	0	0	0	0	0	0	0	1	0
	Pedicia sp.	0	0	0	0	0	1	0	0	0	0
	Dicranota sp.	0	0	2	0	0	0	0	1	0	0
	Limnophora sp.	1	0	0	0	0	0	0	0	0	0
	Atherix sp.	0	1	1	0	0	3	0	0	0	0
	Eukiefferiella sp.	0	2	2	4	0	1	0	9	6	7
	Cricotopus sp.	0	2	6	3	2	1	0	2	3	1
	Orthocladius sp.	3	2	0	9	0	5	3	0	0	2
	Macropelopia sp.	0	1	3	2	0	7	0	9	0	9
	Diamesa sp.	3	2	0	9	0	0	6	2	2	1
	Polypedilum sp.	4	1	9	4	5	0	5	1	0	13
ARACHNIDA	Hydracarina (1 species)										
TRICLADIDA	Polycelis nigra	6	11	7	5	3	0	2	3	4	1
	Phagocata vitta	1	0	0	2	0	3	2	2	0	3
Total Number of Invertebrates		35	46	49	39	22	36	29	36	31	44

5F. Composition of the macroinvertebrate communities in ten sample units taken in April 1986 at stations 1, D, X and F in the Darley Brook and at station 4, together with the total number of invertebrates in each sample unit.

STATION 1

PLECOPTERA	Diura bicaudata										
	Chloroperla torrentium										
	Nemoura cambrica										
	Protonemura meyeri										
	Amphinemura sulcicollis										
	Nemurella picteti										
EPHEMEROPTERA	Baetis rhodani										
TRICHOPTERA	Plectrocnemia conspersa	9	7	2	3	6	7	9	14	12	3
	Rhyacophila dorsalis	0	1	0	1	0	1	0	1	0	0
	Hydropsyche instabilis										
	Odontocerum albicorne										
	Sericostoma personatum										
	Agapetus fuscipes										
	Drusus annulatus										
	Goera pilosa										
COLEOPTERA	Agabus guttatus (larvae)	2	1	0	2	0	1	4	2	1	0
	Agabus guttatus (adults)	0	0	2	0	0	3	1	0	0	0
	Agabus biguttatus (larvae)	0	1	1	2	0	1	2	2	0	1
	Agabus biguttatus (adults)	0	1	1	0	2	0	0	0	1	1
	Elmis aenea (larvae)										
DIPTERA	Pedicia sp.										
	Dicranota sp.										
	Limnophora sp.										
	Atherix sp.										
	Eukiefferiella sp.	9	0	14	1	8	10	19	10	0	15
	Cricotopus sp.	7	6	0	6	0	1	9	8	8	0
	Brillia sp.	0	2	1	2	0	0	0	0	3	0
	Zavrelimyia sp.	7	8	0	2	4	3	5	6	2	8
	Macropelopia sp.	6	6	0	5	3	4	4	7	9	0
	Pentaneura sp.	6	0	5	4	0	8	0	3	7	0
	Diamesa sp.	0	6	2	8	6	4	4	13	9	0
	Polypedilum sp.	0	15	3	5	0	3	9	9	0	1
ARACHNIDA	Hydracarina (1 species)										
TRICLADIDA	Polycelis nigra										
	Phagocata vitta	0	0	1	1	2	1	2	0	2	0
HIRUDINEA	Erpobdella octoculata										
GASTROPODA	Potamopyrgus jenkinsi										
Total Number of Invertebrates		46	54	32	42	31	47	68	75	54	29

		STATION D									
PLECOPTERA	Diura bicaudata										
	Chloroperla torrentium	0	0	0	1	0	0	0	0	0	0
	Nemoura cambrica										
	Protonemura meyeri										
	Amphinemura sulcicollis										
	Nemurella picteti										
EPHEMEROPTERA	Baetis rhodani										
TRICHOPTERA	Plectrocnemia conspersa	0	1	3	6	1	4	0	1	3	2
	Rhyacophila dorsalis										
	Hydropsyche instabilis										
	Odontocerum albicorne										
	Sericostoma personatum										
	Agapetus fuscipes										
	Drusus annulatus										
	Goera pilosa										
COLEOPTERA	Agabus guttatus (larvae)	0	0	0	1	0	0	1	2	0	0
	Agabus guttatus (adults)	0	0	0	0	0	0	1	0	1	0
	Agabus biguttatus (larvae)	0	0	0	0	0	1	1	0	0	0
	Agabus biguttatus (adults)	0	0	0	1	0	0	0	0	0	0
	Elmis aenea (larvae)										
DIPTERA	Pedicia sp.										
	Dicranota sp.										
	Limnophora sp.										
	Atherix sp.	0	0	0	0	0	0	0	1	0	0
	Eukiefferiella sp.	1	0	2	4	3	1	0	0	0	2
	Cricotopus sp.	7	0	1	0	0	0	9	0	3	4
	Brillia sp.	9	8	6	3	0	18	4	1	9	9
	Zavrelimyia sp.	4	0	8	0	9	0	0	5	0	1
	Macropelopia sp.	0	6	0	7	6	1	0	0	3	0
	Pentaneura sp.	6	4	0	0	7	0	4	0	0	3
	Diamesa sp.	0	7	4	1	0	1	0	0	2	2
	Polypedilum sp.	0	3	0	2	0	0	1	9	0	1
ARACHNIDA	Hydracarina (1 species)										
TRICLADIDA	Polycelis nigra										
	Phagocata vitta	0	1	6	3	5	2	2	0	2	1
HIRUDINEA	Erpobdella octoculata	0	0	1	0	0	0	0	0	0	1
GASTROPODA	Potamopyrgus jenkinsi										
Total Number of Invertebrates		27	30	31	29	31	28	23	19	23	26

		STATION X									
PLECOPTERA	Diura bicaudata										
	Chloroperla torrentium	0	0	0	1	0	0	0	0	0	1
	Nemoura cambrica	0	1	0	0	0	0	0	0	0	0
	Protonemura meyeri										
	Amphinemura sulcicollis										
	Nemurella picteti										
EPHEMEROPTERA	Baetis rhodani	6	1	0	6	4	10	10	10	6	3
TRICHOPTERA	Plectrocnemia conspersa	0	1	0	2	1	1	0	3	3	0
	Rhyacophila dorsalis	0	0	0	1	0	0	0	0	0	0
	Hydropsyche instabilis										
	Odontocerum albicorne	0	0	0	0	1	1	2	0	0	0
	Sericostoma personatum	0	0	0	0	0	1	0	0	1	0
	Agapetus fuscipes										
	Drusus annulatus										
	Goera pilosa										
COLEOPTERA	Agabus guttatus (larvae)										
	Agabus guttatus (adults)										
	Agabus biguttatus (larvae)										
	Agabus biguttatus (adults)										
	Elmis aenea (larvae)	0	0	0	0	0	1	0	1	0	0
DIPTERA	Pedicia sp.	0	0	0	1	0	0	0	0	0	0
	Dicranota sp.	0	0	0	0	0	0	0	1	1	0
	Limnophora sp.	0	2	0	0	0	0	0	0	0	0
	Atherix sp.	0	0	0	0	0	0	0	0	0	1
	Eukiefferiella sp.	4	1	8	5	0	7	4	0	9	1
	Cricotopus sp.	3	1	4	0	7	6	2	5	4	2
	Brillia sp.	0	5	7	5	7	5	9	0	5	4
	Zavrelimyia sp.	7	0	9	9	0	12	10	4	5	7
	Macropelopia sp.	5	11	5	1	7	0	12	0	18	7
	Pentaneura sp.	0	0	1	1	0	2	0	1	0	3
	Diamesa sp.	3	3	1	0	4	0	0	1	0	2
	Polypedilum sp.	0	7	9	2	3	4	0	16	0	5
ARACHNIDA	Hydracarina (1 species)	0	0	0	0	0	0	1	0	0	1
TRICLADIDA	Polycelis nigra										
	Phagocata vitta	2	3	2	2	1	0	0	4	1	0
HIRUDINEA	Erpobdella octoculata										
GASTROPODA	Potamopyrgus jenkinsi										
Total Number of Invertebrates		30	36	46	36	35	50	50	46	53	37

STATION F

PLECOPTERA	Diura bicaudata										
	Chloroperla torrentium										
	Nemoura cambrica										
	Protonemura meyeri										
	Amphinemura sulcicollis										
	Nemurella picteti										
EPHEMEROPTERA	Baetis rhodani	11	12	4	11	3	5	8	6	14	9
TRICHOPTERA	Plectrocnemia conspersa	0	0	4	3	5	1	4	4	0	1
	Rhyacophila dorsalis	0	1	0	2	0	1	1	1	2	0
	Hydropsyche instabilis										
	Odontocerum albicorne										
	Sericostoma personatum	0	0	0	0	0	0	1	1	0	1
	Agapetus fuscipes										
	Drusus annulatus										
	Goera pilosa										
COLEOPTERA	Agabus guttatus (larvae)										
	Agabus guttatus (adults)										
	Agabus biguttatus (larvae)										
	Agabus biguttatus (adults)										
	Elmis aenea (larvae)	0	1	0	0	0	0	0	0	0	0
DIPTERA	Pedicia sp.										
	Dicranota sp.	1	0	1	1	0	0	0	0	0	0
	Limnophora sp.										
	Atherix sp.	0	0	0	0	0	0	1	0	0	0
	Eukiefferiella sp.	0	4	0	0	0	5	0	3	0	2
	Cricotopus sp.	0	0	1	0	3	1	0	2	0	0
	Brillia sp.	1	7	0	4	0	5	1	0	0	4
	Zavrelimyia sp.	7	0	0	0	5	2	0	6	0	3
	Macropelopia sp.	3	0	4	0	4	2	0	4	0	2
	Pentaneura sp.	0	0	1	0	0	0	2	2	0	0
	Diamesa sp.	0	3	0	0	1	0	1	0	0	0
	Polypedilum sp.	9	0	0	2	0	0	8	0	6	0
ARACHNIDA	Hydracarina (1 species)	0	0	0	0	0	0	0	1	0	1
TRICLADIDA	Polycelis nigra										
	Phagocata vitta	1	0	2	0	0	0	2	2	2	0
HIRUDINEA	Erpobdella octoculata	1	1	0	0	0	0	0	0	0	1
GASTROPODA	Potamopyrgus jenkinsi	0	0	0	0	1	0	0	0	0	0
Total Number of Invertebrates		34	29	17	23	22	22	29	32	24	24

		STATION 4									
PLECOPTERA	Diura bicaudata	2	2	3	3	17	4	1	2	3	6
	Chloroperla torrentium	18	13	17	5	20	13	2	10	24	9
	Nemoura cambrica	0	0	0	0	0	0	0	0	0	1
	Protonemura meyeri	1	0	0	0	0	0	0	0	0	2
	Amphinemura sulcicollis	2	1	4	12	7	2	1	7	3	1
	Nemurella picteti	0	0	0	0	0	1	3	0	2	0
EPHEMEROPTERA	Baetis rhodani	0	0	0	1	0	0	0	2	0	0
TRICHOPTERA	Plectrocnemia conspersa	0	1	1	3	2	1	0	0	0	4
	Rhyacophila dorsalis										
	Hydropsyche instabilis	17	1	19	6	12	5	1	8	9	4
	Odontocerum albicorne	1	2	0	0	0	1	0	0	0	1
	Sericostoma personatum	1	0	0	1	1	0	1	2	0	0
	Agapetus fuscipes	0	0	1	0	0	0	1	0	2	0
	Drusus annulatus	2	3	12	3	9	4	1	1	5	0
	Goera pilosa	0	0	0	0	6	0	1	0	0	1
COLEOPTERA	Agabus guttatus (larvae)										
	Agabus guttatus (adults)										
	Agabus biguttatus (larvae)										
	Agabus biguttatus (adults)										
	Elmis aenea (larvae)										
DIPTERA	Pedicia sp.	0	2	0	0	1	0	1	1	0	0
	Dicranota sp.	3	4	1	2	2	1	3	1	2	1
	Limnophora sp.	1	0	0	0	0	1	0	0	1	0
	Atherix sp.	0	2	0	2	0	1	3	0	2	0
	Eukiefferiella sp.	0	1	0	1	0	4	0	1	0	3
	Cricotopus sp.	0	2	1	0	0	5	2	0	1	0
	Brillia sp.	2	0	4	0	5	0	3	0	1	3
	Zavrelimyia sp.	0	0	0	2	0	0	3	0	0	0
	Macropelopia sp.	0	0	3	0	0	3	0	0	0	5
	Pentaneura sp.	0	0	2	2	0	0	1	0	0	0
	Diamesa sp.	0	0	0	0	0	2	0	2	0	0
	Polypedilum sp.	2	0	4	3	0	0	4	0	0	0
ARACHNIDA	Hydracarina (1 species)										
TRICLADIDA	Polycelis nigra	4	2	0	6	4	0	1	0	0	1
	Phagocata vitta	0	1	2	11	8	4	2	4	0	3
HIRUDINEA	Erpobdella octoculata										
GASTROPODA	Potamopyrgus jenkinsi										
Total Number of Invertebrates		56	37	74	63	94	52	35	41	55	45

5G. Number of coleopteran larvae in ten sample units taken either monthly or every two months from December 1983 to November 1985 at station 1.

1983	6/12	0	0	0	1	1	0	2	0		
1984	23/1	2	0	0	3	0	1	2	0		
	20/2	0	0	0	2	1	0	1	0		
	20/3	1	0	0	1	0	1	0	0	0	
	25/4	3	5	2	0	0	1	1	0	1	1
	21/5	4	3	2	3	2	4	2	3	4	0
	18/6	5	9	3	2	2	2	5	2	1	2
	18/7	3	5	4	2	10	9	5	11	8	2
	20/8	0	1	1	1	1	2	0	2	3	2
	24/9	0	2	0	0	1	0	0	0	0	0
	19/10	0	0	0	0	4	2	0	0	1	0
	21/11	3	1	0	0	0	2	0	0	0	0
	13/12	0	0	2	0	4	1	1	2	1	0
1985	17/1	1	0	1	0	2	0	1	1	0	0
	15/3	1	1	1	3	0	1	2	2	0	0
	17/5	3	7	3	4	6	2	3	2	2	8
	18/7	7	5	1	2	3	1	3	2	2	3
	18/9	1	0	0	2	4	0	0	3	0	0
	20/11	0	2	1	2	1	0	1	0	2	2

5H. Number of coleopteran adults in ten sample units taken either monthly or every two months from December 1983 to November 1985 at station 1.

1983	6/12	0	0	0	0	1	0	0	1		
1984	23/1	0	1	1	0	0	1	0	0		
	20/2	0	0	0	4	0	0	0	0		
	20/3	1	0	0	2	0	0	2	0	0	
	25/4	0	0	1	0	1	1	0	0	0	1
	21/5	2	0	0	1	1	1	0	0	4	0
	18/6	0	1	0	6	2	4	0	0	0	1
	18/7	1	1	1	0	2	4	2	2	3	1
	20/8	0	1	0	0	0	4	3	0	2	0
	24/9	0	0	0	0	7	0	0	0	0	0
	19/10	0	0	0	0	0	0	4	0	0	0
	21/11	1	2	0	0	0	0	0	1	0	0
	13/12	0	2	0	0	1	0	0	1	1	0
1985	17/1	0	0	0	0	1	0	0	0	1	0
	15/3	0	0	1	0	1	0	1	0	0	0
	17/5	1	0	1	0	0	0	1	1	0	0
	18/7	3	1	1	0	0	0	2	2	0	3
	18/9	2	0	0	0	0	1	0	0	0	2
	20/11	0	0	0	1	0	0	0	2	0	0

APPENDIX 6

6A. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual larvae (instars II to V) of P. conspersa collected at stations 1 and 4 in September 1984.

STATION 1			STATION 4		
Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)	Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
II	2653	0.0838	II	816	0.0724
	4666	0.0490		705	0.0508
	3424	0.0172		762	0.0571
	4812	0.0384		873	0.0514
	4292	0.0584		907	0.0431
	3063	0.0494			
	3258	0.0251			
	3969	0.0544			
III	1297	0.2814	III	420	0.1761
	1187	0.2769		364	0.3610
	2386	0.1519		390	0.1563
	2381	0.1749		375	0.3171
	2666	0.1628		289	0.4384
	1718	0.2096		299	0.3649
	1351	0.3841		258	0.3936
	1525	0.3736		339	0.2342
	2143	0.2375		294	0.3151
	1404	0.2382		409	0.2177
IV	550	0.8012	IV	97	1.9997
	1237	0.3314		148	1.4473
	421	0.7796		158	1.1406
	957	0.6374		131	1.2818
	400	1.5500		109	1.7624
	527	1.0697		157	1.8427
	1024	0.9741		168	1.2055
	618	1.4634		114	1.4541
	474	1.6199		177	1.3032
	1193	0.6622		104	1.6860
V	191	5.5774	V	86	1.6262
	308	4.8651		51	2.5340
	289	1.9578		49	4.1178
	122	4.4864		44	9.7952
	325	2.2798		111	5.2487
	123	1.9447		113	4.9665
	249	7.1472		38	10.5587
	424	2.3780		73	7.9014
	165	3.1677		67	7.5647
	379	7.3609		65	6.7890

6B. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual larvae (instars II to V) of P. conspersa collected at stations 1 and 4 in March 1984.

STATION 1			STATION 4		
Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)	Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
II	1296	0.0204	II	236	0.0363
	1147	0.0393		202	0.0469
	1209	0.0344		356	0.0298
	1050	0.0314		374	0.0274
	1256	0.0271		195	0.0337
III	497	0.2675	III	79	0.2365
	726	0.1380		34	0.3538
	548	0.2317		125	0.1285
	461	0.2846		145	0.1521
	279	0.2512		149	0.1015
	310	0.2967		88	0.2948
	401	0.2156		103	0.2240
	470	0.2359		73	0.2665
	550	0.0898		64	0.2706
	677	0.0554		123	0.2318
IV	305	0.6654	IV	37	1.1542
	289	0.4841		29	1.4493
	213	0.6109		90	0.5124
	410	0.3604		23	1.4563
	206	1.0273		42	0.8812
	81	1.3594		46	0.7689
	239	0.5585		34	1.6723
	193	1.2232		27	1.6645
	150	1.2629		53	0.4008
	196	1.0646		40	0.4517
V	122	1.0444	V	24	1.2818
	80	3.1783		19	6.2815
	89	4.6848		25	2.8526
	76	3.7809		23	3.0386
	95	5.0272		30	3.9738
	167	2.8940		26	3.9752
	109	3.3357		11	3.8618
	91	3.4989		10	5.1251
	77	1.8435		17	5.5895
	68	5.2067		24	2.0935

6C. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual larvae (instars III to V) of P. conspersa collected at station 1 in July 1984.

Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
III	4108	0.1641
	3820	0.2203
	4217	0.3012
	4339	0.1719
	2008	0.4352
	1789	0.5207
	3652	0.2569
	4287	0.2419
	3121	0.6529
	2136	0.7138
IV	1042	1.3641
	836	1.5156
	792	1.4014
	632	0.9165
	1225	1.6067
	1117	1.7255
	726	3.5482
	1509	2.1158
	1681	0.7187
	1162	1.8991
V	456	8.2980
	431	7.3743
	482	2.8742
	247	7.0150
	268	22.0437
	321	6.6642
	525	2.7203
	448	10.3171
	283	9.7365
	273	8.6006

6D. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual larvae (instars II to V) of P. conspersa collected at station 1 in January 1985.

Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
II	2596	0.0206
	3120	0.0524
	2747	0.0365
	3252	0.0239
	2506	0.0344
	2858	0.0484
	2325	0.0539
	2590	0.0450
	2709	0.0253
	2453	0.0488
III	1399	0.2342
	1453	0.2407
	1060	0.3675
	1084	0.2651
	1298	0.1895
	957	0.2342
	1146	0.1640
	1006	0.1489
	949	0.1851
	1361	0.2061
IV	277	1.0229
	549	0.4638
	692	0.5174
	162	1.1813
	555	1.5220
	213	1.1061
	331	0.6603
	827	1.1003
	152	0.5859
	207	0.5211
V	57	3.8095
	251	2.9789
	72	1.7136
	70	8.9466
	203	1.4850
	80	2.0160
	108	2.3947
	154	1.4187
	131	2.0616
	189	0.9508

6E. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual larvae (instars III to V) of P. conspersa collected at station 1 in May 1985, and unstarved or starved for 48 hours at 10°C.

UNSTARVED			STARVED		
Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)	Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
III	6635	0.2133	III	3016	0.2299
	4523	0.5474		3155	0.2104
	5968	0.3537		2422	0.3754
	5387	0.5673		2874	0.3861
	6305	0.2963		2731	0.2510
IV	589	1.2561	IV	1037	0.9068
	391	3.2597		549	2.1356
	1551	1.0442		1617	0.4049
	2383	1.4146		241	1.9842
	1543	0.9444		255	1.3249
	881	1.7863		192	1.5129
	866	1.5228		240	0.9348
	1679	1.1390		314	0.5792
	1744	0.9816		602	0.1883
	1005	0.9707		339	0.9806
V	919	4.2083	V	189	5.4939
	140	7.2479		266	7.4536
	660	8.8405		96	8.9189
	929	3.6544		211	9.5322
	612	5.5565		389	4.3899
	119	13.7794		276	1.8774
	583	6.1303		473	3.2735
	631	6.1355		283	3.2884
	726	8.4364		49	4.5934
	617	5.2843		49	9.3689

6F. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual instar V larvae of P. conspersa collected at station 1 in May and November 1984 and in March 1985.

Date	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
1984		
21/5	460	3.7358
	220	7.8314
	513	5.9730
	319	7.6248
	193	3.8088
	210	6.5913
	512	2.9590
	421	2.2022
	283	12.9766
	313	5.3829
21/11	98	2.9321
	305	4.0666
	169	3.0594
	238	2.4092
	208	2.9951
	90	1.3549
	138	5.0879
	387	2.9577
	289	7.7199
	299	2.2929
1985		
15/3	242	1.8642
	215	10.1889
	99	1.4652
	97	4.8975
	114	3.3258
	208	3.4842
	144	1.4618
	311	3.8043
	295	5.5456
	305	7.1017

6F. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual instar V larvae of P. conspersa collected at station 4 every two months from May 1984 to May 1985 (data for September 1984 is given in Appendix 6A).

Date	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)	Date	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
1984			1985		
21/5	79	10.5248	17/1	16	3.1678
	48	4.6870		34	2.8135
	38	7.2495		29	2.8930
	28	18.8755		9	5.8045
	33	8.5923		40	1.9038
	81	2.5880		30	2.9613
	85	5.3010		14	3.1043
	35	7.8279		39	1.8130
	72	2.4333		52	1.0747
	52	7.5044		10	5.7614
18/7	105	7.6238	15/3	21	3.3060
	99	5.9355		85	5.4684
	108	22.8462		75	1.9467
	83	10.4232		77	1.5321
	60	17.8901		27	3.8072
	114	13.0849		14	4.7903
	64	18.5411		67	2.7680
	154	3.6658		59	5.0533
	120	4.3013		17	3.0422
	78	15.6389		66	2.9084
21/11	78	7.4324	17/5	77	4.5588
	39	3.1325		97	6.2506
	15	2.2589		26	8.1876
	73	6.2530		51	1.5102
	40	5.4517		62	5.2701
	66	2.5157		89	3.7897
	18	7.8376		19	8.9075
	12	7.7838		57	4.1097
	39	3.6462		74	7.5506
	47	5.0236		40	5.4689

6G. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual instar V larvae of P. conspersa collected at stations 1, D, X, F and 4 in July 1985.

Station	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
1	183	3.5768
	212	5.5862
	227	9.7798
	291	8.3815
	321	1.4012
	200	3.8916
	297	2.3232
	156	5.0082
	312	3.2469
	386	7.4297
D	169	2.0213
	264	2.4124
	170	3.0515
	190	3.9388
	140	5.0917
	275	4.9976
	361	2.7067
	138	10.2360
	83	3.4346
	162	3.2267
X	123	4.4531
	91	2.3186
	78	14.3806
	109	13.2481
	118	3.8990
	128	7.0555
	116	8.6670
	66	9.3496
	95	4.5154
	79	2.8081
F	28	6.0387
	53	3.3010
	75	20.6817
	59	6.2395
	50	25.7694
	99	7.8248
	77	6.1858
	56	14.8902
	104	11.4459
	54	18.9719
4	37	5.0369
	80	9.5334
	100	3.1288
	98	3.8863
	37	7.9318
	60	7.3265
	75	6.3925
	64	5.2522
	52	5.2179
	93	6.4298

6H. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual instar V larvae of P. conspersa collected from the Aylesbeare stream in May and November 1985 and March 1986.

Date	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
1985		
16/5	43	2.8310
	40	5.4843
	33	6.2403
	18	12.9540
	36	4.4554
	23	9.0032
	17	11.0886
	18	8.3616
	26	5.7025
	35	4.5557
19/11	24	3.4594
	22	1.6135
	11	5.4021
	12	3.9910
	23	2.8981
	10	9.5845
	15	3.9488
	20	2.9325
	15	4.8717
	25	2.3104
1986		
11/3	23	10.6406
	21	16.4618
	28	9.6221
	20	20.5129
	43	8.2278
	45	7.9093
	54	4.6809
	37	9.3364
	24	19.0259
	31	14.4855

6I. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual instar V larvae, pharate pupae (PP), pupae (P) and adults (A) of P. conspersa collected at station 1 in July 1984.

Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)	Instar	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
V	456	8.2980	P (FEMALE)	66	17.9212
	431	7.3743		76	12.7755
	482	2.8742		124	16.377
	247	7.0150		84	5.9879
	268	22.0437		118	14.6294
	321	6.6642		107	13.3631
	525	2.7203		57	19.5072
	448	10.3171			
	283	9.7365			
	273	8.6006			
PP	132	8.3688	A (MALE)	109	3.4975
	98	13.5904		161	5.4621
	152	7.4917		144	4.4439
	136	7.8039		161	3.4086
	210	7.7589		104	6.6404
				171	4.7155
				103	4.6142
				58	6.9528
				23	4.3268
				38	4.1256
P (MALE)	169	8.5407	A (FEMALE)	93	12.8719
	75	7.8608		84	11.4722
	168	8.6553		90	9.3585
	74	9.5242		116	6.6349
	102	11.4208		57	10.8355
	209	6.6407		132	5.5515
	59	6.3620			

6J. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual prepupae of P. conspersa collected at station 1 in May 1985

Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
73	6.3107
112	8.2937
93	6.6648
114	5.9053
126	5.2149
99	7.3023
104	8.2273

6K. Concentration of copper ($\mu g\ g^{-1}$) and dry weight (mg) in individual female pupae and shed larval skins of P. conspersa collected as instar V larvae at station 1 in July and August 1985.

	Specimen	Copper Concentration ($\mu g\ g^{-1}$)	Dry Weight (mg)
1.	female pupa	9.3596	60
	shed larval skin	0.4890	523
2.	female pupa	11.6397	78
	shed larval skin	0.5883	561
3.	female pupa	7.1049	72
	shed larval skin	0.4672	541
4.	female pupa	7.7519	57
	shed larval skin	0.3700	494
5.	female pupa	6.9283	82
	shed larval skin	0.4392	480
6.	female pupa	9.0428	57
	shed larval skin	0.5115	517
7.	female pupa	5.5257	69
	shed larval skin	0.3651	608
8.	female pupa	7.7652	67
	shed larval skin	0.3808	507
9.	female pupa	6.2470	77
	shed larval skin	0.3131	589
10.	female pupa	8.2060	80
	shed larval skin	0.3853	516

6L. Concentration of copper ($\mu g\ g^{-1}$) and dry weight (mg) in chironomid and coleopteran larvae collected in ten sample units at station 1 in January and July 1986.

JANUARY			JULY		
Sample Unit	Copper Concentration ($\mu g\ g^{-1}$)	Dry Weight (mg)	Sample Unit	Copper Concentration ($\mu g\ g^{-1}$)	Dry Weight (mg)
chironomid larvae			chironomid larvae		
1	107	2.0408	1	763	13.2478
2	378	2.4807	2	1731	4.3416
3	434	1.7268	3	849	8.1855
4	490	3.1155	4	1143	3.2523
5	518	2.2147	5	1054	7.2798
6	286	1.8469	6	766	10.7993
7	765	1.8156	7	1237	5.4933
8	286	2.9242	8	1044	7.1194
9	380	2.3889	9	1144	8.4035
10	422	2.5981	10	807	6.5448
coleopteran larvae			coleopteran larvae		
1	459	3.5419	1	286	29.6310
2	252	18.6119	2	295	22.4964
3	-	-	3	219	29.7000
4	177	12.9775	4	745	2.1673
5	298	14.2410	5	295	29.5530
6	121	8.6260	6	248	27.3220
7	-	-	7	424	14.3859
8	159	15.2756	8	359	9.9876
9	292	15.3727	9	389	16.5449
10	149	7.4823	10	302	18.9920

6M. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual instar V larvae of P. conspersa collected from the Porthtowan stream in May 1985.

Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
143	10.4062
241	7.0155
167	5.2690
244	8.9933
92	15.1089
125	2.4427
156	8.7122
114	11.1439
156	6.4233
122	12.7478

APPENDIX 7

7A. Weekly progress of the transfer experiment, showing the fate of the 70 larvae of P. conspersa in each of the fed (F) and unfed (U) treatments of the Aylesbeare stream (population A) and Darley Brook (population B) larvae. Note that 'enclosed' refers to those larvae which had constructed pupal cases; 'removed' refers to larvae taken for copper analysis.

Time (number of days after commencing transfer)	Fate of larvae	Population A (Aylesbeare stream)		Population B (Darley Brook)	
		Treatment F	Treatment U	Treatment F	Treatment U
7	dead	1	1	2	1
	escaped	0	0	0	1
	enclosed	0	0	0	0
	removed	10	10	10	10
	remaining	59	59	58	58
14	dead	3	0	0	0
	escaped	1	0	0	0
	enclosed	1	1	0	1
	removed	10	10	10	10
	remaining	44	48	48	47
21	dead	2	3	1	1
	escaped	0	0	0	1
	enclosed	0	3	1	0
	removed	10	10	10	10
	remaining	32	32	36	35
28	dead	2	6	2	5
	escaped	0	2	0	4
	enclosed	1	1	1	2
	removed	10	10	10	10
	remaining	19	13	23	14
35	dead	1	1	0	0
	escaped	0	0	0	0
	enclosed	0	1	1	1
	removed	10	11	10	13
	remaining	8		12	
42	dead	1		2	
	escaped	0		0	
	enclosed	1		1	
	removed	6		9	

7B. Concentration of copper ($\mu g\ g^{-1}$) and dry body weight (mg) in individual instar V larvae of P. conspersa taken weekly from the fed (F) and unfed (U) treatments of the Aylesbeare stream (population A) and Darley Brook (population B) larvae.

Population A (Aylesbeare stream)

Time (number of days after commencing transfer)	Treatment F		Treatment U	
	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
7	40	10.9615	32	8.0155
	66	3.1814	20	12.0119
	65	4.2496	35	7.8199
	59	6.0443	40	5.2755
	49	9.9628	16	12.6849
	55	5.2044	25	8.2424
14	61	5.5416	66	4.0889
	50	13.7649	19	10.7483
	76	3.7035	112	2.2492
	74	6.4359	29	8.3273
	61	6.6274	39	4.3558
	53	5.7610	38	5.7566
21	40	8.3121	43	9.3632
	56	7.2089	24	10.6982
	52	9.8182	34	9.9896
	63	5.1208	56	3.5739
	68	4.8456	48	5.2194
	39	10.3337	32	9.0937
28	58	6.4371	75	9.3392
	30	14.7090	74	3.7159
	34	13.1355	62	7.0166
	38	13.2048	53	9.4584
	50	10.5472	62	6.1123
	41	15.6909	90	1.9312
35	40	11.2897	130	2.5430
	65	11.2022	151	1.6627
	54	12.4884	100	6.0787
	54	14.1839	137	3.0937
	71	13.7247	120	6.4323
	82	9.9917	149	2.9887
42	37	20.5339		
	70	10.5514		
	68	12.1493		
	46	13.3106		
	52	14.1844		
	40	16.7293		

Population B (Darley Brook)

Time (number of days after commencing transfer)	Treatment F		Treatment U	
	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)	Copper Concentration ($\mu g\ g^{-1}$)	Dry Body Weight (mg)
7	266	6.3191	321	3.0039
	142	8.2446	190	11.5323
	103	13.4059	196	5.1186
	196	5.2772	407	2.5932
	128	8.3097	355	3.8394
	376	5.0231	286	3.3537
	278	3.9802		
	166	7.7699		
	79	9.3418		
	126	8.0290		
14	262	5.0168	220	11.4729
	326	3.8950	328	2.8685
	191	6.2701	257	2.6983
	214	5.8237	360	2.7950
	170	7.2021	261	5.2464
	197	6.0132	446	1.8302
21	91	9.6990	169	9.6395
	303	5.4705	280	4.0069
	163	9.8014	397	2.1106
	88	10.3994	292	4.5978
	140	9.6916	343	2.5917
	74	12.0418	311	4.0290
28	88	11.0725	251	6.6906
	162	7.5497	387	1.9149
	216	7.5364	277	2.1439
	55	13.0084	366	3.7897
	108	11.2438	139	9.5222
	80	14.0934	501	1.5780
35	86	12.0602	190	2.2190
	116	9.5146	452	1.9135
	59	13.9518	415	4.7414
	114	11.8490	388	4.1282
	89	10.8289	265	3.6692
	56	19.5918	200	3.3136
42	99	13.1238		
	113	8.8083		
	60	18.8908		
	72	12.2549		
	68	18.4147		
	125	8.1959		

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THE MEASUREMENT OF COPPER IN INDIVIDUAL AQUATIC INSECT LARVAE

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ABSTRACT

Using graphite furnace atomic absorption spectroscopy a programme has been developed to measure copper concentrations in single specimens of the larvae, and also pupae and adults, of the caddis fly Plectrocnemia conspersa. Each larval instar from a copper contaminated stream contained significantly more copper than the same instar in a control stream. Highest concentrations (over 1100$\mu g g^{-1}$) were recorded in young larvae, and there was an exponential decrease in larval copper concentration with increasing body weight for both populations. Significantly lower concentrations were found in pupae and adults.

INTRODUCTION

The analysis of the metal content of aquatic organisms is invaluable in the quality assessment of surface water, not least of all because of the difficulty of predicting biological availability of metals from physical and chemical data.

The use of plants and animals as monitors of metal contamination has received more attention in marine and estuarine systems than in freshwater. Recently (1,2) it has been shown that metal analysis of insects serves as a useful means of monitoring heavy metal pollution in freshwater ecosystems; furthermore insects are usually prominent in soft waters, and are almost invariably the dominant group of invertebrates in metal enriched or contaminated streams (1,3,4).

In view of this it is surprising that so little work has been undertaken on the metal concentrations of insects. No doubt this is due to their small size, and most workers have pooled a number of individuals to allow analysis by flame atomic absorption spectroscopy (flame AAS). However this yields little or no information on metal levels in an individual, or on the variation associated with a particular mean concentration.

In graphite furnace atomic absorption spectroscopy (graphite furnace AAS) the flame is replaced by an electrothermally-heated hollow graphite tube. This technique offers particular advantages in situations where sample size is limited (5), and both sensitivity and selectivity are comparable to that associated with neutron activation analysis, which has also been used in the investigation of metal concentrations of aquatic insects (6).

Although graphite furnace AAS has been used to measure copper levels in pooled aquatic insect samples (7,8) and in micro-organisms (9), this paper is, to the authors knowledge, the first account of copper concentrations in single specimens of aquatic insects.

MATERIALS AND METHODS

Sample collection and preparation

Darley Brook drains water from a disused copper mine (Nat. Grid Ref. SX 265723) on Bodmin Moor, Cornwall. After flowing approximately 1 Km an uncontaminated stream enters the Brook, which then flows another 2 Km before its confluence with the River Lynher. Larvae of the predatory net-spinning caddis Plectrocnemia conspersa (Curtis) were collected from the copper rich head waters, just below an adit (Site 1) where there was an invertebrate community of reduced diversity, and also from the uncontaminated stream (Site 2) which supported a diverse invertebrate community. Water quality data for these sites is given in Table 1.

TABLE 1. Monthly mean and ranges in concentrations of selected water quality characteristics recorded from 19 monthly samples collected at sites 1 and 2, 1984-1985. (Metal levels were determined from 0.45 µm filtered samples and measured by flame AAS).

PARAMETER	SITE 1	SITE 2
COPPER (mgl^{-1})	0.85 0.73-0.93	<0.04
CALCIUM (mgl^{-1})	1.42 1.04-2.37	2.81 2.07-4.02
MAGNESIUM (mgl^{-1})	1.08 0.86-1.27	0.91 0.74-1.07
HARDNESS (mgl^{-1})	7.96 6.13-10.68	10.77 8.21-14.19
CONDUCTIVITY K25 (μScm^{-1})	86 52-126	90 52-127
pH	5.6 5.0-6.1	6.4 5.7-7.4
DISSOLVED OXYGEN (mgl^{-1})	9.31 7.67-11.02	10.55 8.60-12.87
% OXYGEN SATURATION	85 70-106	96 85-110

To avoid cannibalism each larva was placed in a separate cell in a compartmentalised tray filled with water taken at the site of collection, and starved for 48 hours at 10°C to remove the contaminating effects of any ingested material. The larvae were separated into instars from head width measurements, and then frozen.

The larvae were subsequently freeze dried (600 Pa pressure, -55°C, 24 hours) and weighed on a Cahn 29 automatic electrobalance (Cahn Instruments Inc., Cerritos, USA). They were then digested in approximately 4 ml of Aristar grade nitric acid (BDH Chemicals, Poole, Dorset) at 110°C for 4 hours, and made upto 5 ml with distilled deionized water.

Graphite furnace AAS operating conditions

Table 2 gives details of the programme devised to analyse the individual larval digests using an Instrumentation Laboratory Video 12 Atomic Absorption Spectrometer with Furnace Atomizer 655 (Instrumentation Laboratory Inc., Andover, USA).

TABLE 2. Graphite furnace AAS operating conditions

WAVELENGTH	: 327.4 nm	LAMP CURRENT	: 3.5 mA
SLIT WIDTH	: 1.0 nm	INJECTION SIZE	: 25 µl
PURGE GAS	: Nitrogen	WORKING RANGE	: 1 TO 50 $ngml^{-1}$
DEUTERIUM BACKGROUND CORRECTION			
NON-COATED GRAPHITE TUBES			
PHASE	TEMPERATURE (^{o}C)	RAMP (X5 sec)	
1	100	7	
2	200	8	
3	750	5	
4	900	6	
5	1800	0	
6	1800	1	

RESULTS

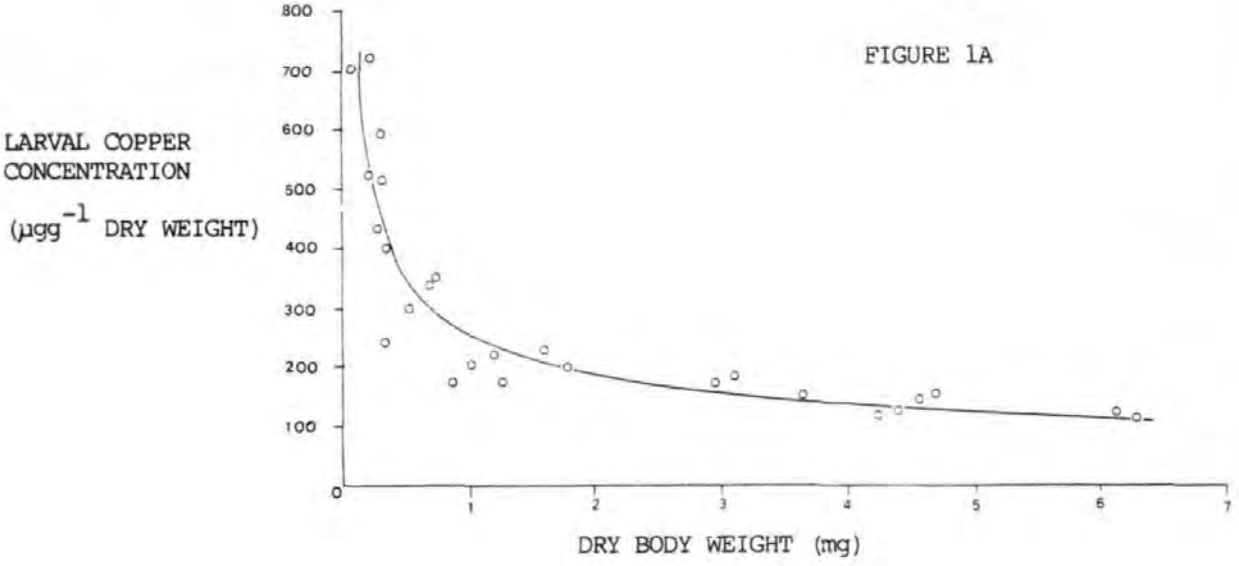
During this investigation it was found that larvae of P.conspersa from site 1 consistently contained elevated copper concentrations, reflecting the high copper levels in the water. Whilst the copper content of the larvae appears to vary seasonally, this forms part of a wider investigation, the results of which will be published elsewhere; it is more instructive here to consider results from larvae collected on just one sampling occasion, hence the copper concentrations in 51 individual larvae from sites 1 and 2 for April 1984 are summarised in Table 3.

TABLE 3. Concentration of copper (μgg^{-1}) in larval instars III to V of P.conspersa at sites 1 and 2, April 1984, showing range, mean, standard error (S.E.) and number (n) of larvae which were individually analysed.

INSTAR	SITE 1				SITE 2			
	n	MEAN	S.E.	RANGE	n	MEAN	S.E.	RANGE
III	6	581	46	432-720	8	106	14	48-177
IV	10	264	25	173-401	9	39	3	27-54
V	10	149	10	113-202	8	18	3	10-31

Each larval instar of P.conspersa from site 1 has a very much higher copper concentration than the same instar at site 2. Within each site there is also a significant ($p<0.01$) difference in the copper concentrations between the instars, with minimal overlap of the ranges. The lowest concentration is shown for the Vth (final) instar larvae, and the highest in instar III. Furthermore concentrations of over 1100 μgg^{-1} and 300 μgg^{-1} have been recorded in the much smaller instar II larvae at sites 1 and 2 respectively.

When the copper concentration for each of the larvae is plotted against dry body weight for the April 1984 samples, as in Figure 1, it can be seen that there is an exponential decrease in copper concentration with increasing body size, and this is evident at both sites.



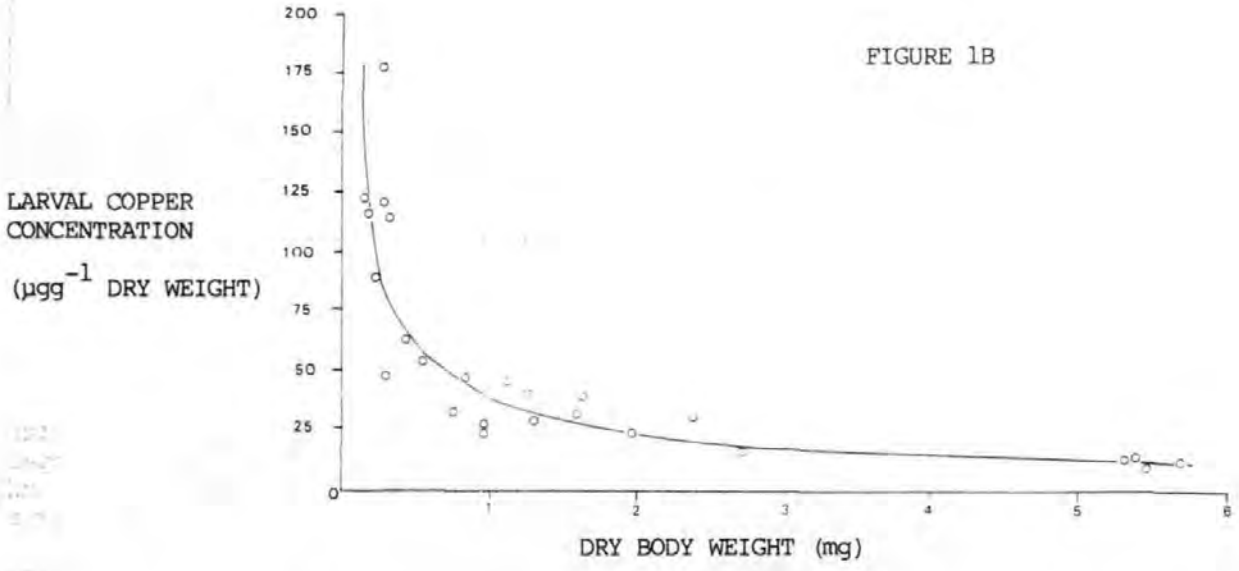


FIGURE 1. Relationship between dry body weight (mg) and copper concentration (µgg^{-1}) in individual larvae (instars III to V) of P.conspersa collected in April 1984 at (A) Site 1, and (B) Site 2.

This relationship was further examined using linear regression analysis, with both variables $\log_{10}$ transformed. The resulting equations are given in Table 4, and show a highly significant negative correlation between weight and concentration.

TABLE 4. Regression constants for the equation Y=a+bX, where Y=$\log_{10}$ larval copper concentration ($\mu g g^{-1}$) and X=$\log_{10}$ dry body weight (mg) for P.conspersa, collected in April 1984 at Sites 1 and 2.

SITE	NUMBER OF LARVAE	REGRESSION CONSTANTS a	b	CORRELATION COEFFICIENT r	SIGNIFICANCE LEVEL
1	26	2.41	-0.440	-0.934	$p<0.001$
2	25	1.59	-0.662	-0.932	$p<0.001$

Larvae with full digestive tracts exhibited a similar copper-weight relationship, and contained significantly ($p<0.01$) higher copper levels than the 48-hour starved larvae.

Single specimens of pupae and adults of P.conspersa from site 1 have also been analysed, and Table 5 summarises whole organism concentrations for pupae and adults as well as for final instar larvae collected in July 1984. There is a significant ($p<0.001$) decrease in copper concentration between instar V larvae and the post-larval instars, which is currently being investigated.

TABLE 5. Copper concentrations ($\mu g g^{-1}$) and weights (mg) of adults, pupae and instar V larvae of P.conspersa collected in July 1984 from site 1 (n=number of individuals).

		n	MEAN	STANDARD ERROR	RANGE
ADULTS	-COPPER	16	103	11	23-171
	-WEIGHT	16	6.5570	0.7482	3.4086-12.8719
PUPAE	-COPPER	14	106	12	57-209
	-WEIGHT	14	11.3976	1.1913	5.9879-19.5072
INSTAR V	-COPPER	10	373	32	247-525
	-WEIGHT	10	8.5644	1.7002	2.7203-22.0437

DISCUSSION

The results presented in this paper, made possible by a technique developed with graphite furnace AAS, have yielded detailed information on the variation of copper concentrations between individuals, between different stages of the life cycle and between different populations of an aquatic insect exposed to different water qualities.

It has been shown in this investigation that larvae of P.conspersa accumulate copper from the water, with levels measurable even in larvae taken from site 2, where the water copper concentration is below the detection limit of flame AAS ($<0.04 mg l^{-1}$). In another investigation (10) on the River Hayle, Cornwall, some correlation was found between the copper concentration in 'free-living' Trichoptera and that in water.

Graphite furnace AAS has also facilitated (with a comparatively small number of larvae) an examination of the relationship between larval copper concentration and dry body weight. Smock (6) observed two general relationships between metal concentration and size in aquatic insects. Most metals (Co,Cr,Fe,Sb,Sc) showed an exponential decrease in body concentration with increasing size, indicating surface adsorption as an important mode of metal accumulation. For the other metals (K,Mn,Na) size had no significant effect, or at most, there was a slight increase in concentration with increasing size, suggesting absorption as the more important factor determining metal concentration.

The exponential decrease in larval copper concentration with increasing body weight shown here for P.conspersa is similar to that in other insects (6), and suggests some form of surface to volume phenomenon. It is hoped that the precise nature of this effect will be elucidated by the use of ^{64}Cu and ultrastructural studies which are now being employed to extend this investigation.

It has been shown in this investigation that ingested material is also important in determining the metal concentration of the larvae. Similar observations have been recorded in the mayfly Stenonema modestum (6) and in the aquatic cranefly Tipula spp (11). It is possible to quantitatively differentiate between the various copper fractions in P.conspersa; ingested material accounts for approximately 60% of the whole-body copper content (non-starved instar V larvae), 30% is associated with the body wall (starved instar V larvae) and 10% is absorbed in the tissues (non-aquatic adults). These figures are similar to those calculated by Smock (6) for chromium in S.modestum and S.interpunctatum.

Metal analysis by flame AAS is dependent on having sufficient tissue with a concentration above the detection limit, restricting its use to insects with large population densities, and generally to large insects or older stages and adults (10,12). Such limitations are overcome by graphite furnace AAS, although the technique is more time consuming and more prone to contamination than flame AAS. In the present study it has enabled the range of copper concentrations in several stages of the life cycle of an aquatic insect to be assessed, and it has provided information on the partitioning of copper within the larvae. Furthermore graphite furnace AAS greatly improves the precision of the data, and also increases the information on the impact of a metal on a population in spatial and temporal terms, both of which are essential in any monitoring programme.

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